Abstracts

Circadian clocks and feeding behavior

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Biological functions display daily rhythms, including feeding/lipogenesis during the active period and fasting/lipolysis during the resting period. Such a temporal organization is controlled by a circadian timing system made of interconnected endogenous clocks and oscillators. The master circadian clock, located in the suprachiasmatic nuclei of the hypothalamus, is mainly reset by light and synchronizes peripheral oscillations. The secondary clocks/oscillators, present in many brain regions and peripheral organs (e.g. liver and white adipose tissue), can be shifted by meal timing, as modulated by temporal restricted feeding, while the suprachiasmatic clock is relatively impervious to the impact of meal time. However, timed calorie restriction (i.e. when only a hypocaloric diet is given every day) is able to modify the suprachiasmatic clockwork and to modulate synchronisation to light, via increased phase-shifting effects of light. High-fat feeding also affects the suprachiasmatic clockwork and modulates synchronisation to light, via reduced shifting effects of light.

Secondary clocks in the brain outside the suprachiasmatic nuclei are differentially influenced by meal timing. Circadian oscillations can be either highly sensitive to feeding cues (i.e. their phase is shifted according to meal schedule) in some structures (e.g. paraventricular hypothalamic nuclei and cerebellum) or hardly affected in others (e.g. hippocampus). Furthermore, the circadian anticipation of meal time relies on cerebellum integrity.

These data indicate that feeding cues can markedly modulate the timing of the circadian system, not only at the periphery, but also within the brain. The light-entrainable clock in the suprachiasmatic nuclei, which drives the sleep-wake cycle, is only sensitive to nutritional cues associated with metabolically challenging conditions. The cerebral clocks sensitive to meal time, such as those in the metabolic hypothalamus and cerebellum, define a network of coupled meal-entrainable oscillators controlling the feeding cycle.

Numerous metabolic processes, like adipogenesis, are regulated by transcriptional networks shared by circadian clocks. Obesity and diabetes are associated with circadian alterations. Conversely, circadian dysfunctions, either due to impaired clockwork (e.g. knock-out of clock genes) or impaired synchronisation (e.g. chronic jet-lag or shift-work), are associated with increased metabolic risks. A chronic desynchronisation can trigger fat overload, leading to a so-called 'chronobesity'.

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Morbidly obese patients and drug: the clinical pharmacologist's view

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Drug lipophilicity, organ blood flow , tissue binding, drug plasma protein binding and the ionization state are the ABCs of pharmacokineticists. Most of us will consider that a lipophilic drug administered to an obese patient will lead to an increase of the peripheral distribution and perhaps to a tissue accumulation. However, we have to admit that few studies are performed in obese patients and even more rarely on morbidly obese. Meanwhile we all teach these concepts hoping that they are generally true. Science is hard and unsympathetic with us and reading the results of studies should encourage us to be more circumspect. Indeed, even if lipophilicity could be an important determinant for some drugs, others are less well predicted by this physicochemical property. Obesity is linked to a number of co-morbidities (i.e. heart insufficiency, hypertension) or to modification of the way of life (decrease in exercise), which could also affect pharmacokinetic parameters. The volume of distribution changes in the obese as well as clearance is drug-specific. So, should we have to recommend performing pharmacokinetic studies in obese patients for all drug development? This question has to be discussed as well as which design should be used, full profile and non-compartmental analysis or population pharmacokinetic modeling? Furthermore, which metric should be test, body weight, body mass index, body surface area…? All these issues will be addressed but all should not be definitively settled.

Is obesity a disease or only an adaptation?

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Obesity is increasing all around the world at an epidemic rate. Although France is not the most affected country, the last OBEPI study shows that 14.5% of the population was obese and 31.9% overweight. In fact, only 50% of the population has a normal body weight.

Increasing body weight is an adaptation to the large changes in life-style that are occurring with a decrease in physical activity and the development of sedentary behaviours as well as changes in food habits patterns. This leads to a positive energy balance and the body has no other solution than to increase fat mass. The increase in obesity prevalence is problematic, as this condition is associated with health complications such as diabetes and cardiovascular diseases, more particularly when the excess body fat is stored in the deep abdominal region. Cut-off values for obesity, de?ned as a body mass index (BMI, kg = m^2)>30 come from the epidemiological observations of the large increase in mortality risk above this level. However, we can found some people with large obesity and lacking of metabolic alteration usually associated to obesity (obese metabolically normal), subjects only prone to mechanical complications of obesity. On the other hand, patients with total lack of fat, as seen in lipoatrophic diabetes, have also important metabolic complications. Understanding the underlying mechanisms leading to ectopic fat accumulation are of major importance to detect subjects for whom weight gain will be particularly deleterious. However on a Public Health perspective, labelling obesity as a disease is a necessary step in a campaign to combat obesity. Prevention by deep changes in lifestyle is mandatory as many of chronic diseases such as diabetes, cardio-vascular diseases, cancer could be prevented by better management of body weight.

Mechanisms and consequences of human adipose tissue inflammation

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Obesity, defined as an excess of white adipose tissue mass, is considered a sterile and chronic inflammatory state, characterized by increased circulating levels of inflammatory factors as cytokines and chemokines. It is now widely recognized that the adipose tissue it-self is a site of inflammation in obesity. In this talk, I will provide examples to show how transcriptomic analysis increased our knowledge of obesity-linked adipose tissue pathology. Our initial studies detected major alterations in inflammation-related genes, including increased expression of macrophage markers, in obese adipose tissue. Macrophage accumulation was confirmed by immunohistochemistry, with a more marked effect of obesity in visceral than subcutaneous fat depot. A positive relationship between the number of macrophages in visceral fat and the severity of non-alcoholic hepatic histopathology was found in a large population of obese subjects (Tordjman et al, *J Hepatol*, 2009; 51: 354–62). This suggests that macrophage accumulation contributes to the well-known association between abdominal obesity and metabolic complications. The mechanisms relaying immune cell infiltration in hypertrophied adipose tissue are the focus of intense research. We contributed to show the importance of chemokines, including CCL5/RANTES, which promote monocyte diapedesis and macrophage survival (Keophiphath et al, *Arterioscler Thromb Vasc Biol*, 2010; 30: 39–45). Further evaluation of transcriptomic interactions characterizing human adipose tissue showed a strong relationship linking inflammation to extracellular matrix components. Indeed, the obese adipose tissue displays large fibrotic areas, with distinct pattern and composition according to tissue anatomic location (Divoux et al, *Diabetes*, 2010; 59: 2817–25). Various cell types, including macrophages, mast cells and pre-adipocytes were detected in fibrotic bundles. Isolated human pre-adipocytes acquire a pro-fibrotic phenotype when cultured with activated macrophage conditioned media (Keophiphath et al, *Mol Endocrinol*, 2009; 23:11–24) suggesting their contribution to fibrosis deposition in the inflammatory microenvironment of obese adipose tissue. Thus, the inflamed adipose tissue undergoes complex and interrelated alterations in obesity. The challenging task of determining their causes and consequences is a prerequisite for new therapeutic approaches targeting adipose tissue homeostasis.

Obesity and cancer: therapeutics aspects

S Hamza, B Guiu, P Hillon

The knowledge of the mechanisms involved in the relationship between obesity and cancer may help us to better understand the therapeutic aspects in cancer prevention and treatment in overweight patients. The mechanisms involved in carcinogenesis related to obesity are for a large part in connection with an excess of visceral fat. Visceral fat is responsible not only for an increased risk of cancer but also for a worsened severity and in some cases for loss of drug efficacy. These deleterious effects are essentially due to dysfunctional visceral adipose tissue. Changes in the physiological functions of adipose tissue lead to insulin resistance, chronic inflammation and altered secretion of adipokines.

Insulin resistance due to inflammation and free fatty acid excess contributes to the increased risk of cancer in obese people. Insulin resistance induces insulin secretion by pancreatic beta-cells and increases bio-availability of IGF-1, responsible for cellular growth and decreased apoptosis. The role of Insulin resistance explains the protective effect of drugs increasing insulin sensitivity like metformin, against hepatocellular carcinoma risk.

Altered secretion of adipokines in obese patients results from pro-inflammatory cytokines, leptin, VEGF and plasminogen activator inhibitor-1 (PAI-1) hypersecretion, and decreased adiponectin secretion. Adipokin abnormalities are involved in different carcinogenesis steps: cellular proliferation and apoptosis, angiogenesis, and extracellular alterations through the activation of matrix metalloproteinases leading to tumour growth, progression, and metastasis. Some of these mechanisms will be potential therapeutic targets in Human cancer in the future. They should explain the decreased efficacy of anti-angiogenic drugs described in high visceral fat volume patients suffering from metastatic colorectal and renal cancer.

The fight against visceral fat excess is based on nutritional recommendations and physical activity. In the future, some new antiangiogenic agents specifically inhibiting angiogenic receptors of visceral adipose tissue, could take a predominant place in obesity treatment. These drugs that are effective on weight loss in obese animals, are currently evaluated in patients suffering from metastatic prostate cancer whose severity seems to be strongly related to visceral obesity.

Melanopsin as a sleep modulator: non circadian effects of light on sleep and alertness in nocturnal and diurnal rodents

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Light can influence sleep and alertness either indirectly through a well-defined circadian pathway involving the suprachiasmatic nucleus (SCN), or directly through poorly understood non-visual, non-circadian mechanisms. Three photoreceptive cell types, rods, cones, and melanopsin-expressing intrinsically photosensitive retinal ganglion cells (ipRGCs), are responsible for light detection in the retina. Melanopsin ipRGCs are crucial for conveying non-visual light information to the brain. The overall goal of the presentation is to demonstrate in rodents how light, beyond its effects on circadian entrainment, can exert via melanopsin, a direct influence on sleep and alertness as well as on sleep homeostasis.

To do this we extensively analyzed under various light dark regimen sleep and the EEG in mice lacking melanopsin and/or the circadian drive. Light exert a direct acute and sustained sleep promoting effect whereas darkness facilitates alertness. We also demonstrated that melanopsin affects sleep homeostasis and that the non-circadian direct effects of light interact with the circadian drive. Our extensive analysis suggest that the direct effects of light contribute to half of sleep wake architecture, thus representing a key sleep regulatory mechanism. We also addressed this question translationally in diurnal rodents (arvicanthis ansorgei, first validation of a diurnal rodent as a model for sleep studies) in order to understand how the non-circadian effects of light switch between nocturnal and diurnal species. Anatomical study looking at c-Fos induction in response to light suggests that the sleep promoting (i.e. galanin-positive) neurons of the ventrolateral preoptic (VLPO) or the SCN may act as possible relays mediating the direct effects of light on vigilance states.

Based on our findings, we propose that the direct non-circadian effects of light and melanopsin represent a key sleep regulatory mechanism working in interaction with the circadian and homeostatic drives.

Genetics, adenosine and sleep-wake systems: how coffee keeps us awake

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Adenosine is importantly involved in regulating vigilance after prolonged wakefulness and EEG delta activity during sleep. By blocking adenosine receptors, caffeine promotes vigilance and reduces sleepiness. Human pharmaco-genetics provides a powerful approach to elucidate physiological processes of sleep-wake regulation, and to identify molecular mechanisms underlying individual differences in response to stimulants. We found that a single nucleotide polymorphism (SNP) in the adenosine A2A receptor gene (*ADORA2A*) contributes to individual sensitivity to caffeine effects on sleep. We further examined the impact of genetic variation of *ADORA2A* and sleep deprivation on vigilant attention, subjective sleepiness, EEG, and individual responses to caffeine and modafinil. The effects of eight different SNP variants of *ADORA2A* were examined in healthy young men. The carriers of a distinct *ADORA2A* haplotype (referred to as haplotype HT4) showed higher vigilance during prolonged waking than carriers of non-HT4 haplotype alleles. Caffeine failed to counteract the consequences of sleep loss on psychomotor speed and EEG delta activity in the carriers of haplotype HT4. Modafinil, which does not interfere with A2A receptors, mitigated the effects of prolonged wakefulness irrespectively of *ADORA2A* haplotype. These findings highlight the importance of genetic factors in studying pharmacological interference with sleep-wake regulation. They demonstrate that genetic variation of *ADORA2A* affects psychomotor response speed and modulates the effects of caffeine after sleep deprivation. In conclusion, A2A receptors contribute to the neurobehavioral and neurophysiological consequences of prolonged wakefulness, and may provide a promising target for the pharmacological improvement of impaired waking performance associated with sleep loss.

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02-O001

Obstructive sleep apnea increases sympathetic outflow independent of lesser neurovascular transduction

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Objectives: We tested the hypothesis that greater resting sympathetic activity in OSAS would relate to lesser sympathetic neurovascular transduction.

Background: Patients with obstructive sleep apnea syndrome (OSAS) have elevated sympathetic outflow independent of obesity or hypertension. However, greater outflow may not result in reduced limb blood flow, suggesting lower sympathetic neurovascular transduction that may cause elevated outflow for appropriate hemodynamic control.

Methods: We assessed resting sympathetic outflow and sympathetic neurovascular transduction in newly diagnosed OSAS without comorbidities ($N = 10$) and in age-matched ($N = 10$) and young ($N = 10$) healthy controls. Sympathetic activity was directly measured (microneurography) at rest and in response to sustained isometric handgrip exercise. Neurovascular transduction was derived from the relationship of sympathetic activity and blood pressure to leg blood flow during exercise.

Results: Sympathetic activity in OSAS was almost 2× the age-matched and 3× the younger controls. Neurovascular transduction was not different between OSAS and age-matched controls, but was lower in both compared to younger controls. Among all subjects, resting activity was related to transduction ($r^2 = 0.12$, $P = 0.04$), however this relation was much stronger without those with OSAS ($r^2 = 0.55$, $P < 0.01$).

Conclusions: Greater sympathetic activity in OSAS does not appear to derive solely from lesser neurovascular transduction. Hence, other potential mechanisms associated with OSAS likely result in greater sympathetic outflow. However, elevated outflow without lesser transduction may underlie the prevalent development of hypertension in this population.

02-O002

Sleep continuity in conscious hypercapnic critically ill patients

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Background: Patients in Intensive Care Units suffer from severe sleep alterations including reduced sleep time and intense sleep fragmentation. Very few studies have examined clinical consequences of these sleep alterations. A major issue for analyzing sleep in ICU patients is that sleep EEG and sleep cycle organization significantly differ from patterns observed in ambulatory patients. For instance, hypnograms often display polyphasic sleep with multiple naps distributed throughout the nycthemere, contrasting with monophasic sleep of ambulatory patients. Theses specificities prompt the search for new parameters for sleep quantifications in ICU sleep recordings.

Noninvasive ventilation (NIV) is the treatment for hypercapnic exacerbations in patients with chronic respiratory failure. Nevertheless, the failure rate remains relatively high, and many patients experience late NIV failure, despite an initial improvement.

Objective: The main objective of our study was to determine whether sleep continuity was associated with late NIV failure in patients with hypercapnic respiratory failure.

Methods: We performed, on the third day, 17-h polysomnographies in consecutive conscious and non sedated ICU patients admitted for acute hypercapnic exacerbation requiring NIV.

Sleep continuity was assessed by calculating the duration of each sleep bout. Then, we calculated the sleep time spent in very short sleep bouts (<10 min), in short naps (10–30 min) and in longer sleep bouts (>30 min).

Results: Polysomnographies of 16 patients were analyzed; Six patients had a poor outcome and 10 had a favorable outcome. Usual parameters (total sleep time, arousal index) were similar in both groups. In contrast, sleep time spent in very short sleep bout (<10 min; expressed as a % of total sleep time [TST]) was significantly higher in patient with poor outcome (84% [58–99] vs. 54% [45–69]; $P < 0.05$, respectively). In addition, the % of TST spent in sleep bouts lasting more than 10 min but <30 min was significantly higher in patient with good outcome (32% [20–38]) vs. 16% [8–23], $P < 0.05$, respectively). Age, arterial pH, pCO_2 and disease severity were similar in both groups.

Conclusion: Sleep continuity assessed by the time spent in very short sleep bouts and in short naps could be alternative parameter for sleep quantification in ICU patients.

02-O003

Early cardiovascular disease markers in 'healthy' OSA patients are correlated with sympathetic activity

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Rationale: Obstructive sleep apnea syndrome (OSAS) is associated with high Sympathetic activity (SA) and a subsequent risk of hypertension and atherosclerosis. Objectives were to assess SA and its impact on early cardiovascular disease markers.

Methods: In 25 otherwise healthy OSA and 13 aged-matched controls we measured SA by direct peroneal microneurography: Muscle Sympathetic Nerve Activity (MSNA). All subjects underwent a full polysomnography, 24 h Ambulatory Blood Pressure Monitoring (ABPM), arterial stiffness by Pulse Wave Velocity (PWV), vascular reactivity by Peripheral Arterial Tone (PAT) and early atherosclerosis by arterial carotid Intima Media Thickness (IMT). Preliminary data in seven OSA and seven controls were obtained after 6 month (CPAP for OSA).

Results: OSA patients (BMI 25.9 ± 2.8 kg/m^2; AHI 38.8 ± 20.4/h; Age 53 ± 11 years) and healthy controls (BMI 23.9 ± 2.6 kg/m^2; AHI 7.9 ± 5.8/h; Age 48 ± 13 years). They were comparable for all but for BMI ($P < 0.05$). At baseline SA was higher in OSA than in controls 39.8 ± 9.4 vs. 30.6 ± 7.3 bursts/min $P < 0.01$). SA was correlated with OSAS severity (mean nocturnal SaO_2, −0.401, $P < 0.05$). SA also correlated with cardiovascular markers, vascular reactivity PAT (−0.354, $P < 0.05$), systolic and diastolic office (0.464, $P < 0.01$) and ABPM blood pressure, and IMT (0.464, $P < 0.01$). Six-month CPAP treatment induced a significant reduction in SA -10.3 ± 8.1 vs. 1.5 ± 5.9 bursts/min ($P < 0.01$).

Conclusion: Early cardiovascular disease markers are correlated with SA, supporting the fact that SA is likely to play a significant role in the cardiovascular morbidity of OSA patients.

02-O004

Automated sleep apnea syndrome recognition from ECG recordings in heart failure patients

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Introduction: Sleep Apnea Syndrome (SAS) is associated with cadiovascular morbidity. The prevalence of this syndrome in heart failure population reaches 50%. The gold standard for diagnosis is polysomnography wich is an expensive procedure. Thus, efforts are made to develop alternative methods to detect SAS. A promising method, validated in subjects without cardiac pathologies, consists to analyse the Ecg-Derived Respiration (EDR) recorded overnight. The goal of the present study was to evaluate the possibility to diagnose SAS using EDR method in patients suffering from cardiac failure.

Methods: Thirty nine ($n = 39$) heart failure subjects with complete overnight polysomnography diagnosis (AHI < 15: $n = 11$, 15 ≤ AHI < 30, $n = 15$, AHI ≥ 30, $n = 13$; central, obstructive and mixed apneas) with an ECG recording, were included (age: 63.8 ± 16.6 years, weight: 82.4 ± 22 kg, height: 172.1 ± 6.3 m). An algorithm based on R-peak artefacts rejection and Fourier spectral analysis of the EDR signal was developped to determine the presence of SAS (choosen threshold: AHI > 15 for SAS+). The method was validated on the 70 recordings of the MIT apnea database and then applied to the set of heart failure patients.

Results: The developped method indicates an accuracy = 97.1% for the MIT database. For heart failure patients, the method permitted to recognize 27 out of 28 SAS+ and nine out of 11 SAS- (sensibility = 96.4%, specificity = 81.8%, accuracy = 92.3%). Interestingly enough, it was possible to diagnose seven out of eight SAS+ patients with a pacemaker and two out of two SAS+ patients with atrial fibrillation. Also, detection was possible for central and obstructive apnea syndrome.

Conclusions: The EDR method to detect SAS is thus applicable in heart failure patients, and could be easily implemented in routine 24 h-ECG analysis for preventive diagnosis. Further studies in larger population should specify explanations for diagnosis mistakes to increase the accuracy.

02-O005

In sleep apnea syndrome, nonalcoholic fatty liver disease (NAFLD) is associated with the severity of intermittent hypoxia and more severe endothelial dysfunction

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Introduction: Nonalcoholic fatty liver disease (NAFLD) begins with the aberrant accumulation of triglycerides in the liver, which in some individuals elicits an inflammatory response that can progress to cardiovascular complications, cirrhosis and liver cancer. Although NAFLD is strongly associated with obesity and insulin resistance, its pathogenesis remains poorly understood. In morbidly obese, intermittent hypoxia has been demonstrated as a contributing factor. NAFLD has not been investigated in an unselected obstructive sleep apnea (OSA) population. Beyond liver biopsy, there are non invasive validated tools allowing a screening of NAFLD in large samples of patients.

Aims: (i) To use non-invasive blood tests (Steatotest®, NASHtest® and Fibrotest®) to evaluate steatosis, NASH and fibrosis stage in a large cohort of OSA patients; (II) To assess endothelial function as measured by peripheral arterial tone (PAT) in OSA with or without NAFLD.

Patients and methods: Two hundred and twenty-six subjects referred for suspicion of OSA were included (men: 55%, median age: 56 years, mean BMI: 34 kg/m^2).

Results: 61.5% of OSA patients exhibited advanced steatosis as defined by a Steatotest® ≥S2. By multivariate analysis, triglycerides ($P < 0.0001$), insulin resistance (HOMA>3 ($P = 0.0004$)) and intermittent hypoxia as measured by cumulative time spent <90% of SaO2 (CT90) ($P = 0.01$) were independent factors for liver steatosis. Thirty-eight percent of OSA displayed possible or probable NASH (N1 or N2 with NASHtest®). In univariate analysis, CT90 was significantly associated with NASH ($P = 0.035$) but this became non significant in multivariate analysis. Endothelial function was more impaired in OSA patients with advanced steatosis ($P = 0.04$) and possible or probable NASH ($P = 0.013$). Office systolic blood pressure was also significantly more elevated in those with advanced steatosis and possible or probable NASH ($P = 0.01$ and $P = 0.004$, respectively).

Discussion/conclusion: In a large unselected population of OSA patients, the severity of intermittent hypoxia was independently associated with steatosis. Endothelial dysfunction was more severely impaired in OSA patients demonstrating NAFLD. This is the first demonstration of the involvement of this mechanism in cardiovascular consequences of OSA. The interest of a systematic evaluation by non invasive biomarkers of NAFLD in severe OSA patients deserves further studies.

Free radicals: mirages or realities in cardiovascular physiopathology?

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Free radicals (FR) can be defined as molecules containing one or more unpaired electrons in molecular orbitals. This unpaired electron(s) gives a considerable degree of reactivity to the FR. Reactive oxygen species (ROS) including a number of chemically reactive molecules are predominantly implicated in causing cell damage, they also play a major physiological role in several aspects of intracellular signaling and regulation. Not only oxygen, but also nitrogen can be reactive species: RNS. ROS/RNS are known to play a dual role in biological systems such as cardiovascular field. In addition, some reactive species were practically forgotten in biology: hydrogen, thiyl, hydrosulphides, nitrogen dioxide, carbonate radicals. Recently, it has been reported that these FR were potentially important oxidants in physiological environments. Several factors control the reactivity of radicals and can provide the strategies to convert highly reactive species into more persistent species that are easier to detect in an experiment. Oxidative stress is the primary event, whereas lipid peroxidation products are second messengers that convey to the cell information about the initiating oxidative event. In membranes, polyunsaturated fatty acids (PUFA) are highly susceptible to oxidation and readily undergo peroxidation by enzymatic or free radical chain reaction mechanisms, yielding numerous electrophilic species. If lipids peroxides are not neutralized by endogenous antioxidants, they will fragment unsaturated aldehydes (acrolein; 4-hydroxynonenal: HNE; and 4- hydroxyhexanal: HHE). NO and nitrite can react with FA to yield NO_2-Fas. They are highly reactive electrophiles capable of covalently modify proteins, DNA and other macromolecules, similar to ROS/RNS. An important question has recently been raised regarding the relationship between FR and gaseous signaling molecules, nitric oxide (NO), carbon monoxide (CO) and hydrogen sulfide (H_2S) in the cardiovascular system. It is evident that these three 'gasotransmitters' perform a variety of homeostatic functions. The lack of evidence to prove the benefits from usage of antioxidant vitamins to prevent several oxidative- stress-related diseases, has led to boost new strategies. The development of inhibitors against the main sources of ROS generation offers an alternative approach to conventional antioxidant therapies.

03-O006

BNP and BMP4, two novel targets of aldosterone in hypertension

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Arterial hypertension (AH) is associated with a cardiovascular remodelling in which the Renin-Angiotensin-Aldosterone system plays a key role. Several studies suggest that the cardiac fibrosis that develops during AH results from an imbalance between profibrotic (CTGF, TGFß, inflammation) and antifibrotic (BNP, BMP4) pathways, in which the role of aldosterone is not yet established. Our aim was to determine, in a context of AH, the role of intracardiac aldosterone in the development of myocardial fibrosis.

We developed a model of double-transgenic mice (AS-Ren) with cardiac hyperaldosteronism (AS mice) and systemic hypertension (Ren mice). Cardiac fibrosis level was threefold higher in 9-month old

hypertensive mice when compared to control mice, and cardiac hyperaldosteronism further enhanced the fibrosis level. The expression of CTGF (×1.9; $P < 0.05$) and TGF-ß (×1.5; $P < 0.01$) was similarly increased in both Ren and AS-Ren mice compared to WT and AS mice, respectively. However, hyperaldosteronism combined with AH favored the presence of macrophages (CD68+ cells), enhanced the transcription of MCP-1 (×1.43; $P < 0.01$), osteopontin (×2.6; $P < 0.05$) and of galectin 3 ($P < 0.05$) particularly in the fibrotic area. It is noteworthy that galectin 3 expression was related to the preferential expression of collagen 1 vs. collagen 3 in AS-Ren mice (×1.7 and ×0.8 respectively). Besides, the AH-induced BNP was completely blunted in AS-Ren hearts. BMP4 mRNA and protein were significantly inhibited in AS-Ren hearts (−40% and −20% respectively vs. Ren). The MR-antagonist eplerenone given for 1 week restored the BNP expression in AS-Ren mice. The inhibitory effect of aldosterone on BNP and BMP4 was verified in vitro on cultured cardiomyocytes. Finally when AH was induced in WT and AS mice by Ang II infusion for 2 months the mRNA profiles of CD68, CTGF, BNP and BMP4 were similar to those observed in Ren and AS-Ren mice, respectively.

These data demonstrate that a cardiac hyperaldosteronism inhibits the AH-induced expression of the anti-fibrotic factors BNP and BMP4 and thus worsens the AH-associated cardiac fibrosis.

03-O007

Bariatric surgery induces an improvement of left ventricular function and differential effects on cardiac ectopic fat deposition

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Objectives: we thought to investigate the effect of bariatric surgery induced weight loss on cardiac ectopic fat (epicardial and myocardial fat) using 3T magnetic resonance imaging (MRI) in severely obese subjects.

Background: Heart disease is the one of the leading cause of mortality and morbidity in obese patients. Cardiac ectopic fat deposition has been related to an increased heart risk. Whether a sustained weight loss can modulate epicardial fat, a marker of visceral adiposity, or myocardial fat is unknown.

Methods: Twenty three morbidly patients underwent ^{1}H-magnetic resonance spectroscopy (MRS) to determine myocardial triglyceride content (MTGC), MRI assessment of epicardial fat volume (EFV), anthropometric, biological, and visceral abdominal fat (VAT) assessments at baseline and 6 months after bariatric surgery.

Results: Bariatric surgery reduced BMI significantly from 43.1 ± 4.5 to 32.3 ± 4.0 kg/m^2, subcutaneous fat from 649 ± 162 to 442 ± 127 cm^2, VAT from 190 ± 83 to 107 ± 44 cm^2, and EFV from 137 ± 37 to 98 ± 25 mL, all $P < 0.0001$, whereas there was no significant change in MTGC: 1.03 ± 0.2, at baseline vs. 1.1 ± 0.2%, $P = 0.85$. A significant reduction in left ventricular mass (118 ± 24 vs.101 ± 18 g) and cardiac output (7.1 ± 1.6 vs. 5.4 ± 1.0) was also observed and was linked to weight loss. Surprisingly, the loss in epicardial fat was less important than that of VAT, and was not correlated to the percentage of BMI or visceral fat loss suggesting that the two depots decreased significantly, but in

different proportions according to patients ($P = 0.007$ for paired *t*-test). In some patients we observed thus a 'resistance' to epicardial fat loss, which was not associated with sleep apnea syndrome, or baseline hypertension.
Conclusion: Six month bariatric surgery modulates differently cardiac ectopic fat deposition with a significant increase in epicardial fat and no change in myocardial fat. Structural abnormalities of epicardial fat could participate in epicardial fat loss 'resistance'.

03-O008
Hypertension dictates specific structural rearrangements in AT1- Alpha2-receptors heterodimers
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Background: Arterial hypertension (HTA) represents a major risk for cardiovascular diseases. It is mainly characterized by an increased Sympathetic Nervous System (SNS) activity reflected by elevated levels of norepinephrin (NE) leading to concomitant Renin-Angiotensin System (RAS) activation. At sympathetic ending level, NE release is mostly controlled by presynaptic G Protein Coupled receptors (GPCR) like angiotensin type 1 receptors (AT_1R) which positively modulates the NE release or α_2-adrenergic subtypes A and C receptors (α_{2A}-AR, α_{2C}-AR) the most prominent inhibitory receptors. Molecular mechanisms for SNS hyperactivity still remain poorly explored.
Methods: Several works previously described signaling crosstalk between AT1 and α_2-AR to regulated NE release but results are conflicting. In this context, and because both receptors could form receptor's dimers, we hypothesized that NE release could be regulated by a direct interaction between α_2-AR and AT_1R through heterodimerization.
Results: Co-immunoprecipitation and BRET experiments showed that AT_1R interacts specifically and constitutively with both α_{2A}-AR and α_{2C}-AR. Fluorescence complementation experiments further demonstrated that both heterodimers localized to the plasma membrane. Of interest, we showed that specific agonists and antagonists of the two receptors induced different conformational changes within pre-formed AT1/α_2-AR dimers. Intriguingly, while NE and AngII individually promoted disctinct structural rearrangements of the AT1/α_2-AR dimer, concomitant presence of the two agonists (mimicking pathophysiological HTA conditions) stabilized a new and third conformation of the complex. All together, these results highlight the existence of multiple conformations of AT1/α_2-AR dimers depending on the ligands context. The presence of different conformations questioned now about the activation profile associated, in terms of G protein coupling, downstream signaling pathways and finally NE release that will be studied in the future.
Discussion: This work shows for the first time the existence of heterodimerization between angiotensin type 1 and α_2-adrenergic subtypes A and C receptors. Moreover, HTA conditions seem to stabilize a specific conformation of the dimer potentially associated with dysregulated activity. As these receptors represent major modulators of NE release, AT1/$\alpha_{2A/C}$-AR heterodimers could provide new pharmacological sympatholytic target.

03-O009
Influence of statin therapy on leukocyte telomere length in patients with acute myocardial infarction
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Background: Statin treatment is the major therapy of post myocardial infarction (MI) and is a potential protector of leukocyte telomere length (LTL). Furthermore, telomeres are shorter in several cardiovascular diseases including outcomes of acute MI. Moreover, Finkel-Biskis-Jinkins Osteosarcoma (*c-Fos*) could be a reliable and a LDL-independent marker of anti-inflammatory statin treatment. Therefore, we aimed to investigate the relationship between prior statin therapy, LTL, c-Fos transcript and a biomarkers of oxidative DNA lesions than could explain the attrition of LTL by inflammation: 8-oxoguanine DNA glycosylase (OGG1).
Methods. From 278 consecutive patients admitted for an acute MI < 24H after symptom onset, blood samples were collected on admission. LTL was assessed by Q-PCR, and *c-Fos* and OGG1 mRNA levels were measured by Q-RT-PCR. Patients under prior chronic statin therapy were compared with patients without statin therapy.
Findings. While patients on statin therapy were older, their mean LTL was strikingly longer than patients not under statin therapy ($P = 0.008$). In contrast, *c-Fos* and OGG1 mRNA levels were similar for the two groups. LTL decreased with increasing age ($P = 0.004$), increasing *c-Fos* ($P = 0.039$), and OGG1 mRNA levels, ($P < 0.001$). Statin therapy remained associated with longer LTL, even after adjustment for confounding factors ($P = 0.001$), and in younger patients (≤64 year). Even in cohorts matched for propensity scores for statin use, LTL was markedly longer in patients under statin therapy ($P = 0.026$). The other independent determinants of LTL, in addition to age, were *c-Fos* and OGG1 transcripts.
Interpretation. Our prospective study showed that statin therapy was associated with longer LTL. Given the compelling evidence for a causal role of telomere shortening in coronary artery disease, these data bring to light opportunities to identify new targets for early primary preventive treatment strategies. Moreover, our study raised *c-Fos* and OGG1 as new relevant biomarkers of LTL.

03-P001
Flow-mediated vasodilation in brachial artery is impaired in asymptomatic diabetic patient with coronary stenosis
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Objectives: To investigate if flow-mediated dilation (FMD) impairment, which precedes overt atherosclerosis, is associated with silent myocardial ischemia (SMI) and asymptomatic coronary artery disease (CAD) in type 2 diabetes.
Research design and methods: Forearm FMD, measured by ultrasonography; SMI (abnormal stress myocardial scintigraphy and/or stress dobutamine echography) and CAD (coronary angiography in the patients with SMI) were assessed in 118 asymptomatic type 2 diabetic patients fulfilling the French Language Association for the Study of Diabetes and Metabolic Diseases and Society of Cardiology (ALFEDIAM-SFC) criteria for screening for SMI.
Results: SMI was present in 60 patients, 25 of whom had CAD. Baseline brachial artery diameters were similar in the three groups (SMI−: 0.368 ± 0.064; SMI+/CS−: 0.392 ± 0.064; SMI+/CS+: 0.379 ± 0.056 cm; NS). Increase in brachial flow was similar whatever the ischemic status (SMI−: 365 ± 222; SMI+/CS−: 352 ± 208; SMI+/CS+: 300 ± 194%) but FMD was lower in the patients with SMI (SMI+: −0.1 ± 4.6% vs. SMI−: 2.1 ± 3.4%, $P < 0.05$), with a higher prevalence of paradoxical vasoconstriction (SMI+: 50.0 vs. SMI−: 29.3%, $P < 0.05$); as in the patients with or without CAD (CAD+: −0.9 ± 4.7% vs. CAD−: 1.5 ± 3.9%, $P < 0.01$; paradoxical vasoconstriction: CAD+: 64.0 vs. CAD−: 32.6%, $P < 0.01$). Cox regression analysis considering parameters predicting SMI or CAD in univariate analyses with a $P < 0.10$ show that paradoxical vasoconstriction (odds ratio 2.5 [95% confidence interval 1.1–5.6], $P < 0.05$) and nephropathy (OR 2.3 [1.0–5.1], $P < 0.05$) were independently associated with SMI; and paradoxical vasoconstriction (OR 4.2 [1.5–11.4], $P < 0.001$), nephropathy (OR 3.2 [1.2–8.7], $P < 0.05$) and LDL-cholesterol (OR 1.7 [1.0–1.8], $P < 0.05$) with CAD. When FMD rather than paradoxical vasoconstriction was considered, only FMD was associated with SMI and CAD (same Results: OR 0.89 [0.80–0.99], $P < 0.05$).
Conclusions: These data show for the first time that FMD is independently associated not only with SMI but although with asymptomatic CAD and may constitute a non invasive marker of these conditions.

03-P002
Acyl-carnitine regulates intracellular calcium homeostasis in mouse cardiomyocytes
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Carnitine, a molecule brought mainly by diet, is essential in the long chain fatty acid (LCFA) catabolism. Association of LCFA with carnitine forms intermediate metabolites, acyl-carnitine (AC), necessary to the mitochondrial oxidative metabolism. First described in 1973 by Engel and Angelini, a disturbance of serum carnitine and AC are implicated in the progression of cardiomyopathy. While some perturbations of the metabolism can account for the development of the disease, the precise mechanisms involved are not fully understood. We aimed to determine the effects of carnitine and conjugated AC on cardiomyocytes intracellular calcium handling. We investigated, using a confocal microscope, the effects of different concentrations of palmitoyl-L-carnitine (PC) on calcium (Ca^{2+}) signaling. Ca^{2+} transients were measured on freshly isolated cardiomyocytes from C57BL/6 mice incubated in presence of different PC concentrations (0, 3, 10 µM). Under field stimulation, we first measured the dynamic variations of cytosolic calcium (Ca^{2+} transients) at 1 Hz, using the Ca^{2+} dye fluo-4-AM. Then, we recorded the activity of type 2 ryanodine receptor (RyR2) by measuring spontaneous Ca^{2+} release from the sarcoplasmic reticulum (Ca^{2+} sparks). We observed that acute PC application reduced and affected the kinetics Ca^{2+} transient dose-dependently. The rate of release and relaxation were slowed down. In addition, PC also increase Ca^{2+} sparks frequency dose-dependently indicating sarcoplasmic reticulum Ca^{2+} leak. Altered Ca^{2+} handling was prevented by the anti-oxidant, N-acetyl-cystein (NAC), application. These results suggest that AC regulate intracellular Ca^{2+} homeostasis through ROS production. This regulation might be involved in the cardiomyopathy associated with disturbance of carnitine homeostasis either directly and/or consequently to the modulation of the excitation-contraction coupling.
Ref:. Engel AG, Angelini C. Carnitine deficiency of human skeletal muscle with associated lipid storage myopathy: a new syndrome. Science. 1973;179 (76):899–902

03-P003
Aldosterone protects the insulin-activated AKT pathway in heart of diabetic type 2 mice
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Introduction: The pathogenesis of diabetic cardiomyopathy is not fully elucidated.Numerous factors may contribute to the development of heart failure in a diabetic context, including the scarcity of microvasculature and neurohumoral dysregulation, particularly the renin-angiotensin-aldosterone-system. We have previously shown that a modest increase of intracardiac aldosterone prevents the development of cardiomyopathy in mice with type 1 diabetes, possibly through a prevention of cardiac capillary dropout (Messaoudi et al, Faseb J 2009). This study aimed to determine the pathophysiological effects and to study transduction pathways of insulin in the case of a cardiac hyperaldosteronism with or without type 2 diabetes (T2D).
Methods: Three week-old mice overexpressing aldosterone synthase (AS), and their wild-type (WT) littermates were fed a high fat, high sucrose diet or a standard

diet ad libitum. After 4 months of diet, glucose and insulin tolerance tests were performed. In addition, to analyze the signaling pathways dependent of the insulin receptor and insulin growth factor receptor, some of these mice received a dose of insulin (1 UI/kg body weight) 30 min before the sacrifice.
Results: After 4 months of HFHSD, both WT-D and AS-D mice had hyperglycemia (+55%, 56%, $P < 0.05$ vs. WT and AS, respectively) and body overweight (+20%, $P < 0.001$; +30%, $P < 0.05$ vs. WT and AS, respectively). Both WT and AS displayed glucose intolerance and insulin resistance, but these T2D signs were less prominent in AS-D. Echocardiography did not show any cardiac dysfunction in WT-D and AS-D mice. The RT-PCR revealed an increase in expression of the markers of oxidative stress and inflammation in WT-D group only. Surprisingly, VEGFa and insulin receptor substrate 1 (IRS1) mRNAs were upregulated in AS-D mice (+44%, $P < 0.05$ vs. WT-D; +20% $P < 0.05$ vs. AS). Besides, NOS3 (Nitric Oxide Synthase 3) and IRS2 were increased in both diabetic groups. In basal conditions, as shown by the decreased p-AKT/AKT ratio on Western blot the AKT activity was decreased in hearts of WT-D mice only. Interestingly, acute stimulation of AKT by insulin revealed an increase of the p-AKT/AKT ratio in AS-D only.
Conclusion: The results indicate that in mice developing T2D the AKT signaling pathway in heart could be stimulated by insulin only when cardiac hyperaldosteronism was present. This aldosterone-dependent activation of the AKT pathway might play a role in the induction of VEGFa in heart.

03-P004
Role of the mitochondria on the paradoxical effect of red wine polyphenols on endothelial cells
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Red wine polyphenol (RWPC) extracts has been reported to possess vasoprotective properties that involve nitric oxide (NO) release from endothelial cells via a redox-sensitive pathway. Besides, the molecular target of RWPC to release NO has been recently revealed and it involves the activation of the estrogen receptor alpha (Era). Paradoxical effects of RWPC have been shown with regard to angiogenesis. Indeed in a rat model of postischemic neovascularization, low-dose is pro- whereas high dose is anti-angiogenic. NO and ERa are key regulators of mitochondrial function. Furthermore, angiogenesis is a highly energetic process associated with mitochondrial biogenesis. However, whether RWPC induces changes in mitochondrial function has never been addressed and it is the aim of this study.
The effects of RWPC at low concentration (10^{-4} g/L, LCP) and high concentration (10^{-2} g/L, HCP) after 48 h time exposure were investigated on human endothelial cells. Mitochondrial respiration, expression of biogenesis factors and DNA content was assessed using oxygraphy and qRT-PCR, respectively. In vitro capillary formation using Matrigel® was performed. The mechanism involved with respect to ER using the ER-antagonist fulvestrant and the ERa selective agonist PPT was studied. The involvement of both NADPH oxidase and NO synthase was addressed using apocynin and L-NA respectively.
LCP, but not HCP, increased mitochondrial respiration. The effect of LCP was associated with an increase of both expression of several mitochondrial biogenesis factors (Nrf-1, Nrf-2, ERRa, Tfam, PolG) and mitochondrial DNA content whereas HCP had no effect on these parameters. PPT displayed the same pharmacological profile than LCP. All the effects of LCP and PPT on mitochondrial respiration are prevented by fulvestrant, apocynin and L-NA. Finally, LCP and PPT promoted in vitro capillary elongation that was also prevented by fulvestrant, apocynin and L-NA.
The present study highlights the implication of the axis ER, NADPH oxidase and NOS pathways on both increase mitochondrial function and capillary elongation in response to RWPC at low concentration. They may explain the paradoxical effect of RWPC depending on the concentration with respect to angiogenesis, mitochondria being key targets for its pro-angiogenic properties.

03-P005
Thrombomodulin activates ErbB signaling to inhibit PAR-1-induced monocyte adhesion
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In the cardiovascular system, thrombin does ambivalent functions owing to its receptors, thrombomodulin (TM) and protease-activated receptor-1 (PAR-1). These two receptors have opposite actions on coagulation, inflammation and cell growth. PAR-1 belongs to the family of G protein coupled receptors. TM is a membrane glycoprotein constituted of a large ectomain with lectin C-like region, six epidermal growth factor (EGF)-like domains and a Ser-Thr rich sequence, a transmembrane domain and a short cytoplasmic tail. In highly proliverative cells, thrombin and PAR-1 mediate the transactivation of EGF receptor (EGFR) through release of heparin-binding EGF (HB-EGF) by a metalloproteinase. We previously demonstrated that TM is able to activate EGFR in endothelial cells (J Biol Chem 2005, 280:35999).
To test the hypothesis that thrombin and TM activate a specific EGFR signaling to down-regulate the PAR-1 pathway, we investigated the mechanism by which TM modulates the downstream EGFR effectors: ERK1/2, p38 and Src.
Analysis of protein phosphorylation by western blot indicated that PAR-1 and TM stimulated ERK1/2 and p38 through distinct pathway in human endothelial cells. PAR-1 activated ERK1/2 by an intracellular pathway that was independent of metalloproteinase activity, HB-EGF and EGFR. In contrast, TM induced phosphorylation of mitogen activated protein kinases (MAPK) through an extracellular signal that implicated metalloproteinase and EGFR. We next provided evidence that soluble TM fragments were involved in the extracellular activation of EGFR. Thrombin stimulated the metalloproteinase-dependent release of three types of TM fragments: the full ectodomain and two short fragments containing at least the EGF2-5 domains. The TM ectodomain stimulated phosphorylation of EGFR, ErbB2 and their downstream mediator Src. Fluorescence labeling with phospho-specific ERK1/2 antibody revealed that TM down-regulated the nuclear localization of PAR-1-activated ERK1/2. RT-PCR analysis showed that TM signaling also prevented PAR-1 from inducing expression of adhesion molecules. Such anti-adhesive signal of TM - was confirmed by static adhesion assay with monocytes and endothelial cells.
These results demonstrated for the first time that thrombin induces release of TM fragments to stimulate EGFR and ErbB2 and down-regulate PAR-1 pro-inflammatory signaling.

03-P006
Pravastatin reverses the membrane cholesterol reorganisation induced by myocardial infarction within lipid rafts in circulating monocytes
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Objective In acute myocardial infarction (MI), there is activation of CD14+/CD16− cells associated with membrane reorganisation. Given that all patients are treated with statins post-MI, we aimed to evaluate effect of pravastatin on distribution of cholesterol within monocyte lipid rafts in acute MI patients
Material and Methods Monocytes from healthy donors and acute MI patients were cultured with or without 4 μM pravastatin. Lipid rafts were extracted by Lubrol WX, caveolae and flat rafts were separated using a modified sucrose gradient. Cholesterol level and caveolin-1 expression in lipid rafts were determined.
Results In healthy donors, cholesterol was concentrated in flat rafts (62 ± 4% vs. 13 ± 2%, $P < 0.001$). While monocytes from MI patients presented similar cholesterol distribution in both caveolae and flat rafts. Cholesterol distribution was higher in flat rafts in healthy donors, compared to MI patients (62 ± 4% vs. 40 ± 3%, $P < 0.001$), with less distribution in caveolae (13 ± 2% vs. 36 ± 3%, $P < 0.001$). Pravastatin reversed the cholesterol distribution in MI patients cells between flat rafts (40 ± 3% vs. 66 ± 3%, $P < 0.001$) and caveolae (36 ± 3% vs. 18 ± 1%, $P < 0.001$).
Conclusions MI redistributes cholesterol from flat rafts to caveolae indicating monocyte membrane reorganisation. In vitro pravastatin treatment restored basal conditions in MI monocytes, suggesting another pleiotropic effect of statins.

03-P007
Arterial stiffness correlates with autonomic cardiovascular dysfonction
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Background: Primary (multiple system atrophy, Parkinson's disease, pure autonomic failure...) or secondary (diabetes mellitus, renal failure...) autonomic failure (AF) affects patient's quality of life and is a risk factor cardiovascular morbidity and mortality. Abnormal arterial stiffness has been reported in patients with orthostatic hypotension but the relationship between AF and arterial stiffness remains poorly reported. The aim of our study was to evaluate the relationship between AF and artery compliance assessed by pulse wave velocity (PWV) and central blood pressure (CBP).
Methods: Thirty-four consecutive patients referred to the Autonomic Unit of the Toulouse University Hospital underwent autonomic testing based on changes on heart rate and blood pressure (recorded using Nexfin® digital photoplethysmograph) during five standardized tests (deep breathing, 30/15 ratio, Valsalva manoeuvre, isometric handgrip and 80° head-up tilt test). Severity of AF was quantified using Ewing score (from 0 to 5). Carotid-femoral PWV and CBP were assessed by artery aplanation tonometry (Sphygmocor®).
Results: Based on autonomic testing, 15 patients suffered from significant AF (AF+, Ewing score >1) whereas 19 patients were devoided of AF (AF-, Ewing score ≤1). Age, sex, BMI and AF etiology (diabetes, multiple sclerosis and Parkinson's disease) were not significantly different between groups. AF+ patients had a significantly higher PWV when compared to AF- group (10.1 ± 0.7 vs. 8.3 ± 0.5 m/s; $P < 0.05$). PWV was significantly correlated to Valsalva ratio ($r^2 = 0.2809$; $P = 0.0045$) and to changes in diastolic blood pressure during handgrip ($r^2 = 0.1994$, $P = 0.0372$). PWV was also correlated to CBP parameters (Aortic systolic pressure, aortic pulse pressure and aortic pressure).
Discussion: We show for the fist time a direct relationship between PWV and cardiovascular autonomic dysfunction. These results suggest that that arterial stiffness could, at least in part, account for abnormal responses during standard autonomic testing and that its assessment should be systematically achieved in AF patients. Moreover, since some drugs have been shown to reduce PWV, evaluation of their ability to modulate AF severity/progression could be of interest in patients with both AF and arterial stiffness.

03-P008
Improvement of insulin sensitivity by imidazoline-like drugs in rats
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Introduction: Insulin resistance plays a pivotal role in the 'metabolic syndrome', defined as a cluster of cardiovascular and metabolic disorders leading to increased cardiovascular risk. We demonstrated recently that a selective imidazoline I1 receptor ligand, LNP509, improves glucose tolerance independently of any centrally-mediated sympathetic inhibition in old rats displaying impaired glucose regulation. The objective of the present study was to identify the mechanisms involved in the beneficial metabolic action of this drug.
Methods: Fourteen months-old male Wistar rats were treated for 1 week with LNP509 (10 mg/kg/day ip; $n = 8$) or vehicle ($n = 9$). Glucose regulation was then assessed in anesthetized animals challenged with glucose intravenous injection. Pancreatic morphology (number of Langerhans islets) and function (expression level of the glucose transporter Glut2) were studied to evaluate insulin secretory

function. Plasma concentration of adiponectin, an insulin-sensitizing hormone, and expression level of the glucose transporter Glut4 in adipose tissue were determined to assess insulin sensitivity.
Results: LNP509 did not significantly modify fasting glucose and insulin (7.0 ± 0.2 vs. 6.3 ± 0.4 mM and 3.1 ± 0.5 vs. 2.1 ± 0.4 µg/L in treated and control animals, respectively). Glucose tolerance and insulin secretion were evaluated following glucose injection (0.5 g/kg). Compared to control animals, LNP509-treated rats displayed significantly accelerated glucose clairance, as assessed by a lower area under the curve of glycemia over time (4040 ± 162 vs. 4615 ± 183; $P < 0.05$). In these animals, peak insulin after glucose injection was unchanged but insulin secretion over time was lower (area under the curve = 4380 ± 383 vs. 6172 ± 974), indicating improved sensitivity of insulin-sensitive tissues. The plasma concentration of adiponectin was significantly increased in treated rats (2.04 ± 0.21 mg/L) compared to control rats (1.48 ± 0.11 mg/L). Histological analysis revealed no change in the number of Langerhans islets in the pancreas of animals receiving LNP509. Finally, expression levels of the glucose transporters Glut2 and Glut4 were not modified by treatment.
Conclusion: These data suggest that imidazoline I1 receptor ligands do not modify insulin secretion but increase insulin sensitivity of target tissues, thus improving glucose regulation. Future studies will aim to identify the cellular and molecular mechanisms involved in these beneficial metabolic actions.

Role of cell plasticity in progression and reversal of renal fibrosis
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The need for novel insights into the mechanisms of progression of renal disease has become urgent during the last several years because of the increasing incidence of chronic renal disease worldwide. Independent of the underlying disease, the subsequent progression of renal fibrosis is mainly characterized by an exaggerated synthesis and abnormal accumulation of extracellular matrix proteins by mesenchymal cells within the kidney. These cells are mainly myofibroblasts deriving from a variety of renal cells such as vascular smooth muscle, mesangial, resident stem, tubular epithelial, vascular endothelial cells or pericytes. The appearance of myofibroblasts is a reversible process, as suggested by studies in experimental models showing regression of renal fibrosis during therapy with antagonists and/or blockers of the renin-angiotensin system. An additional factor that can also affect the mechanisms of progression/regression of fibrosis is the plasticity of podocytes controlling glomerular filtration. The challenge for the years to come is to identify targets and treatments inducing the re-establishment of normal phenotype in renal cells and thus, allowing therapy of chronic renal disease.

'Calcium-sensing' in the kidney: the calcium-sensing receptor CaSR and beyond
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The kidney plays a major role in calcium metabolism by matching the urinary calcium excretion and the input of calcium from the intestine and/or bone; thereby, it largely determines the calcium concentration value in the extracellular fluid. Changes in extracellular calcium (ECF) concentration profoundly affect renal function: an increase in ECF calcium concentration increases urinary calcium, sodium, water and acid excretions.
For several years, we aim at unraveling the role of the calcium-sensing receptor CaSR expressed in the renal tubule. CaSR is mainly expressed in the basolateral membrane of the thick ascending limb cells in the loop of Henle. Its inhibition increases calcium absorption across the epithelium, does not alter NaCl absorption or the transepithelial voltage but increases the paracellular pathway permeability to calcium. This permeability is also controlled by the parathyroid hormone. We have been unable to detect an effect of CaSR on Mg, water, NaCl or acid transport in the kidney. Preliminary data suggest that the effects of extracellular calcium on acid transport could involve another receptor, GPRC6A. The identity of the receptors that could be involved in the effect of calcium on NaCl and water transports remains unknown.
In summary, the available data indicate that, in the kidney, CaSR only affects calcium transport. The effects of calcium on the renal tubule are more complex than originally thought.

04-O010
The essential role of AKT2 in podocytes
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In chronic kidney disease (CKD), the loss of functional nephrons results in the overload of the remaining ones. This leads to a metabolic and mechanical stress triggering, in turn, further nephron loss. The molecular pathways engaged to counteract this stress are still unknown. The aim of this study was to evaluate the potential role of AKT proteins in the adaptation of glomerular cells to nephron reduction.
On that purpose, we combined different models of nephron reduction (subtotal nephrectomy, uninephrectomy and aging nephropathy), glomerular isolation through laser capture microdissection and *Akt1* and *Akt2* mutant mice. Finally, we evaluate glomerular AKT activation in renal transplant recipients with low or conserved kidney function and assessed the impact of the immunosuppressive drug sirolimus, which has been shown to inhibit AKT activation in certain non renal cells, on AKT activity.
We showed that the activation of the serine/threonine kinase AKT2 plays an essential role in the survival of podocytes and in their adaptation to nephron reduction, without affecting tubular cells. We demonstrated that this function is isoform specific. In fact, glomerular lesions and albuminuria were dramatically increased in $Akt2^{-/-}$, but not in $Akt1^{-/-}$ mice. Mechanistically, AKT2 deficiency acts by preventing the triggering of the compensatory genetic program whichs involve MDM2, GSK3 and RAC1 functional regulation. This is associated with increased apoptosis and foot processes effacement in $Akt2^{-/-}$podocytes. Remarkably, these data are relevant to human CKD where AKT2 activation by mTOR complexe 2 is essential for podocyte survival in patients with severe nephron reduction. More importantly, we provide evidence that AKT2 activation in podocytes could serve as a prognostic marker to predict the deleterious proteinuric effect of sirolimus.
Our results show a novel function for AKT2 in podocytes and identify a potential therapeutic target for the maintenance of glomerular function in CKD.

04-O011
Dysfunction of B-intercalated cells impairs renal sodium and potassium conservation in type I distal tubular acidosis
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Inactivating mutations of the vacuolar H^+-ATPase in human lead to renal distal tubular acidosis (dRTA), a disease often associated with a salt and potassium loosing nephropathy of unknown origins. The distal nephron, composed of principal cells (PCs), α- and β- intercalated cells (ICs) plays a key role in the renal control of salt, K^+, water and acid-base balance. PCs are considered to be the main conduit for Na^+, K^+ and water. ICs were initially thought to be exclusively involved in acid-base regulation until recent observations showing that β-ICs can absorb NaCl. Here we show that mice with disruption of the B1 subunit of the proton pump ($Atp6v1b1^{-/-}$ mice), which is expressed in both types of ICs, display, beside insufficient acid excretion by α-ICs, a renal loss of sodium, chloride, potassium and water causing dehydration, hypokalemia and polyuria. Despite extracellular fluid volume depletion, cortical collecting ducts (CCDs) isolated from $Atp6v1b1^{-/-}$ mice did not absorb NaCl as a consequence of reduced protein expression of the epithelial sodium channel ENaC and the chloride/HCO_3^- exchanger pendrin in CCDs. Expression of the water channel AQP2 and the maxi K channel BKCa was decreased and increased, respectively. Higher prostaglandin E_2 (PGE_2) level was observed in urines from $Atp6v1b1^{-/-}$ mice. Blockade of PGE_2 synthesis in vivo by indomethacin greatly improved urine output and plasma potassium concentration in $Atp6v1b1^{-/-}$ mice. AQP2 and BKCa expression was normalized. ENaC expression was normalized in CCDs but not in MCDs, where its expression remains higher. In microperfused CCDs isolated from wild-type mice, inactivation of the basolateral proton pump in β-ICs by bafilomycin A1 induces ATP-dependent luminal PGE_2 release through activation of luminal purinergic receptors. The data demonstrate that β-ICs participate in the regulation of Na^+ balance directly by reabsorbing NaCl and by controlling ENaC activity in neighboring PCs through released paracrine factors ATP and PGE_2 into the tubular fluid. The data highlight the importance of intercalated cells in paracrine control of renal Na^+, K^+ and water balance.

04-O012
Distal convoluted tubule (DCT) specific transcripts identified using DCT large scale sampling
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The renal distal convoluted tubule (DCT) is important for the renal control of ion homeostasis and blood pressure. Although several DCT-specific ion transporting proteins have been identified, only little is yet known about the molecular mechanisms that regulate DCT function. We hypothesized that gene products that are specifically expressed in the DCT might be of particular importance for the control of DCT cell function. Here, we used Complex Object Parametric Analysis and Sorting (COPAS) to isolate DCTs in large scale for the identification of a DCT transcriptome. A renal tubule suspension was obtained from transgenic mice expressing EGFP specifically in the DCT. The tubules were then separated by COPAS in three fractions (i.e. all tubules, EGFP-positive DCTs and EGFP negative (non-DCT tubules). Real-time PCR and Western blot analysis confirmed the significant enrichment and derichment of known DCT-specific marker molecules in the EGFP-positive and EGFP-negative samples, respectively. Subsequent microarray analysis (Agilent) revealed about 400 genes that are being more than 20-fold enriched in EGFP-positive tubules compared with EGFP-negative tubules. Aside from the known DCT-specific gene-products parvalbumin, NCC and TRPM6, many novel DCT enriched gene products were identified. Among them, Slc16a7 (MCT2) and its accessory protein GP70 (embigin) sticked out. Immunohistochemistry confirmed the significant expression of these two gene products in the DCT. Thus, transcriptomic analysis of COPAS-sorted renal tubules allows the identification of novel DCT enriched gene products and may provide a pool of novel candidate genes for DCT-specific functions and diseases.

04-O013
Increased expression of the serine-threonine kinase L-WNK1 in the Distal Convoluted Tubule leads to the development of hyperkalemic hypertension
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Background: Mutations in the serine-threonine kinase WNK1 are responsible for Familial Hyperkalemic Hypertension (FHHt), an autosomal dominant form of hypertension associated with hyperkaliemia and hyperchloremic metabolic acidosis. WNK1 gives rise to a long ubiquitous isoform, L-WNK1, and a shorter isoform

lacking a functional kinase domain and expressed exclusively in the distal nephron, KS-WNK1. FHHt mutations are large deletions of the first intron of the gene but the consequences of these deletions on the expression of L-and KS-WNK1 and on renal ion transport are unclear.
Methods: In order to elucidate the mechanisms underlying the deregulation of renal ion handling in WNK1-related FHHt patients, and thereby the role of WNK1 on blood pressure and ion homeostasis, we generated 'WNK1-FHHt' knock-in mice harbouring a deletion of the first intron of WNK1.
Results: WNK1-FHHt mice display high blood pressure, hyperkalemia and hyperchloremic metabolic acidosis. We show that L-WNK1 expression is increased in the Distal Convoluted Tubule (DCT) and, to a lesser extent, in the connecting tubule (CNT), whereas KS-WNK1 expression is not modified upon deletion of *WNK1* first intron. As expected from in vitro studies, the modification of L-WNK1 expression leads to increased expression and activity of the sodium-chloride cotransporter NCC. In addition, the blood pressure and metabolic disorders are reversed by administration of hydrochlorothiazide. In order to understand how NCC is activated by L-WNK1 increased expression, we quantified the phosphorylation of the SPAK kinase, which has been shown to phosphorylate NCC, upon its own activation by phosphorylation by WNK1. Surprisingly, SPAK phosphorylation is not modified by L-WNK1 overexpression in the DCT.
Conclusions: Our study demonstrates that, by increasing L-WNK1 expression in the DCT, the deletion of WNK1 first intron leads to the stimulation of NCC activity and the development of FHHt. The mechanisms by which NCC is activated in WNK1-FHHt mice and the contribution of L-WNK1 activation in the CNT remain to be defined.

04-P030
The inhibition of the expression of Notch3 receptor protects against glomerulonephritis
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Recent studies showed that the de novo activation of Notch3 receptor is involved in the vascular remodeling during pulmonary hypertension (Nat Med 2010). Moreover, our team identified that Notch3 is important in the regulation of the renal vascular tone (Hypertension 2011).
The aim of this work is to study the involvement of Notch3 receptor in the mechanisms of progression of experimental crescentic glomerulonephritis (GN). GN was induced in mice that were treated either with the Notch3 DNA antisense or with the scrambled sequence for 9 days. In the end of the protocol, mice were sacrificed and tissue, urine and plasma samples were obtained and used for subsequent analysis. The mRNA and protein expressions of Notch3 were significantly reduced in the renal cortex of mice that received Notch3 DNA antisense compared to scrambled injected mice ($P < 0.05$).
The two groups progressed to chronic renal disease, but the mice injected with Notch3 DNA antisense were relatively protected compared to scrambled group as evidenced by the values of plasma urea ($P < 0.05$) and proteinuria ($P < 0.05$). This improvement of renal function was accompanied by fewer deposits of fibrin within glomeruli and less peritubular and glomerular inflammation. Moreover, the inhibition of Notch3 was associated with blunted activation of growth factor signaling pathways well known to be involved in the development of the GN such as the *PDGFRb* and *HB-EGF* expressions.
Our study shows that the activation of Notch3 is involved in the remodeling occurring in the kidney during the GN especially by promoting proliferative and pro-inflammatory pathways.
These results imply that Notch3 activation is involved in the physiopathology of CKD, at least in this model, and suggest that inhibiting the activation of Notch3 could be a novel therapeutic approach.

04-P031
The calcium receptor CaSR and parathyroid hormone determine the paracellular pathway permeability to calcium in the thick ascending limb
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Introduction and objective: Renal tubular calcium absorption is a major determinant of urinary calcium excretion and, together with net bone calcium release, of blood calcium concentration. In the kidney, calcium is reabsorbed in the proximal tubule, the cortical thick ascending limb (cTAL) of the loop of Henle and the distal/connecting tubule. Renal tubular calcium absorption is negatively altered by the extracellular calcium concentration, an effect that likely involves the calcium-sensing receptor (CaSR); conversely, it is positively altered by parathyroid hormone (PTH). Both factors act in the cTAL where calcium is reabsorbed paracellularly thanks to a lumen-positive transepithelial voltage and a measurable permeability of the paracellular pathway to calcium. The objective was to determine the mechanism(s) by which CaSR and PTH both control calcium absorption in the cTAL.
Materials and methods: The rate of transepithelial calcium, sodium and chloride absorption and paracellular passive permeability for calcium by the cortical thick ascending limb of the loop of Henle (cTAL) was measured in invitro microperfused cTAL from male Sprague-Dawley rats weighting 70–95 g. Calcium, sodium and chloride concentrations were measured by microfluorometry, microcoulometry, and ion-exchange chromatography, respectively. Transepithelial voltage was measured by a differential electrometer.
Results: An allosteric CaSR inhibitor (NPS2143, 1 μM in bath) reversibly increased calcium absorption (4.9 ± 0.43–7.2 ± 0.54 pmol/mm/min) without altering the transepithelial voltage or sodium and chloride absorptions. In addition, NPS2143 reversibly increased the paracellular pathway permeability to calcium (2.2 ± 0.2–3.4 ± 0.5 10^{-6} cm/s). Parathyroid hormone (3.10^{-10} M in bath) reversibly increased calcium absorption (4.2 ± 0.3–6.1 ± 0.8 pmol/mm/min) without altering the transepithelial voltage. In addition, when added in the presence of PTH, NPS2143 still reversibly increased calcium absorption (by 0.40 ± 0.01 pmol/mm/min) but its effect was smaller in the absence of PTH (2.25 ± 0.29 pmol/mm/min).
Conclusion: Both CaSR and PTH directly alter calcium absorption in the cTAL. Both affect the paracellular pathway permeability to calcium. CaSR and PTH share, in part, common pathways for their action on the paracellular permeability.

04-P032
Bone and vascular disease in patients with chronic kidney disease: potential role of fibroblast growth factor 23
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Background: The hormone fibroblast growth factor 23 (FGF23) is involved in mineral homeostasis but may also have a role in vascular calcification and bone mineralization. Previous studies related to FGF23 and vascular and bone outcomes have been restricted to dialysis patients. The aim of the present study was to establish whether or not plasma FGF23 levels are associated with aortic and coronary calcification, arterial stiffness and bone mineral density in patients with early as well as late stages of CKD.
Methods: One hundred and fifty-three patients with CKD stages 2–5D were included in a cross-sectional study. In addition to routine biochemistry and intact FGF23 determinations, aortic and coronary calcification and stiffness and bone mineral density (BMD) were assessed by multislice spiral computed tomography and automated pulse wave velocity (PWV).
Results: Plasma intact FGF23 levels were elevated in CKD patients; the elevation preceded that of serum phosphate in early-stage CKD. Patients with elevated FGF23 levels had higher aortic and coronary calcification scores than patients with lower FGF23 levels. Multivariate linear regression analysis indicated that only age ($P < 0.001$) and FGF23 ($P = 0.008$) were independently associated with aortic calcification score. Plasma FGF23 was neither associated with PWV nor with BMD.
Conclusions: Our data suggest that plasma FGF23 is an independent biomarker of vascular calcification in patients with various CKD stages including early stages. The association between vascular calcification and FGF23 levels appears to be independent of BMD. It remains to be seen whether this association is independent of bone turnover and bone mass.

04-P033
Circadian expression of renal H,H-ATPase type 2 contributes to stability of plasma K+
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Maintenance by the kidney of stable plasma K^+ values is crucial, as the latter control muscle and nerve activity. Inability to do so, induced hyper- or hypokalemia both related to heart dysfunctions, muscle weakness and neurological disorders.
Since renal K^+ excretion is regulated by the circadian clock we aimed to identify the ion transporters involved in this process.
In control mice, the renal mRNA expression of the H,K-ATPase type 2 (HKA2) is 25% higher during rest compared to the activity period. Conversely, under dietary K^+ restriction, HKA2 expression is approximately 40% higher during the activity period. This reversal suggests that HKA2 contributes to the circadian regulation of K^+ homeostasis. Compared to their wild-type littermate (WT), HKA2-null mice on a normal diet have a 2-fold higher K^+ renal excretion during rest. Under K^+ restriction, their urinary K^+ loss is 40% higher during the activity period. This inability to excrete K^+ 'on time' is reflected in plasma K^+ values, which vary by 12% between activity and rest periods in HKA2-null mice but remain stable in WT. Analysis of the circadian expression of HKA2 regulators suggests that Nrf2 but not progesterone contributes to its rhythmicity. Therefore, HKA2 acts to maintain the circadian rhythm of urinary K^+ excretion and preserve stable plasma K^+ values throughout the day.

04-P034
Metformin inhibits renal gluconeogenesis in Zucker Diabetic Fatty (ZDF) rats: a cellular metabolomic study with 13C-lactate and carbon 13 NMR
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Metformin, one of the most widely used antidiabetic drugs, has been shown to decrease hyperglycemia in diabetic patients and diabetic animal models. This effect results in part from the inhibition of hepatic gluconeogenesis. However, renal gluconeogenesis has also recently been shown to be increased in diabetic subjects suggesting that this process may significantly contribute to systemic gluconeogenesis and, therefore, should be inhibited. This is why the objective of the present study was to investigate in vitro the effect of metformin on renal lactate gluconeogenesis by using our cellular metabolomic approach.
For this, renal proximal tubules from fed ZDF rats, a good model of type 2 diabetes in which intrinsic gluconeogenesis has been shown to be augmented, were isolated with a collagenase method. Then, the tubules were incubated with 5 mM L-[1-13C]-, or? ?L-[2-13C]-, or L-[3-13C]lactate or 5 mM L-lactate + 25 mM NaH13CO3 as substrates. At the end of the incubation period, substrate utilization and product formation were measured by both enzymatic and carbon 13 NMR methods.
Metformin dose-dependently inhibited lactate gluconeogenesis (IC50 = 1.15 mM). The addition of 1 mM metformin also inhibited the removal of 13C-lactates and the production of 13C-glucose and 13CO2 without changing the fixation of 13CO2.

Combination of enzymatic and NMR measurements with a mathematical model of renal lactate metabolism previously validated showed that metformin caused an inhibition of fluxes through lactate dehydrogenase (by 27%), glucose-6-phosphatase (by 40%), pyruvate dehydrogenase (by 23%) and citarte synthase (by 21%). By contrast, metformin did not alter fluxes through pyruvate carboxylase and phosphoenolpyruvate carboxykinase, pyruvate kinase and alanine aminotransferase.
It is concluded that renal lactate gluconeogensis is very sensitive to the inhibitory effect of metformin and that this effect does nor result from the inhibition of fluxes through all key-gluconeogenic enzymes involved in this pathway.

04-P035
Is vascular mineralocorticoid receptor involved in cyclosporine a nephrotoxicity?

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Background: Cyclosporine A (CsA) nephrotoxicity is one of its most frequent adverse effect but its physiopathology remains unclear. Pharmacological blockade of the Mineralocorticoid Receptor (MR) has been reported to prevent CsA nephrotoxicity in the rat by modulating the expression of vaso-active factors (Perez-Rojas et al. AJPRP 2005). We have recently shown that MR is expressed in the endothelium and the vascular smooth muscle of the renal vasculature (Nguyen Dinh Cat et al. FASEB J 2010) Moreover genetic manipulation of MR expression in the endothelium (Nguyen Dinh Cat et al. FASEB J 2010) or the smooth muscle (unpublished data) alters vascular function. Our working hypothesis is that the activation of vascular MR plays a key role in CsA nephrotoxicity.
Methods: We studied the effects of pharmacological blockade of MR on acute CsA nephrotoxicity in wild-type female mice under low salt diet: control, Ctrl (vehicle), CsA (CsA 100 mg/kg/day) and CsA+Can (CsA + canrenoate 30 mg/kg/day in tap water). The same protocol (without pharmacological blockade) was applied to transgenic mice (MR knock-out in vascular smooth muscle cells).
Results: Body weight loss was higher in mice under CsA than control ($P < 0.05$). Kidney function, attested by creatinine clearance and blood urea nitrogen, did not reach the statistical significience between groups but mice under CsA experienced histological damages (proximal tubular vacuolizations) widely prevented by MR antagonism/knock-out. Canrenoate prevented the increase of renal excretion of NGAL, a biomarker of renal damage, observed in CsA. Moreover, NGAL is widely overexpressed in renal epithelial cells (immunolocalization) under CsA and this is partially prevented in KO mice.
Conclusions: In conclusion, we demonstrate that pharmacological MR antagonism has beneficial effects on histological alterations in a mouse model of acute CsA nephrotoxicity. Genetically modified mouse model encountered same findings and will allow us to delineate the mechanisms responsible for the reported beneficial effect of pharmacological MR blockade.

04-P036
Why is plasma creatinine higher in Blacks regardless of renal filtration?

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Introduction: Plasma creatinine (P_{Cr}) is the best estimate of renal function and numerous formulas based on its value have been developed in order to approach the glomerular filtration rate (GFR). Ethnic coefficients have been defined to take into account long-recognized differences in P_{Cr} regardless of GFR in Black individuals. Whether extrarenal metabolism of creatinine significantly contributes to creatinine excretion in individuals remains elusive, and higher muscular mass and differences in tubular secretion of creatinine have been held responsible for this observation. We sought to evaluate the relevance of these two mechanisms.
Patients and results: We measured GFR with urinary clearance of Cr51-EDTA (mGFR), P_{Cr} (enzymatic), urinary creatinine excretion, lean body mass (LBM) with DEXA, total body water (TBW) with bioimpedance spectroscopy, in 119 Blacks and 119 Caucasians (C) paired for sex, age, body weight, body mass index (BMI), height and mGFR; protein mass (PM) was calculated as (LBM-TBW). Tubular secretion of creatinine (T_{Creat}) was calculated using mGFR, creatinine clearance and P_{Cr} values.
Results: Urinary creatinine excretion was higher in Black (9.8 vs. 8.4 mmol/24 h) as was also T_{Creat} (3.6 vs. 2.8 mmol/24 h, $P < 0.05$). After adjustment for weight, body composition analysis indicated a higher bone mass in Blacks but no difference in LBM (48.4 vs. 48.9 kg, P = ns), PM (10.5 vs. 9.7 kg, P = ns) nor creatinine generation. After adjustment for sex and creatininuria, P_{Cr} was similar in the two groups (152.9 vs. 154.5, $P = 0.8$), but body weight was higher (80.9 vs. 74 kg, $P < 0.01$) with a non significant trend for lean body mass (51.6 vs. 49.1, $P = 0.051$) and protein mass (11.3 vs. 9.7 kg, $P = 0.08$) in Blacks vs. Caucasians. Creatine Phospho Kinase (CPK) were higher in black (251 vs. 123 UI/L, $P < 0.01$).
Conclusion: We confirm that plasma creatinine is higher in Blacks vs. Caucasians with similar GFR. Higher muscular mass in Black does not account for this difference as it has been shown in black patients on hemodialysis (1), nor does the tubular secretion of creatinine. Higher CPK values suggest intrinsic differences in extrarenal creatinine metabolism.
Reference: 1. Hsu J et al. *Clin J Am Soc Nephrol* 2008; 3: 992–997.

04-P037
Improvement of urinary exosomal preparation for proteomic analysis

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Objective: Growing number of studies point to the importance of exosomes, intraluminal vesicles (ILVs) secreted by most cell types, in the intercellular communication and cell signalling. Recently urinary exosomes attracted the attention of nephrologists and researchers as a potential source of biomarkers for renal diseases (1). On the other hand, investigation or urinary exosomes may unveil some of the basic phenomena underlying these disorders. Large scale proteomics studies of urinary exosomes indicate the presence of at least 1000 proteins, including transmembrane and cytosolic proteins specific of the tubular cells, and ubiquitary proteins involved in exosomes biogenesis. These studies suggested that the major pitfall of proteomics analysis is the abundance of a highly glycosylated protein, the Tamm Horsfall protein (THP). The objective of our study is to overcome this issue and to standardize a preparation of urinary exosomes applicable in clinical studies. We aim to reproducibly identify low abundance proteins as they could be potential markers for renal diseases.
Methods: Exosomes were isolated from 50 mL of first morning urine by differential centrifugation (2) and extracted proteins then separated by isoelectric focusing OFF-Gel. Total proteins were identified by NanoLC-LTQ-Orbitrap mass spectrometry (MS).
Results: HP confinement at the acidic fractions was observed by Western blot and MS detection. This pH based fractionation allowed us to identify approximately 1200 exosomal proteins. The time necessary for this identification was five time shorter than the one reported by others (2). Among the identified proteins, several are specific of different parts of nephron, mostly proximal tubule. Many specific proteins from the mechanism of exosome biogenesis, such as ESCRT (endosomal sorting complexes required for transport), were also identified.
Conclusions: Optimization of the protocol for identification of proteins allow to compare urinary samples from patients affected from renal diseases in time. Furthermore, profiles from different renal diseases (cystinuria and renal cancer) will also be compared to healthy donors.
References: 1. Pisitkun, T., R.F. Shen, and M.A. Knepper. Proc Natl Acad Sci USA, 2004; **101** (36): 13368.
2. Gonzales, P., et al. Journal of the American Society of Nephrology, 2009; **20** (2): 363.

Somatropic axis adaptation during pubertal development

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One of the major events occurring during pubertal development is a great increase in growth velocity. Many interactions between the sex steroids (estradiol or testosterone) and several actors of the somatropic axis (somatostatin, GHRH, growth hormone (GH), IGF I …) have been described by the past.
One of the most remarkable phenomenons during puberty is a large increase of the GH secretion which is sexually dimorphic. Furthermore, in animal models, these different patterns of secretion between males and females lead to the activation of specific pathways and the expression of specific target genes. In the same way, the impact of exogenous steroids on IGF I secretion is also sexually dimorphic, as oral estradiol is responsible of a GH resistance.
Many arguments highlight the major role of IGF I in pubertal growth. A strong increase of IGF I is observed in both sexes with a maximal level at 14 (girls) and 16 (boys) years old which finely corresponds to the peak of growth velocity. Animal models of partial inactivation of IGF type 1 receptor (IGF-1R) show a shorter size at the adult age, which is explained by a decreased growth velocity during the phase of sexual maturation. This observation is sexually dimorphic with males which are more severely affected. In humans with heterozygous mutation of IGF-1R, a growth retardation is observed at birth and during childhood, and puberty can sometime be delayed. The few patients with IGF I mutations are severely short at birth and can present a pubertal delay with small testicular volumes, highlighting the role of IGF I in gonadal function.
Apart of its role in growth, IGF I is also implicated in bone mineralization during pubertal development. The peak of bone acquisition occurs between 11 and 14 (girls) and 13 and 17 (boys) years old, which correspond to the increased period of IGF I secretion. Several animal models of inactivation of igf-1 or igf-1r show a decreased bone mineral density (BMD), as described for the first patient with an IGF I mutation. Finally, several studies suggest an association between BMD and some polymorphism in the IGF I promoter.

Early postnatal Nutrition Programs the Somatotropic axis through Epigenetic mechanism

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Our previous findings suggest that differential activation of the IGF-1R programs the somatotropic axis activity. Such studies have used transgenic mouse models carrying brain specific igf-1r knockout or physiologically, by a transient decrease of nutrition during the early postnatal period. We studied GHRH and somatostatin (SRIH) neurons that demonstrate a persistent alteration in our models to determine molecular mechanisms involved in the programming of the GH axis activity. Effects occurring at the transcriptional level strongly suggest epigenetic influences in

programming neuronal expression with changes of histones marks and DNA methylation. Accordingly, ChIP against histone H3 acetylated on the lysine 9 (H3K9ac) or histone H4 monomethylated on the lysine 20 (H4K20me1) highlight increased enrichment of the SRIH promoter in adult mice previously restricted during early postnatal period as compared with control. These changes are associated with a decreased frequency of cytosine methylation in the CpG island present in the SRIH promoter and correlate well with the persistent increased transcription. In contrast, no modifications were observed in the promoter of GHRH. We infused epigenetic modifiers intracerebroventrically (icv) for 14 days in order to understand implication of epigenetic mechanisms in the control of SRIH and GHRH expression. Icv infusion of deacetylase inhibitor Trichostatin A (TSA) in adult, control mice was performed to mimic the restricted phenotype regarding epigenetic status. In agreement with our hypothesis, control mice infused with TSA show increased association of H3K9ac and H4K20me1 with the SRIH promoter. In contrast, restricted mice icv-infused with the methyl donor L-methionine have a strongly decreased association of H4K20me1 with the SRIH promoter. Gene expression levels are consistent with the revised epigenetic status: normal mice infused with TSA show increased SRIH and a trend for decreased GHRH mRNA levels compared with saline-infused littermates. Restricted mice infused with methionine show decreased levels of SRIH associated with a strong increase of GHRH mRNA levels as compared with saline-infused littermates.

05-O014

Gelatinase B (MMP-9) immunoexpression in the Libyan jird (Meriones libycus) coagulating gland during seasonal cycle of reproduction and after castration

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An immunohistochemical study of gelatinase B (MMP-9) using the indirect method with streptavidin-biotin-peroxydase was carried out on the coagulating gland of the Libyan jird (*Meriones libycus*) in order to verify their involvement during the seasonal cycle of reproduction. The animals were collected from their natural habitat (Béni-Abbès, Algerian Sahara) during breeding period (spring and early summer) and resting phase (late summer, autumn, late winter). Several *Meriones* castrated since 1 month at spring, were also studied. During the active period, the MMP-9 is highly expressed in both epithelial cells and secretion, with a slight immunoexpression in smooth muscle cells (SMCs). MMP-9 immunostaining decreased insignificantly in the epithelial cells and intensified slightly in the SMCs during the resting phase and after castration, similar results were observed in the ventral prostate of adult Wistar rat castrated for 21 days (Justulin et al., 2010). No immuno-response of MMP-9 was observed in the extracellular matrix. The profile of expression of this gelatinase seemed unaffected by the total absence of testosterone in castrated animals, and by seasonal hormonal fluctuations. These later were characterized by a weak testicular androgen contents and a minimal plasmatic testosterone in the non-breeding period, and by a high value in the active season (Boufermes, 1997; Mataoui, 1999). This result demonstrated the MMP-9 involvement in the seasonal tissue remodeling of the coagulating gland during the recrudescence in active period and its involution during both resting season and after castration; so, MMP-9 could be an enzymatic component of seminal plasma because of its presence in secretion.

05-O015

Metabolic syndrome and type 2 diabetes: risk factor screening in Algerian subject with or without diabetic family related

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It is described that the type 2 diabetes mellitus pathogenesis is related to several factors. Many studies shown that metabolic syndrome (MetS) is the most important risk factor to diabetes emergency. In Algeria, the epidemiological study estimates at 7.3% the type 2 diabetes prevalence.

Objective: The aim of this study is (I) isolate the most important risk factor between the MetS markers; (II) establish the relationship between MetS and type 2 diabetes family.

Subjects and methodology: One hundred no diabetic subjects were included in the study related or not to diabetic family: 43 MetS subjects with type 2 diabetes parental (1st group), 27 MetS subjects without type 2 diabetes parental (*2nd group*) and 30 no MetS subjects but with type 2 diabetes parental (*3rd group*). Ten healthy volunteers participated in the study. All research was conducted over 12 months at diabetologia department hospital. Diabetes screening was achieved by Oral glucose tolerance testing (OGTT). Metabolic parameters were determined by spectrophotometry. Insulin by radioimmunoassay. Insulin sensitivity was also assessed by the homeostasis model assessment (HOMA) approach (glucose × insulin/22.5).

Results: The fasting glucose was normal in all three groups. In *the 1st* and *the 2nd* groups there is a significant hyperinsulinemia and to a lesser extent in the 3rd group (72%, 66% and 57%, respectively). The HOMA model confirms an acute insulin resistance (79%, 75% and 65% increase respectively). Hypertriglyceridemia was observed only in the 2nd group. HDL dyslipidaemia has been identified in women *of the 2nd* group (<0.39 g/L). Our study seems to confirm that the genetic predisposition to type 2 diabetes is not the only factor, but environmental factors identified by MetS markers are also to considerate. This study has revealed the same metabolic alterations in subjects screened, they have one or more MetS risk factors with or without diabetes family.

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05-O016

The diabetic phytovigilance, what does in Morocco?

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Introduction Diabetes is an epidemic disease of the millennium; it imposes a heavy economic charge especially among populations of emerging countries and poor. In Morocco, the diabetic patients are generally forced to seek alternatives to drugs, such as herbal medicine.

This work aims to describe the profile of diabetic patients using herbal medicine and to list the adverse effects of these plants

materiel and methods: This is a prospective study over 3 months (March-May 2011), including all patients with diabetes type 2, men and women, adults, hospitalized or consultant to the endocrinology department. Children, type 1 diabetes and women with gestational diabetes were excluded.

The demographic, clinical, para clinical and therapeutic data were collected by the farm and then analyzed using SPSS version 17. The results are expressed as numbers, percentages or means ± standard deviation. The group of patients who use medicinal plants, and group who do not use were compared by chi-square.

Result: Our study included 129 diabetic patients, hospitalized or consultant in the Department of Endocrinology of CHU-Fez, the average age of our patient was 56 ± 10 years. The female is dominant with 69%. Fifty-one percent of patients are not secularized, and 85% of them live in urban areas. The use of herbal medicine was noted in 61% of our population. Among the 79 patients that use plants, 87% use herbal medicine because of its effectiveness, and only 4% because of its cost.

In our study 37 plants were used (average of 2.7 ± 2.15 plants for each patient). The plant is most commonly used is fenugreek (Halba), followed by sage (Salmia), olive (Zaytuna), dill (chebt), oregano (zaatar) and wormwood (chiba).

The use of plants has resulted in 12.7% of cases of adverse reactions recorded by the patients themselves adverse effects observed in patients using plants differ from one patient to another, the gastrointestinal disorders that are most common.

Conclusion Serious efforts should focus on awareness of the dangers of using these plants, especially those with a known potential for toxicity. The implementation of a performance system of phytovigilance is mandatory to eradicate if not reduce the danger of the plants.

11-O017

Tenofovir exposure through breast feeding: serum concentrations in neonates and clinical follow-up

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Objective: The prevalence of chronic viral hepatitis B during pregnancy is estimated between 0.1% and 6%. Since recommendations for immunoprophylactic treatment in neonates born to infected mothers, several studies have shown that breast-feeding does not increase the risk of postnatal mother-to-child transmission in these properly immunized newborns. Tenofovir disoproxil fumarate (TDF) is a prodrug of the active nucleotide analogue tenofovir (TVF) used in HIV and HBV infections, including during pregnancy. Although the available data on human milk transfer indicate that only very limited amounts of TVF are excreted into breast milk, clinical data on the safety of breast-feeding for the newborn of TDF-treated patients are lacking. Lyon Pharmacovigilance Center is regularly asked about the compatibility of breast-feeding in HBV-positive TDF-treated women. Our experience regarding TDF during breast-feeding is described.

Patients and method: After individual assessment, clinical and biological surveys were proposed to each patient. To monitor infant exposure to tenofovir through breast-feeding, serum level measurement in the newborn was systematically proposed after 2–3 weeks of breast-feeding. A clinical follow-up was also performed.

Results: Since 2010, a follow-up has been obtained from four patients that exclusively breast fed five neonates (one pair of twins) for a mean duration of 1.8 months. Two neonates were premature with a gestational age of 33 weeks. All mothers have been treated with tenofovir 245 mg/day throughout pregnancy for chronic viral hepatitis B. Associated drugs in one mother included hydroxychloroquine and corticosteroids. Serum tenofovir levels were measured in all five neonates. Tenofovir was undetectable (lower limit of detection <0.005 mg/L) in 4, and close to the limit of detection in one (0.0055 mg/L). Short-term follow-up (4 months of age) of two infants exclusively breastfed during 3 months did not show any adverse outcome regarding standard developmental growth parameters.

Conclusion: This is the first study investigating tenofovir exposure in infants exposed through breast-feeding. The results confirm previous information suggesting that tenofovir is probably safe during breast-feeding. Until more data are available, careful infant monitoring is recommended especially in infants with renal dysfunction.

11-O018

Psychomotor effects of in utero exposure to psychotropic medications: a comparative study in EFEMERIS database

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Some studies have assessed the risk of malformations and perinatal adverse reactions associated with in utero exposure to psychotropic drugs. Conversely, little is known about neurodevelopment of children exposed to these drugs during pregnancy.

Objective: The objective of the present study was to compare psychomotor development between children exposed in utero to psychotropic medications and children unexposed to these drugs in utero.

Method: EFEMERIS is a database including prescribed and delivered drugs during pregnancy (data from Caisse Primaire d'Assurance Maladie of Haute-Garonne) and outcomes (data from Maternal and Infant Protection Service and from Antenatal diagnostic Centre). Women delivered from 2004 to 2008 in Haute-Garonne and registered in the French Health Insurance Service were included into EFEMERIS database. We compared neurodevelopment at 9 months and 2 years (data from compulsory medical examinations registered by Maternal and Infant Protection Service) between children exposed to psychotropic medications (anxiolytics, antidepressants, neuroleptics and antiepileptics) during the second and/or third trimesters of pregnancy and children unexposed to these drugs.

Results: Four hundred and ninety-three (1.5%) newborns exposed during the second and/or the third trimesters of pregnancy to psychotropic drugs were compared to 32, 303 controls. Two hundred and ninety-eight (0.9%) infants were exposed to anxiolytics, 207 (0.6%) to antidepressants, 83 (0.3%) to antiepileptics and 81 (0.3%) to neuroleptics. Prematurity rate was similar in the two groups. Exposed newborns have no more malformation than controls.

Exposed infants have more motor difficulties at 9 months (8.8% vs. 6.1%; $P = 0.01$) and 2 years (1.8% vs. 0.4%; $P = 0.003$) than controls and more negative items about mental development at 2 years (2.2% vs. 1.0%; $P = 0.04$). After adjustment on mother age, prematurity and malformations, the relative risk of abnormal motor development increased (RR = 1.6 [1.1–2.2] at 9 months and RR = 4.8 [2.1–11.0] at 2 years]). More abnormal psychomotor development was observed in children exposed to antiepileptics (mainly sodium valproate), neuroleptics or antidepressants in particular.

Conclusion: The present study found an association between psychotropic drug prescription during second and third trimesters of pregnancy and abnormal psychomotor development. However, these results must be discussed according to other confounding factors, like mothers diseases, social environment

11-O019

Usefulness of pharmacovigilance databases for drugs in pregnancy: a study on antihypertensive drugs

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Aim: Antihypertensive drugs are prescribed to 5% of pregnant women and consecutive risks for the foetus need to be evaluated. We investigated a methodological approach for more systematically use pharmacovigilance data in relation to pregnancy. For this purpose we analysed adverse effects (AE) reported for foetus or newborns in association with maternal administration of antihypertensive drugs, restricted to those preferentially used: adrenergic beta-antagonists (ABA) and calcium channel blockers (CCB).

Material and methods: Potential AE for foetus or newborns were reviewed in scientific literature for all ABA ($n = 18$) and CCB ($n = 12$) available in France. In parallel, queries and analysis of fetal or neonatal AE were performed in two pharmacovigilance databases: the French Pharmacovigilance Database (BNPV) up to March 2011 and OMS international pharmacovigilance database (Vigibase) up to May 2011.

Results: Neonatal AE may occur with ABA (hypoglycemia, cardiorespiratory depression). Intrauterine growth retardation has been reported with ABA or CCB, but probably due to underlying maternal hypertension. An increase in malformations rate has not been highlighted. In BNPV, 280 cases of AE were found. Cases involving ABA ($n = 73$) described neonatal hypoglycemia ($n = 33$), bradycardia ($n = 23$), respiratory distress ($n = 9$). Nine deaths, six malformation cases, 11 growth retardations and two prematurities were reported. Cases with CCB ($n = 9$) included three fetal or neonatal distress. In Vigibase, 538 cases were identified with ABA and 314 with CCB. AE described with ABA as a single agent ($n = 184$) were neonatal effects of ABA (14%), prematurity (10%), growth retardation (11%), congenital abnormalities (43%) and deaths (16%). Among cases involving CCB as a single agent ($n = 70$), neonatal troubles (17%), prematurity (20%), congenital malformations (31%) and deaths (16%) were reported.

Discussion: Analysis of databases led to similar description than data derived from literature, leading in part to the validation of the query's methodology. Similar AE were reported in the two databases that appeared to be complementary: the french database contains more precise descriptions and international database more cases. Such comparison will allow further development of adequate queries in databases.

11-O020

Tamoxifen exposure and pregnancy: what is known about the potential risks?

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Introduction: Tamoxifen is an antineoplasic agent with anti-estrogenic properties, but also an estrogen-like effect, used to treat breast cancer. Women who delay pregnancy into their thirties or desire a child after the diagnosis of cancer risk exposure to tamoxifen during pregnancy.

Methods: We present data collected by 14 French Regional Pharmacovigilance Centres and a point on litterature.

Results: Summary of product characteristics impose 2 months delay between the end of treatment and the beginning of pregnancy, because of the half-life of the molecule and its major metabolite. Moreover, some authors have shown significant concentrations of tamoxifen in different tissues (lungs, heart, ovary, intestinal wall), up to 14 months after the end of treatment. Tamoxifen shows carcinogenic properties in rodents and abnormalities in the reproductive tracts of animals (mostly females) after *in utero* exposure, similar to those observed in humans after diethylstilbestrol (DES). Embryogenesis of external genital tract starts in human around 7th week of gestation.

Precise human data are scarce: Ten published documented cases, with 10 live births; three of which report anomalies (e.g. ambiguous genitalia in a girl, exposed to tamoxifen during the first 20 weeks of pregnancy). In our database, full data were obtained for 26 pregnancies. Mean age for these women was 36 years old (25–46). Two (7%) women were exposed before pregnancy, 14 (54%) during the first trimester, six (23%) during first two trimesters and three (12%) througout pregnancy. Such data are missing for one (4%) case. Spontaneous abortion occurred in one pregnancy (4%), elective abortion in four cases (15%). Medical abortion was decided in four cases (15%), with no anomaly in one foetus and no information for the others. Seventeen pregnancies (66%) ended in live birth without congenital malformation. No relationship between time of exposure and outcome of pregnancy was observed.

Conclusion: Few data are available for human pregnancies exposed to tamoxifen. However, increasing age of pregnant women could expose more women. It's recommended to avoid pregnancy for at least 2 months after a treatment, and any exposure needs individual counselling. Because of potential carcinogenicity, follow-up of *in utero* exposed offspring is recommended.

12-O021

A new cause of valvular heart disease: 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy')

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Ecstasy, an amphetamine-derived drug, is a psychoactive stimulant widely used as a recreational drug. In vitro experiments have suggested that ecstasy can lead to fenfluramine-like proliferation of cardiac valves through activation of 5HT2B receptors. We report the first observation of valvular disease with pathological confirmation after long-term use of ecstasy.

A 33 year old male patient was admitted for shortness of breath and chest pain. The only risk factor reported was active smoking. He reported long lasting exposure to ecstasy (several pills/week since 17 years old). At examination a systolic and diastolic mitral murmur was diagnosed. BP was of 106/70 mmHg. ECG showed sinus rhythm (91 bpm) with a right bundle branch block. Echocardiography revealed a slight left ventricular dilation with an ejection fraction of 55% and typical features of mitral valve disease. There was a left atrial enlargement (area of 42 cm^2) thick mitral valve with decreased opening of the mitral valve leaflets. The mean pressure gradient across the mitral valve was of 14.7 mmHg and the mitral valve areas of 1.56 cm^2. These moderate to severe mitral stenosis features were associated with severe mitral regurgitation (regurgitant orifice area of 0.49 cm^2) with a central jet of blood. Other valves and heart chambers were normal. This patient had pulmonary hypertension (40 mmHg) without right HF. A coronary angiogram showed normal coronary arteries with a decreased cardiac index and post capillary pulmonary hypertension. Mitral valve leaflets and the chordae tendinae were markedly thickened and fibrous. The chordae tendinae were retracted. At histology, the endocardium on both sides was fibrous up to 2 mm-thick. It was stratified with layers of collagen bundles and spindle cells. The density of spindle cells was heterogeneous, but more frequently low. In this abundant extracellular matrix, rare foci contained abundant elastic fibers. No inflammation or myxoïd matrix accumulations were observed. Common diseases or intake of other drugs known to affect cardiac valves were not found.

The present case report suggests that ecstasy could be able to induce valvular heart disease.

12-O022

Is cannabis involved in cardiovascular disorders? Experience from the French Addictovigilance Network

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Travaux réalisés par l'Association Française des Centres d'Evaluation et d'Information sur la Pharmacodépendance – Addictovigilance (CEIP-A)

Aim: Cannabis is known to be associated with neuropsychiatric troubles, and less to complications affecting other specified body systems. Several outstanding cardiovascular complications following cannabis use were reported to the French Network of Addictovigilance Centres (CEIP-A) during the recent years. This study aimed to summarize and evaluate the cardiovascular complications of cannabis reported to the French monitoring system of abuse and dependence.

Methods: We identified all spontaneous reports (NotS) of cardiovascular complications related to cannabis use collected from 2006 to 2010 by the 13 French CEIP-A. Clinical characteristics of these cases and their evolutions were described.

Results: Among the 1979 NotS involving cannabis exposure, 35 were cardiovascular complications. Patients were mainly males (85.7%) with a mean age of 34.3 years old (SD 8.8). Cases were characterised by three cerebral, 10 peripheral and 22 cardiac complications with 20 cases of acute coronary syndromes and two heart rate disorders. 'Acute cerebral angiopathy', 'transient cortical blindness' and 'spasm of cerebral artery' were reported as cerebral complications. Peripheral complications consisted in lower limb or juvenile arteriopathies and in Buerger-like diseases. Polydrug use was notified in eight cases (mainly tobacco and alcohol). The follow-up of these reports identified nine deaths in patients with cardiac complications whereas 19 patients were hospitalised for acute coronary syndrome. Management of hospitalised patients comprised non-invasive and invasive techniques (i.e. cardiac repermeabilisation, limb amputation).

Discussion: Cannabis derivatives were shown to lead to cardiovascular complications through mechanisms involving the autonomous nervous system. These complications represent about 2% of all adverse events related to cannabis use reported to the French system of drug abuse monitoring. Even though these complications are more likely observed among young adults who use stimulant drugs, practitioners should be aware that cannabis may work as other illicit psychoactive drug (cocaine or amphetamine) and may be a potential triggering factor for cardiovascular complications in young people.

12-O023

Identification of drug abuse and dependence cases using the French hospital administrative database

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Objective: To identify definite and possible substance abuse and drug dependence cases in the regional hospital databases

Methods: Cross-sectional study conducted in patients hospitalized in hospitals of our region in 2009. Patients registered in the regional hospital databases with an ICD-10 code, suggesting drug abuse or dependence, were pre-selected. These suspicions of cases were then assessed by a validation committee.

Results: A total of 639 cases were validated. These cases came from 76 hospitals among the 161 (public and private) hospitals of the region. Out of the 531 hospitalisations with a code 'Cannabis', 22 were classified as certain and 81 as possible (neurological effects, respiratory system disorders, cardiovascular and psychiatric complications). Out of the 143 hospitalisations with a code 'Cocaine', 11 were classified as certain and 36 as possible. Out of the 24 hospitalisations with a code 'Psychostimulant', 16 were classified as certain or possible and out of the 13 hospitalisations with a code 'Hallucinogenic substance', five were classified as certain or possible. In the heterogeneous opioid group ($n = 688$), 149 cases were classified as possible abuse or dependence cases or drug-related hospitalisation (especially infectious and pulmonary complications). In the group of 'Polydrug use' (787 hospitalisations), 194 were considered as possible cases, with mostly neurological, infectious, cardiovascular and pulmonary complications. From paediatric cases after exposure during pregnancy, 125 cases of possible substance abuse and dependence were identified.

Discussion: The use of data from the regional PMSI database to identify cases of substance abuse and dependency for some specified substances is possible especially for substances such as cocaine and cannabis. However, it is difficult to identify cases of opiate abuse or addiction, not clearly coded or in case of 'Polydrug use'. The imprecise codification of hospital stays as well as the multitude of codes in the ICD-10 may hinder the creation of a simple algorithm to aid in the identification of cases.

This database could, however, be used in routine for substances such as cocaine, cannabis or cases occurring in neonates after pregnancy exposure.

12-O024

Long term consequences of acute pain on patients receiving methadone or buprenorphine maintenance treatment: a prospective multicenter cohort study

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Objective: To investigate if exposure to an acute painful phenomenon could lead to a decrease of long term retention concerning buprenorphine or methadone maintenance.

Methods: Patients aged 18 or more and under opioid maintenance treatment (OMT) since at least 3 months were included in this prospective multicenter cohort study with a follow-up of 12 months. Patients were considered in the acute Pain group (P) if they were exposed to an acute painful phenomenon at inclusion, the Control group (C) corresponding to patients not exposed at the inclusion. Follow-up was performed by general practitioners in charge of these patients. The primary outcome variable was the OMT retention rate at 12 months. In case of lost of follow-up during the study period, we considered in an intention-to-treat analysis that those patients were no longer under OMT. Statistical analysis was performed with SAS 9.1© software. Comparison between groups used Chi-square tests, and multivariate analysis was performed using logistic regression to identify factors associated to 12 months OMT retention.

Results: Among the 151 patients included (80 in group P), 111 (74%) were male and 54 (36%) had a traumatic pain. The median age was 36 (Interquartile range 30–41). One hundred and four (69%) were buprenorphine users. The groups did not significantly differ for all aspects including chronic pain, anxiety, OMT misuse and usual treatment. The patients in the group P were by definition more often admitted for a traumatic chief complaint (44 patients (55%) in group P and 10 (14%) in group C). The 12 month- OMT retention rate was 42% ($n = 30$) in group C vs. 25% ($n = 20$) in group P, ($P < 0.05$). The multivariate analysis found two factors independently associated with a lower 12 month-OMT retention: Patients of group P vs. group C (OR, 0.5; CI95%, 0.2–0.9; $P < 0.05$), and buprenorphine vs. methadone users (OR, 0.4; CI95%, 0.2–0.8; $P < 0.05$).

Conclusion: Acute pain is associated with significant changes in OMT long term retention. One hypothesis should be an inadequate treatment of the pain. Effective management of pain in these patients requires a heightened awareness of this important issue.

12-P193

Detection and magnitude of methylphenidate abuse and misuse using VigiBase and correlation with data from drug utilisation studies

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Introduction: Methylphenidate (MPH) is a psycho-stimulant approved for the treatment of attention deficit and hyperactivity disorder in USA and more later in European countries. Recently, consistent data suggesting an increase of MPH abuse were identified in France with intravenous administration of crushed tablets. In the same way, a rise in MPH global availability has been identified in France and other European countries.

Objective: To assess the relationship between the levels of consumption of methylphenidate in European countries and MPH misuse or abuse reported to VigiBase, the WHO global ICSR database.

Methods: Data were collected for all Continental European countries for the period 1994–2010. Data on MPH utilization were researched and extracted from national consumption statistics. Using Vigibase, individual case reports of MPH abuse, defined by the WHO-ART term 'drug abuse', were extracted. Trends in MPH abuse reporting were analysed using a bayesian confidence propagation neural network method, providing a statistical indicator, information component (IC) (Norén et al, 2011).

Results: Despite an extensive variability in the consumption levels, there is a common trend of growing MPH utilization in Europe, with a sharp increase since 2005 (+ 525% in Denmark (0.8–4.2 DID), + 167% in France (0.18–0.30 DID), +216% in Germany (1.0–2.16 DID) and +222% in Netherlands (2.04–4.53 DID) between 2005 and 2009). Preliminary results from VigiBase showed an increasing relative reporting rate over time for European reports with methylphenidate and drug abuse using the Information Component (IC), which is computed as the logarithm of a shrunk observed-to-expected ratio.

Conclusion: Analysis of the trends in MPH consumption, together with preliminary findings from Vigibase, is consistent with the existence of a positive relationship between the recent MPH increasing availability and the growing frequency of reported dependence-related ADRs. In a way to better understand and characterize this association, a quantitative in-depth analysis of these preliminary results will be undertaken.

Reference: 1. Norén GN, Hopstadius J, Bate A. Shrinkage observed-to-expected ratios for robust and transparent large-scale pattern discovery. Stat Methods Med Res 2011.

12-P194

Drug use in the French overseas departments and territories in the 2010 OPPIDUM survey

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Objective: Data on drug use in the French overseas territories are sparse. For a few years, some overseas addiction centres have been taking part in the French OPPIDUM (Observation of Illicit Drug and Misuse of Psychotropic Medications) program.

The purpose of this study was to describe the profile of drug users and the characteristics of their psycho-active substances use in overseas areas.

Methods: OPPIDUM is an annual cross-sectional nationwide survey, based on the network of the Centres for Evaluation and Information of Pharmacodependence-Addictovigilance. They recruit, *via* addiction centres, subjects presenting with addiction, drug abuse and/or using opiate maintenance treatment. Through a standardized questionnaire, patients' use of psycho-active substances during the week preceding the interview is collected. The areas were grouped as follows: Pacifique Sud (Polynésie Française, Nouvelle-Calédonie), Antilles-Guyane (Guyane, Guadeloupe, Saint-Martin) and La Réunion.

Results: In 2010, 184 subjects (229 psycho-active substances) were included in the French overseas territories (vs. 4971 subjects (10 107 psycho-active substances) in metropolitan France): 102 (102) in Pacifique Sud, 54 (60) in Antilles-Guyane, 28 (67) in La Réunion. There were 146 men, 38 women (vs. 3839 and 1132 in metropolitan France); mean age was 32.5 years (vs. 33.7 in metropolitan France).

Medicines were used by 19% of subjects in Pacifique Sud, 7% in Antilles-Guyane, 93% in La Réunion (mainly benzodiazepines and opiate maintenance medicines) vs. 87% in metropolitan France. Cannabis was used by 84% of subjects in Pacifique Sud, 52% in Antilles-Guyane, 14% in La Réunion vs. 37% in metropolitan France. Heroin was used by 4% of subjects in La Réunion (no use in Pacifique Sud and Antilles-Guyane) vs. 17% in metropolitan France. Cocaine was used by 52% of subjects in Antilles-Guyane (*crack*: 82% of cocaine use), 4% in La Réunion (no use in Pacifique Sud) vs. 10% in metropolitan France (*crack*: 8% of cocaine use).

Discussion: The 2010 OPPIDUM survey shows that characteristics of drug use are very different between the French overseas areas and metropolitan France and within overseas regions. This survey must be reinforced in these areas, in order to carry out prevention programs that take into account the specificities of drug use.

12-P195
ASOS 2007–2011: focus on fentanyl

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Objective: Long-acting transdermal fentanyl is indicated in severe chronic pain resistant to other opiates, but reimbursed in France only in cancer pain. Short-acting formulations are approved only in breakthrough pain in patients already receiving maintenance opioid therapy for chronic cancer pain. The purpose of this study is to evaluate the use of fentanyl in France through ASOS surveys.

Methods: ASOS is an annual 1-week cross-sectional survey conducted in a representative sample of pharmacies in France, which include patients with a prescription form containing at least one narcotic analgesic. Data of the 2007–2011 ASOS surveys were analyzed for each pharmaceutical form of fentanyl.

Results: Three hundred and thirty-four patients of the 713 (47%) patients included in the ASOS survey in 2007 had a prescription of long-acting transdermal fentanyl, 313/693 (45%) in 2008, 264/586 (45%) in 2009, 254/623 (41%) in 2010 and 279/642 (43%) in 2011. The indication was cancer pain in 41.5% of cases in 2007, 46% in 2008, 33% in 2009, 42% in 2010 and 38% in 2011.

In 2007, 15 patients (2%) among the patients included in the ASOS survey had a prescription of short-acting oromucosal fentanyl, 23 (3%) in 2008, 25 (4%) in 2009, 18 (3%) in 2010 and 19 (3%) in 2011. The indication was cancer pain in only 5.5% of cases in 2010 vs. 39% in 2008. Another opiate drug (OD) was associated in 20% of cases in 2007, 62.5% in 2008, 44% in 2009, 61% in 2010 and 53% in 2011.

In 2009, 2010 and 2011, sublingual fentanyl tablets were prescribed respectively to three patients (with another OD in two patients), two patients (with another OD in one patient) and five patients (with another OD in three patients); when known, the indication was always cancer pain.

Discussion: As the most potent opiate, fentanyl has a major risk of abuse and dependence as well as death from respiratory depression. However, off-label use was frequent in ASOS surveys, particularly for short-acting fentanyl: prescription of short-acting fentanyl without another OD or in other indications than cancer pain. These results confirm other data available in France on fentanyl use.

12-P196
On site-urinary drug screening tests for opiate substitution treatment: a qualitative study among general practitioners in Toulouse (France)

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Purpose: The first general practitioners (GPs) who began to prescribe opiate substitution treatments in France in the mid 90's did not have easy access to on-site urinary drug screening tests. Consequently, they developed a prescription practice without these tools. By promoting access to tests for GPs, recent local initiative in the city of Toulouse may possibly change their way of prescribing opiate substitution treatments.

The purpose of this study was to understand current ways of using tests among GPs in Toulouse, and to describe the characteristics of practitioners who chose or not to use tests.

Materials and method: This study was based on 20 qualitative interviews with GPs working in Toulouse, and on ethnographic observations of medical consultations for opiate substitution treatment. These data have been analyzed in order to provide typologies of test practices.

Results: Three attitudes towards on-site urinary drug screening tests were identified among our sample of GPs: practitioners using tests frequently, GPs wishing to use tests, but encountering material or ethical problems to integrate them into their daily practice, and GPs reluctant to use tests.

Three different ways of using tests were also identified: GPs using tests for diagnosis of opiate addiction to begin a treatment, GPs using tests with the intent to control drug use along a treatment, and GPs introducing innovative practices, in which tests are not used for the information given by their results.

Discussion: This qualitative study showed that urinary tests are not widely used in Toulouse: most GPs from our sample were not interested or not informed about these tools. Historical construction of their practice and their representation of addictions can help us to understand their stance. However, innovative practices with tests show that some GPs are starting to appropriate these tools.

Nevertheless, a comparative study with a larger sample including other locations would certainly help understanding the extent of local specificities in our results, which cannot currently be extrapolated to all French GPs' situations towards urinary tests.

12-P208
Assessment of methadone capsules acceptability in two care centers: a descriptive study

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Background: Methadone capsules form is available in France since 2008. Few data are available on the acceptability of patients regarding this new formulation.

Objectives: To assess the patients' 'feeling' after the switch methadone syrup/methadone capsules and the misuse profile of the methadone capsules.

Methods: A descriptive study using a questionnaire was conducted between March 2011 and October 2011 in two methadone centers. Information about socio-demographic characteristics, drug status, methadone maintenance treatment as syrup and then as capsules form were collected.

Results: Thirty patients (80% men) were included in the study, with a mean age of 39 years [range 23–53]. Most have regular resources (93%) with a stable housing (96%) and a professional activity (53%). The mean age at the start of drug abuse was 15 years [range 12–17]. The first psychoactive drug consumed was cannabis (80%). Alcohol dependence was mentioned for two patients and most were smokers (93%). Illicit consumptions were cannabis (36%) and cocaine (one case). The mean duration of methadone maintenance therapy as syrup was 3 years [range 1–12], for three patients duration was <1 year [15 days–4 months] due to syrup intolerance. A majority of patients (80%) described side effects related to the syrup formulation (nasty taste, disorders linked to sugar, gastric disorders and sweating). Mean dose at the switch to methadone capsules was 70 mg/day [range 15–160]. The main reasons for the switch were practical using and side effects. Concerning methadone capsules, the mean duration was 12 months [range 1–24]. Two patients needed a dose increase. Few patients described a difference in the 'bioequivalence' between the two formulations. Concerning capsules misuse, 23% of patients reported to have seen capsules available at black market, and two have already obtained it without a medical prescription. None of patients tried to solubilize methadone capsules and five tried to snort it.

Conclusion: Access to methadone capsules concerns mainly long-time dependent patients. Patients seem to be satisfied with this new formulation, none of them returned to the syrup. Despite the rule of prescription and dispensation, the capsules availability on the black market seems to be a reality.

12-P209
Management of opioid withdrawal: retrospective study in a cohort of chronic non-cancer pain patients

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Background: Chronic Non-Cancer Pain patients (CNCP) are usually treated with opioid analgesics, but the efficacy seems to be limited [Rome *et al.* 2004]. In addition the use of morphine derivatives exposes the patients to adverse-effects, physical dependence, psychological dependence, hyperalgesia, and tolerance.

Objective: The aim of this retrospective study was to compare patients at admission and discharge from a management of opioid withdrawal hospitalization.

Methods: From May 2008 to May 2011, 63 CNCP patients were admitted to the Pain Medicine Center, for opioid withdrawal. The following data were collected in the medical records: age, gender, hospitalization period, pain diagnoses and severity status (Visual Analogue Scale), use of opioids, depression, outcome and treatment at discharge. Opioid intake was converted to oral morphine equivalents for analysis.

Results: The mean age of the patients (women 63%) was 52 years [range: 29–75]. Low back pain (54%), neuropathic pain (20%) and polyalgia such as fibromyalgia (12%) were the most frequent pain disorders. At admission, 65% of patients have depression and available VAS were between 5 and 8 for 82%, and >8 for18%.

The most frequently identified opioids were morphine (long and short acting morphine sulphate) 43%, transdermal fentanyl 33%, oxycodone 21%, and buprenorphine 2%. An opioid association was used in 11% of cases. At admission, opioid dose were available for 53 patients, the mean daily morphine equivalent dose was 160 mg [3.75–720]. The mean duration of hospitalization was 12.7 days [1–77].

Discontinuation of opioid treatment was total for 34% of patients, 58% of patients have decreased the dose, 2% have no change and 6% have increased the opioid consumption. Discharge treatments associated different therapeutic class: anxiolitics, antidepressants, antiepileptics and antipsychotics. In addition to opioid withdrawal, the management of patients includes patients' educational program and daily relaxation therapy.

Discussion-conclusion: AFSSAPS guidelines about CNCP management recommend morphine sulphate. In our study, fentanyl and oxycodone were mostly used. Comparatively to the study of Rome et al, mean initial daily morphine equianalgesic dose was much higher (74 vs. 160 mg). Reevaluation of opoid treatment needs to be done regularly in order to decrease difficult management of withdrawal.

12-P210
General characteristics of incidents patients substituted with buprenorphine (HDB) in 2008 in PACA-Corse: the impact of the arrival of generic terms in their consumer

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In France since 1996, high dosage buprenorphine (HDB) was marketed as an opioid maintenance therapy and generic forms (GNR) were available since 2006. This study aims to describe patients with HDB with a focus on incident patients and on the repartition between princeps and GNR forms.

This study was conducted using french health reimbursment database, at the regional level (PACA-Corse). All patients with at least one HDB dispensing in 2008 have been included. All dispensing occured in 2008 were collected. Incident patients were defined as subjects who haven't had any HDB delivery before 1st march 2008.

Eight thousand one hundred and thirty-seven patients had at least one HDB dispensing reimbursed in 2008. Among them, 7% had only one dispensing, 83% had an average period between each dispensing lower than 36 days, 5% between 35 and 45 days and 5% beyond 45 days. Ninety-three percent had at least one dispensing of the princeps and 37% of the GNR. Among these patients, 22% were considered as incident. Among these incident patients, 53% had also benzodiazepines dispensing, 21% antidepressants, 15% opioids,15% antipsychotics and 1%

methadone. Concerning the repartition princeps/GNR, 66% patients had only dispensings of princeps, 12% only GNR and 22% had dispensing of princeps and GNR (3% had first princeps and then switch to GNR, 5% had first GNR and then switch to princeps and 14% overlap both form).
This study shows that among incident patients, princeps is always often dispensed and some patients overlap both forms.

12-P211
Methadone maintenance treatment: which benefits in ambulatory patients?

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The impact of methadone maintenance treatment (MMT) on the improvement of quality of life and social insertion remains controversial and insufficiently documented.
Objectives: To evaluate the serological status and social outcome of patients who benefited a MMT, initiated at the Center for drug abuse treatment at NANCY University Medical Center (France). The study was coordinated by the Center for Evaluation and Information on Pharmacodependence and conducted by the county's 'City-Hospital Drug Abuse Network' (*RTVH 54*), in 2009.
Method: Retrospective follow-up study based on two questionnaires, documented by the physicians: one through a retrospective analysis of the patients' medical records before methadone treatment initiation and one, after several years of substitution treatment, based on present clinical and social status of the patient. Follow-up could be assessed only for 73 out of the 100 initially screened patients (55 men, 18 women, mean age: 33 years-old). The variables studied were: 1: serologic status for HIV, VHB and VHC; 2: social insertion, concerning independent housing, financial and marital status.
Results: The 73 patients were evaluated after 37.6 ± 13.8 months methadone maintenance treatment. Whereas more than 25% of the patients were ignorant of their serological status for HIV and VHC before methadone initiation, they were much more aware of their serological conditions while on MMT: 86% knew their status for HIV, 83.5% for VHC and 70% for VHB. Patients also improved their living conditions significantly while on methadone treatment: whereas 28.8% had no personal income before MMT, they were only 7.2% after MMT. Similarily, MMT permitted more independent and self-financed housing (52.1% before vs. 70.6% after MMT). They were also less isolated socially and 47.1% were living with a partner after MMT, whereas they were only 32.9% before MMT.
Conclusion: Methadone maintenance treatment clearly demonstrates its benefits in resocialization and in improvement of quality of life for addicted patients and its interests in a risk limitation policy.

13-O025
A model predicting expected infliximab serum concentrations using only dose and time since last infusion

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Objectives: Infliximab, an anti-TNF-α antibody, has profoundly modified the treatment of several inflammatory diseases but its pharmacokinetics (PK) is highly variable between patients. This variability influences the clinical response. The objective of this study was to describe infliximab pharmacokinetics using routine therapeutic drug monitoring (TDM) data and to develop a model allowing the prediction of infliximab concentrations using only dose and time since last perfusion.
Methods: Between 2005 and 2010, infliximab concentrations were monitored in 655 patients treated with infliximab in Tours university hospital. Infliximab concentrations associated with positive antibodies toward infliximab were removed from the analysis. To describe infliximab pharmacokinetics, a one-compartment PK model was used. A simplified model for TDM (TDM model) was built to devise the expected infliximab concentrations using only dose and time since last infusion. This model described, for each visit, a monoexponential decrease of infliximab concentrations since the last infusion. 2/3 and 1/3 patients were randomly assigned to estimation and validation groups, respectively. In the validation group, infliximab concentrations were predicted using typical PK and TDM model parameter estimates. To validate both PK and TDM models, their respective concentration predictions were compared.
Results: A total of 354 patients were eligible. They were treated for rheumatoid arthritis (RA, $n = 49$), ankylosing spondylitis (AS, $n = 132$), inflammatory bowel disease (IBD, $n = 112$), both AS and IBD (AS-IBD, $n = 7$), Psoriatic rheumatism (PR, $n = 44$) and other diseases (OTH, $n = 10$). Both PK and TDM models described the data satisfactorily and provided estimations of volume of distribution (Vd) and clearance (CL). Using PK analysis, median Vd, CL and half-life (T½) were 6.7 L, 0.32 L/day and 14 days, respectively. Using TDM model, apparent volume (Vd*) and clearance (CL*) were 5.7 L and 0.27 L/day, respectively. The two types of PK parameters were similarly influenced by covariates: Vd and Vd* were influenced by disease whereas CL and CL* were influenced by disease, weight and sex. The validation step showed that the two models provided similar concentration predictions.
Discussion: Infliximab PK is different between SPA, AS and IBD patients. The developed TDM model is precise and accurate and may be used for TDM of infliximab.

13-O026
Dapt-os; a monocentric phase one clinical study to assess the daptomycin penetration in bones

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Objectives: Clinical data suggest that daptomycin may be superior to vancomycin for the treatment of *Staphylococcus aureus* bone and joint infections. However little is known on the penetration of daptomycin in bones and synovial fluid. The aim of our pilot clinical study was to assess the penetration of daptomycin in these tissues.
Methods: This study was conducted in non infected patients undergoing surgery for knee or hip replacement. Prior to surgery, they received a single 8 mg/kg daptomycin dose. Plasma daptomycin concentrations were determined 1 h after the end of daptomycin infusion (Cmax) and during the surgery, around the mean residence time of daptomycin (Cmrt). Daptomycin concentrations were also measured on bone fragments (tibia; femur; acetabulum depending the surgery done) and synovial fluid collected. Daptomycin determinations were realized using a validated diode array-HPLC method (limit of quantification 0.05 mg/L). The bone penetration was considered good if the bone daptomycin concentration was above 1 mg/g, which is the susceptibility breakpoint of clinical isolates of *Staphylococcus aureus* for daptomycin (EUCAST 2011).
Results: Sixteen subjects (median age 69 years, range 55–91) were evaluated. Knee and hip replacement were performed in 10 and six patients respectively. In average, Cmrt, bone and synovial fluid were collected 7.4 h (range 4–12 h) after daptomycin infusion. Mean daptomycin concentrations were respectively of 3.3; 3.4; 9.3 μg/g (in tibia; femur; acetabulum) and 21.6 μg/mL in synovial fluid. Median daptomycin bone concentration in early (<8 h) and late samples (>8 h) were respectively of 2.5 and 4.2 μg/g ($P = 0.0378$).
Discussion: In this study, the bone daptomycin concentrations were above the minimum inhibitory concentration of daptomycin for *Staphylococcus aureus* in all subjects. These results support the use of daptomycin in the treatment of *Staphylococcus aureus* bone and joint infections.

13-O027
Perspectives in colistin therapeutic drug monitoring

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Objective: After being abandoned for many years because of suspected nephrotoxicity, colistin is increasingly used as a last line defence against MDR Gram-negative pathogens. It is administered as a prodrug (CMS) and specific analytical assays have only recently been developed[1]. The present study reports our experience in developing PK tools for an optimized TDM of colistin in critical care patients.
Methods: Results from pharmacokinetic studies in healthy volunteers[2] and patients ($n = 98$) were combined to perform a population pharmacokinetic analysis allowing the determination of main parameters of CMS and colistin[3].
Results: At steady-state, fluctuations of colistin concentrations were limited within the most frequent (8 h) dosing interval and individual average concentrations (Css) could be predicted from: $Css = (CL_{NR}/CL_{NR} + CL_R)\ (Dose/t)\ (1/CL_{coli})$; where CMS renal clearance (CL_R) was set at 0.85 * creatinine clearance and used to select the initial colistin dosing regimen (Dose/t) to reach a target concentration of 2 μg/mL. The initial estimate of CMS non-renal clearance (CL_{NR}) was 24 mL/min and that of colistin clearance (CL_{coli}) 33 mL/min. On the 3rd day of treatment, a blood sample corresponding to the steady-state concentration can be drawn, allowing to refine individual CL_{coli} and so to adjust dosing regimen if necessary.
Conclusion: This two-step TDM procedure using a single drug concentration determination at steady-state was found to be useful in order to optimize colistin dosing regimen in critical care patients. Our next objective is to develop a Bayesian procedure using blood sample collected earlier during the course of treatment.
References:
1. Gobin P et al. Antimicrob Agents Chemother 2010.
2. Couet et al. Clin Pharmacol Ther 2011.
3. Grégoire et al. ECCMID-ICC 2011. Milan (Italy).

13-O028
Pharmacokinetic/Pharmacodynamic modeling of unboosted atazanavir in a cohort of HIV-infected patients

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Background: Atazanavir (ATV) boosted with ritonavir is widely used in HIV-infected patients requiring highly active antiretroviral therapy. Unboosted (u) ATV, administered once daily (QD) or twice (BID) daily, is an attractive option in patients with undetectable HIV viral load and with lipoatrophy and/or metabolic disorders. Data on the pharmacokinetics/pharmacodynamics (PK/PD) of uATV are limited.
Methods: This was a retrospective analysis of data from a prospective cohort of 58 HIV-infected patients treated with uATV (69 ATV-based regimens, 27 QD and 42 BID; 128 uATV steady-state concentrations available) [1]. The NONMEM and Matlab softwares were used to estimate PK parameters and various measures of drug exposure. The relationship between PK parameters and clinical outcome was analyzed using appropriate statistical tests and logistic regression.
Results: Virological failure (VF) and ATV cessation occurred in seven and 14 subjects, respectively. The final PK model was a linear one-compartment model with a fixed absorption lag-phase. A mixture model with two subgroups of smooth and low absorption much improved the fit. Estimated population PK parameters

(relative standard error) were clearance, 13.4 L/h (8.4%); volume of distribution, 71.1 L (12.0%); and fraction of smooth absorbers, 0.49 (21.6%). Interpatient variability for CL and V was 40.7% and 29.7%. All patients who experienced VF were low absorbers of ATV. The absorption rate constant (0.38 ± 0.20 vs. 0.75 ± 0.28/h, $P = 0.002$) and ATV exposure (AUC_{0-24}, 10.3 ± 2.1 vs. 22.4 ± 11.2 mg/h/L, $P = 0.001$) were significantly lower in those patients than in patients without VF. In the logistic regression analysis, a bivariate model including Ka and time above 0.15 mg/L was a significant predictor of VF.
Conclusion: Two subgroups of ATV exposure were identified with a mixture PK model. Such a model may be a surrogate for differences in drug adherence or meal consumption [2]. This study confirms a relationship between ATV exposure and virological outcome in HIV-infected patients.
References:
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2. Andrade A., et al. 18th Conference on Retroviruses and Opportunistic Infection. Boston, February 27–March 02, 2011

13-P471
Ex-vivo pharmacodynamics of tacrolimus on the calcineurin pathway of lymphocytes from healthy donors
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Background: Individualizing transplant patients' immunosuppressive treatment requires the identification of factors involved in the inter-individual variability of the clinical response, in order to compensate as much as possible for this variability by administering a personalized dose. Pharmacodynamic monitoring may help to fine-tune immunosuppression. Knowing the strength and variability of signal translation along the calcineurin pathway, or the stages where the sources of internal (genetic) or external (regulatory) variability are the most significant, would help to select the best set of biomarkers, i.e. those with high specificity for the immunosuppressive effect and most affected by interindividual variability.
Patients and methods: As part of an on-going non-interventional study, 30 healthy volunteers have been enrolled. After ex-vivo incubation of peripheral T lymphocytes with increasing concentrations of tacrolimus, (from 0.1 up to 50 ng/mL), the intracytoplasmic expression of IL2 and IL17 in CD4 and CD8 subpopulations, inhibition of the translocation of NFAT to the nucleus in T lymphocytes, intracytoplasmic expression of the FoxP3 in T regulatory cells, expression of TCR αβ-γδ in T Lymphocytes, and the percentage of T lymphocytes expressing IL-2Rα during proliferation were measured by flow cytometry.
Results: A large inter-individual variability was found for all these biomarkers, both regarding their physiological levels (E_0) and their pharmacodynamic response to tacrolimus (IC_{50}). The NFAT, IL2 and CD25 responses were adequately fitted by an Emax sigmoidal model. The average IC_{50} (ng/mL) were: 1.2 for NFAT; 2.6 for IL2 in CD4 cells and 3.8 in CD8 cell; 4.8 for CD25. At the highest concentration of tacrolimus there were still 20% of CD4 and CD8 T lymphocytes expressing IL2 (E_{max}). The activation marker CD25 was still expressed in 85 and 30% of the cells, and NFAT was still present in the nucleus of 85% and 20% cells, respectively.
Conclusion: The present results suggest that the panel of biomarkers that best reflect the response of the calcineurin pathway to tacrolimus inhibition is comprised of the NFAT translocation to the nucleus or leucocytes, intracytoplasmic IL2 in CD4 and CD8 cells, and expression of CD25 on lymphocyte surface as a marker of activation.

13-P486
Genotype-based quantitative prediction of drug exposure for drugs metabolized by CYP2C9
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Objective: A model to predict quantitatively the impact of CYP2C9 polymorphism on drug exposure has been evaluated based on the approach of Tod et al. [1]. The metrics of interest is the ratio of drug AUC in mutant to wild-type patients. The model was derived to rely the AUC ratio with two characteristic parameters, one for the drug (the fraction metabolized by CYP2C9 in vivo, CR), the other for the genotype (the fraction of activity with respect to the homozygous wild type, FA). Any combination of alleles may be accommodated.
Methods: The primary goal of the analysis was to estimate the CRs and FAs for 24 drugs and five classes of genotypes respectively, including CYP2C9*2 and *3 allelic variants. Data were available for 50 (drug, genotype) couples. A three-step approach was applied. First, initial estimates of CRs and FAs were obtained using data from the literature. Second, an external validation of these initial estimates was carried out, by comparing the AUC ratios predicted by the model equation to the observed values, using a second set of published data. Third, refined estimates of CRs and FAs were obtained by a bayesian orthogonal regression, using all the data and initial estimates of CRs and FAs from step 1. The posterior distributions of the AUC ratios, CRs and FAs were obtained by Monte Carlo Markov chain simulation by using WinBugs 1.4.
Results: With the refined estimates, the mean prediction error of AUC ratios was −0.05, while the mean prediction absolute error was 0.27. The model predicts a six fold increase of AUC for Lornoxicam, Warfarin-S and Acenocoumarol-S in homozygous CYP2C9*3 patients compared to wild type patients. Variations of exposure may be predicted for 100 combinations between drugs and genotypes.
Discussion: The predictive performances of the model were good. This model, already developed for CYP2D6 extends its application to CYP2C9 polymorphism. The method is easy to use and may contribute to the development of personalized medicine based on CYP genotype.
Reference:
1. Tod M, Goutelle S, Gagnieu MC. Genotype-based quantitative prediction of drug exposure for drugs metabolized by CYP2D6. Clin Pharmacol Ther **90**(4): 582–7.

13-P487
Effect of posaconazole introduction on immunosuppressive therapy in allogeneic hematopoietic stem cell allotransplantation recipients developing graft versus host disease
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Background: Recipients of allogeneic hematopoietic stem cell allotransplantation (AHSCT) developing graft-versus-host disease (GVHD) received posaconazole (PCZ) for prophylaxis of invasive fungal infections. Because of P450 cytochrome inhibition, PCZ is responsible for drug interactions, especially with narrow therapeutic range drugs such as immunosuppressive (IS) agents. Nevertheless, drug interactions between PCZ and the different IS therapies used in AHSCT patients developing GVHD and frequently presenting digestive disorders remain to be better documented. This study aimed at determining the effect of PCZ introduction on residual plasmatic concentrations (C_{min}) and administered dosage of IS in those patients.
Methods: The data concerning PCZ and IS treatments were retrospectively analyzed in 32 adult patients presenting a GVHD post AHSCT and receiving PCZ for prophylaxis treatment (200 mg tid). Blood measurements were performed for PCZ and IS C_{min} using validated LC-MS/MS methods. IS C_{min} were monitored before and at 7, 14, 21 and 30 days after PCZ administration.
Results: Among the 32 patients (age: 48.3 ± 11.8 years, BMI: 22.5 ± 3.6 kg/m^2), five received twice PCZ prophylaxy. PCZ C_{min} did not differ in patients treated with cyclosporine ($n = 24$) or everolimus ($n = 9$) (C_{min} = 1.2 ± 0.7 vs. 1.5 ± 0.9 mg/L respectively, $P > 0.05$). After PCZ introduction, everolimus C_{min} increased to 227.5% at D21 without any dosage reduction despite half of the patients presenting C_{min} higher than therapeutic range. Cyclosporine C_{min} slightly increased and dosage reduced (amplitude of 20% for both at D30) without reaching statistical significance. Diarrhea was responsible for a 50% reduction in C_{min} PCZ but had no incidence on IS concentrations of cyclosporine (230 ± 101 vs. 202 ± 89 mg/L in absence or presence of diarrhea) or everolimus (6.39 ± 3.93 vs. 5.86 ± 2.57 ng/mL in absence or presence of diarrhea).
Conclusion: The introduction of PCZ susceptible of increasing IS C_{min} in ASHCT patients developing GVHD was well managed for cyclosporine treated patients whereas everolimus C_{min} were above therapeutic range for half of the patients with no dosage modifications associated. Everolimus dosage adaptation should therefore be performed in those patients. When diarrhea occurred, patients presented a drastic decrease of PCZ plasmatic concentrations, but not of IS, in favor of a specific mechanism of absorption for PCZ.

13-P488
Thiopental and esomeprazole in critically ill patients: drug interaction
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Introduction: Thiopental is a thiobarbiturate given for induction of anesthesia or in case of brain injuries to reduce intracranial pressure and to manage cerebral ischemia. Esomeprazole is a proton pump inhibitor used to reduce stress ulcers, erosions of the stomach and upper gastrointestinal bleeding that are complications in critically ill patients. Esomeprazole wasn't yet marketed when a two-compartment model was developed in critically ill patients [1]. A new model is proposed describing the influence of concomitant administration of esomeprazole on the volume of distribution of thiopental.
Method: Fifty-nine critically ill patients (weight: 16.9–114 kg) aged 5–78 years, admitted in critical care unit for treatment of intracranial hypertension, induced by traumatic (58%), vascular (27%) or other origin (15%) acute brain diseases, were studied. High dose thiopental was administered by continuous infusion. Total mean dose of 295 ± 181.3 mg/kg was given in 96 ± 72 h. Blood thiopental concentrations were determined by a liquid chromatography method. Pharmacokinetic analysis was performed by using a non linear mixed-effect population model.
Result: A one-compartment model with first-order elimination including two covariates: body weight on clearance and volume of distribution, and administration of esomeprazole on volume of distribution were used. The population mean (percent relative standard error [%RSE]) values for clearance (CL), central volume of distribution (V_d) in patients with and without administration of esomeprazole were 5.4 L/h (8.5%), 261.0 L (6.6%) and 132.6 L (10.9%), respectively. The interindividual variabilities (%RSE) of CL and V_d were 50.3% (21.5%) and 24.9% (34.1%), respectively. The residual variability (%RSE) was 7.12 mg (16.7%).
Conclusion: The pharmacokinetic parameters of thiopental in critically ill patients were estimated. These results are comparable to those presented by Russo in patients without esomeprazole. Concomitant administration of thiopental and esomeprazole causes an increase in the volume of distribution of thiopental. A dose adjustment should be made to achieve the target concentrations in patients receiving esomeprazole. Esomeprazole has been reported as an inhibitor of P-glycoprotein which may suggest other potential drug interactions. Further studies on concomitant administration of esomeprazole should be conducted.
Reference:
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13-P501

Impact of renal insufficiency on the pharmacokinetics of nefopam in post-operative analgesia

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Introduction: Nefopam is a non-morphinic central analgesic for which precautions are recommended but without any recommendations for Glomerular Filtration Rate (GFR)-adapted posologies. Our objective was to explore the relationships between renal function alteration and nefopam pharmacokinetics to propose guidelines for practical use.

Patients and methods: Elderly patients hospitalized for broken hips were recruited in a prospective monocentric trial. Nefopam was administered as a 30 min infusion post-operatively. Simultaneously, a 1 min-IV infusion of Iohexol (180 mg/5 mL) was performed, in order to calculate GFR. Blood samples for the determination of Nefopam, desmethyl-nefopam (metabolite) and Iohexol clearance (Cl_{IO}) were drawn between 0 and 1440 min after nefopam administration. Iohexol, Nefopam and desmethyl-nefopam were measured by HPLC-MS/MS. A population approach, implemented in Monolix (V-4.0.1), was used to study the pharmacokinetic profile of Nefopam. Model selection and evaluation was based on usual diagnostic plot, precision and information criteria.

Results: Forty-eight subjects (ie, 452 blood samples) aged 65–99 years (mean:84) were enrolled. A 2-compartment open model, parameterized as clearances (total CL and intercompartmental Q) and volumes (central V1 and peripheral V2), with zero-order input was selected. Inter-individual variability (IIV) was described by exponential terms and residual variability by a combined additive (a = 1.33 µg/L) and proportional (coefficient = 0.08%) error model. Typical values for CL, V1, Q and V2 were respectively 17.3 L/h (IIV: 53.2%), 114 L (IIV: 121%), 80.7 L/h (IIV: 79%) and 208 L (IIV: 63.6%) with relative standard errors of estimation <18%. Individual CL and AUC were derived for each patient by using the Empirical-Bayes-Estimates of the individual parameters. Patients with Cl_{IO}>30 mL/min have higher CL and lower AUC of nefopam than patients with Cl_{IO} < 30 mL/min: 18.5 L/h vs. 13.5 L/h (P < 0.0001), 1152 µg/h/L vs. 1569 µg/h/L (P < 0.01) respectively. This result indicated that a decrease of 30% in the Nefopam dose should be applied for patients with Cl_{IO} < 30 mL/min.

Conclusion: Our model describes adequately the pharmacokinetic of Nefopam in elderly patients. It has confirmed that the clearance of Nefopam is linked to the renal function. Interestingly, a GFR lower than 30 mL/min should associate with a decrease of 30% in the dose. Further pharmacokinetic-pharmacodynamic-model with other co-variables is being studied.

13-P502

Prophylaxis of invasive Aspergillosis: correlation of Aspergillus Galactomannan Antigen detection in serum and residual concentrations of posaconazole

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Objectives: In order to further define the interest of the TDM of posaconazole (PZC), we retrospectively analyzed measurement of residual concentrations and correlated these data with the detection of *Aspergillus* galactomannan antigen (GM) in the same population.

Material and methods: Serum samples were obtained from consecutive patients receiving PZC as prophylaxis of invasive aspergillosis during induction chemotherapy for acute myeloid leukaemia between January 2008 and November 2011. PZC concentrations were measured by HPLC-DAD (Alliance 2695 Waters®, PDA 2996 Waters®). The limit of detection of the method is 0.2 µg/mL. Intraday CV is 5.89% and interday CV 10%, with an accuracy <10%. GM detection was performed using an enzyme-linked immunosorbent assay kit (Platelia *Aspergillus* Ag, BioRad®). A GM index of 0.5 was considered to be positive.

Results: One hundred and fifty five residual concentrations from 59 patients were measured. Fourteen concentrations were undetectable; mean concentration was 1.51 µg/mL. 116/155 (75%) concentrations were ≥0.5 µg/mL. Twelve of the 59 patients had a positive GM detection. None of them had a PZC concentration <0.5 µg/mL at the time of GM positive detection. Five of these 12 patients had a diagnostic of possible or probable invasive aspergillosis.

Discussion: The mean residual PZC concentration we observed is higher than usually described. It is still difficult to define the threshold of effective PZC concentration, even if the FDA has proposed 0.7 µg/mL. Unfortunately, in some patients, PZC concentration was not available at the time of positive GM detection. TDM of PZC in prophylaxis of invasive aspergillosis during induction chemotherapy for acute myeloid leukemia is recommended by ECIL-3. Nevertheless, some authors underline its interest only in case of problems of compliance or absorption. Three patients had a correct PZC residual concentration when GM detection was positive but concentration measured few days or weeks before was below the threshold, suggesting inadequate efficacy of prophylaxis

Conclusion: Our results are limited because of the retrospective aspect of this study. However, they show the interest of measuring PZC weekly or just after a positive GM detection to consider the effectiveness of the molecule on a pharmacokinetic point of view.

14-P266

First candidates of susceptibility to l-dopa induced dyskinesia in parkinson's disease

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Dopaminergic replacement therapy in Parkinson's disease (PD) is hampered by the occurrence of motor complication, L-DOPA-induced dyskinesia (LID). One major hypothesis is that LID result from L-DOPA-induced aberrant transcriptional program in striatum through hypersensitized D1 dopamine receptor involving ERK signalling pathway. Our goal was to determine genes responsible for dyskinesia priming, i.e. the genes deregulated as early as the first injections of L-DOPA, correlated with LID and dependent of the ERK pathway.

Methods: After 3 weeks, mice with unilateral injection of 6-hydroxydopamine in the striatum were treated with 20 mg/kg L-DOPA, with and without the MEK inhibitor, SL327 50 mg/kg. Mice were sacrificed 0, 1, 3 and 6 h post L-DOPA injection, total mRNA was extracted from striata and hybridized on cDNA beadchips (Illumina MouseWG-6 v2.0). Gene expression analysis along time was carried out using BRB array tools (http://linus.nci.nih.gov/BRB-ArrayTools.html). In a *second experiment*, mice were sacrificed 3 h after a second injection of L-DOPA after an abnormal involuntary movement (AIM) specific quotation. Correlation between gene expression and AIM score was analyzed.

Results: We found 805 genes deregulated by L-DOPA along time (F test, P < 0.01). Gene ontology analysis showed an enrichment in genes belonging to the MAPK signalling pathway (enrichment ratio (ER) = 2, P = 0.01), long term depression (ER = 3.1, P = 0.01) and regulation of synaptic plasticity (ER = 3.5, P = 0.03). Among these deregulated genes, 36 were significantly more deregulated in dyskinetic mice vs. non-dyskinetic mice (fold-change >1.2, P < 0.05). Among the 10 top-genes, four were found to be ERK dependent; *Fosb* (P = 0.0001), a target known as involved in LID, *Cdkn1a* (P = 0.0001), cyclin-dependent kinase inhibitor, *Nptx2* (P = 0.01), synaptic protein involved in clustering of (AMPA)-type glutamate receptors and *Sstr2* (P = 0.03), somatostatin receptor 2.

Conclusion: Four genes (*Fosb, Cdkn1a, Nptx2, Sstr2*) were found deregulated in the dopamine-denervated striatum after one injection of L-DOPA, correlated with LID and dependent of the ERK pathway. Among these genes, Nptx2 and Sstr2, involved in glutamate signalling pathways represent excellent candidates for dyskinesia priming.

14-P267

O-demethyl tramadol/tramadol ratio, a new tool to detect CYP2D6 poor metabolisers in post-operative patients: the CYTRAM study

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Object: Tramadol is an analgesic drug metabolized through CYP2D6 in O-demethyl tramadol, 2–4 times more efficient than tramadol for pain relief. Five to 10% of caucasian patients are poor metabolizers (PM) for CYP2D6, implying lower analgesia with tramadol[1]. The aim of CYTRAM was to detect CYP2D6 PM by dosing tramadol and its metabolite in post-operative patients.

Method: We included caucasian adult patients requiring digestive surgery, if post-operative analgesic regimen contained tramadol. Patients treated with CYP2D6 strong inhibitors were excluded. Patients received tramadol from the end of surgery and for at least 2 days. Blood samples were collected the first (H24) and the second (H48) day after surgery. Genotyping CYP2D6 was made on H24 sample, and drug assays were performed on H24 and H48 with high performance liquid chromatography tandem mass spectrometry. After genotyping, patients were separated in two groups: PM and others. Odemethyl tramadol/tramadol (ODT/T) mean ratio were compared for the two groups with Student test. A ROC curve gave the better ratio to identify PM.

Results: CYP2D6 genotyping and H24 O-DT/T ratio were determined in 325 patients, and 242 patients had also a H48 O-DT/T ratio. Genotyping found 26 PM (8%). The mean O-DT/T ratio (SD) was 0.055 (±0.034) for the PM and 0.178 (±0.094) for the other group (P < 0.0001). The better line to detect PM was a ratio ≤0.1 with 88% sensibility and 82% specificity for H24, and 94% sensibility and 80% specificity for H48.

Discussion: When pain relief is inefficient in a patient treated with tramadol, he could be a PM or the pain intensity is too high for mild opioids. With the pharmacologic ratio O-DT/T, we propose a CYP2D6 phenotyping, in order to detect rapidly and with low cost PM, in order to switch faster to morphine instead of increasing posology of tramadol. This new tool could be useful to detect CYP2D6 PM, concerning other drugs like codeine, oxycodone and many psychotropics.

1Enggaard TP et al. The analgesic effect of tramadol after intravenous injection in heathly volunteers in relation to CYP2D6. Anesth Analg 2006;102:146–50.

14-P268
Could CYP4F2 and EPHX1 polymorphisms be new target gene on anticoagulation response to fluindione?

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Background: Genetic variants of the enzyme that metabolizes warfarin, cytochrome P-450 2C9 (*CYP2C9*) and of a key pharmacologic target of vitamin K antagonists, vitamin K epoxide reductase (*VKORC1*), contribute to differences in patient's responses to coumarin derivatives. *VKORC1* and *CYP2C9* genetic variants accounted for about a third of the interindividual variability. Recently, a genetic variant of CYP4F2, enzyme that damages vitamin k, was associated with coumarin derivatives dose requirement. Genetic variants of EPHX1, enzyme with vitamin k binding site, seem to influence patient's response to coumarin derivatives. Fluindione, an indanedione derivative, is the most widely oral anticoagulant used in France. The role of these variants in fluindione response is unknown.
Objectives: To assess whether genetic factors contribute to the variability of fluindione response.
Methods: Four hundred sixty five patients with a venous thromboembolic event treated by fluindione for at least 3 months with a target international normalized ratio (INR) of 2.0–3.0 were studied. *VKORC1*, *CYP2C9*, *CYP4F2* and *EPHX1* genotypes were assessed. INR checks, fluindione doses, and bleeding events were collected.
Results: *VKORC1* genotype had a significant impact on early anticoagulation (INR value ≥2 after the first two intakes) ($P < 0.0001$), on the time required to reach a first INR within the therapeutic range ($P < 0.0001$), on the time to obtain a first INR value >4 ($P = 0.0002$). The average daily dose of fluindione during the first period of stability was significantly associated with the *VKORC1* C1173T genotype: 19.8 mg (± 5.5 SD) for *VKORC1* CC, 14.7 mg (± 6.2 SD) for *VKORC1* CT and 8.2 mg (± 2.5 SD) for *VKORC1* TT ($P < 0.0001$). In patients with two allelic variants of *CYP2C9* or of *EPHX1* the time to achieve a first INR in the therapeutic range was shorter than for patients with wild genotypes. *CYP4F2* TT genotype was found to be a significant predictor variable for the maintenance dose of fluindione explaining 2.4% of dose variability.
CYP2C9, *CYP4F2* and *EPHX1* genotypes did not significantly influence the response to fluindione.
Conclusion: *VKORC1* genotype strongly affected anticoagulation induced by fluindione whereas *CYP2C9*, *CYP4F2* and *EPHX1* genotypes seemed less determining.

14-P269
Association of HLA-G genetic polymorphisms with acute graft rejection and graft survival in renal transplantation

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Objective: HLA-G, a non-classical HLA class I molecule, is an important factor in human transplantation and was shown to influence the outcome in renal transplantation. We aimed to study the effect of *HLA-G* genetic polymorphisms on acute graft rejection and long-term survival in a cohort of kidney transplant recipients recruited from the Kremlin Bicêtre University Hospital.
Material/patients and methods: Two hundred and ninety-eight renal graft recipients were genotyped for *HLA-G -725 C>G* and *HLA-G 3142 G>C* single nucleotide polymorphisms. For acute rejection analysis, fisher's exact test was used to compare allelic frequencies. Survival curve analysis was used to estimate the influence of *HLA-G* polymorphisms on long-term renal graft survival.
Results: Of the 298 renal graft recipients, 259 were of Caucasian origin. Within the Caucasian patients, carriers of the *HLA 3142C* allele showed higher rejection episodes as compared to carriers of the *HLA 3142G* allele (43% vs. 34% respectively, $P = 0.04$), while a tendency for higher rejection episodes were noticed for carriers of *HLA -725G* allele as compared to carriers of *HLA -725C* allele (51% vs. 37% respectively, $P = 0.07$). *HLA -725 C>G* polymorphism was found to influence the graft survival during the first 5 years of follow-up post-transplantation. Patients with the *CC* genotype showed less deterioration in renal graft function as compared to carriers of the G allele (HR: 0.30, 95% CI: 0.13–0.69, $P = 0.005$). No significant association was found for *HLA-G 3142 G>C* on long-term graft survival.
Discussion: *HLA-G* genetic polymorphism appeared in our study to play a role in acute graft rejection as well as graft survival. These results need to be confirmed in larger clinical studies in the future.

14-P270
Effect of a new functional CYP3A4 polymorphism on sirolimus in vitro metabolism and kidney transplant recipients trough levels

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Objective: Recently, a newly discovered polymorphism in *CYP3A4* (rs35599367 *C>T*; *CYP3A4*22*), associated with decreased mRNA hepatic expression and enzymatic activity, was found to be significantly associated with increased cyclosporine and tacrolimus dose-adjusted concentrations in kidney transplant recipients [1]. Sirolimus is another immunosuppressant, substrate of CYP3A4. We investigated the effect of *CYP3A4*22* on (i) sirolimus in vitro hepatic metabolism and (ii) sirolimus trough levels in kidney transplant recipients.
Methods: In vitro experiments consisted of 10-min incubation of sirolimus (100 µg/L) with human liver microsomes (0.1 mg/mL; $n = 31$), prepared from samples obtained from Biopredic International (Rennes, France). Sirolimus metabolism was evaluated from its disappearance rate.
The clinical study included 113 stable kidney transplant recipients from Toulouse University Hospital, switched from a calcineurin inhibitor to sirolimus. Sirolimus trough concentrations were measured at 1, 3 and 6 months after the switch. Microsomes and patients were genotyped for *CYP3A5*3* and *CYP3A4*22* using Taqman® allelic discrimination assays, and classified based on expected metabolic status: extensive (EM; *CYP3A4*1&CYP3A5*1* carriers), intermediate (IM; *CYP3A4*1&CYP3A5*3* carriers) and poor metabolizers (PM; *CYP3A4*22&CYP3A5*3* carriers). Sirolimus was determined in blood and in vitro samples using turbulent-flow chromatography-tandem mass spectrometry.
Results: *CYP3A4*22* frequency was 5.9% ($n = 144$) and conform to the Hardy-Weinberg equilibrium. A linkage disequilibrium with *CYP3A5*3* was found ($D' = 0.99$; $r^2 = 0.25$). Microsomes carrying *CYP3A4*22* ($n = 3$ heterozygotes and one homozygote) metabolized sirolimus at significantly lower rates than non-carriers ($n = 27$) (65.5 [55.2–80.7] vs. 86.1 [53.4–105.2] pmol/mg/min; $P = 0.0411$). However, metabolic rates of EM ($n = 5$), IM ($n = 22$) and PM ($n = 4$) HLM did not significantly differ ($P = 0.1885$).
In kidney transplant recipients, no association was found between sirolimus C_0/dose and patient genotypes for *CYP3A4*22* ($n = 11$ carriers and 102 non-carriers) or patient metabolic status ($n = 19$ EM; 85 IM and 9 PM) at month 1 ($P = 0.8674$ and $P = 0.3476$), 3 ($P = 0.7445$ and $P = 0.8523$) and 6 ($P = 0.8745$ and $P = 0.7370$).
Conclusions: *CYP3A4*22* resulted in a moderate (approximately 20%) but significant decrease of sirolimus in vitro hepatic metabolism. However, no association was found between this allele and sirolimus C_0/dose in renal transplant recipients. This new functional polymorphism in CYP3A4 might not account for a major part of sirolimus pharmacokinetic variability.
[1] Elens et al. Pharmacogenomics (2011)

14-P271
Pharmacogenetics of gemcitabine: impact of CDA genetic polymorphism on treatement efficacy in patients with pancreatic cancer

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Background: Gemcitabine is a mainstay in the treatment pancreatic cancers, either alone or in combination. Gemcitabine elimination pattern is driven by deamination in the liver by cytidine deaminase (CDA). CDA is affected by genetic polymorphism. We demonstrated previously that reduced CDA activity was a marker for early severe toxicities upon gemcitabine intake. We observed too that about 10% of patients displayed particularly elevated CDA activities. Ultra-metabolizer (U.M.) patients could be theoretically at risk of over-detoxifying gemcitabine, with subsequent lack of efficacy. The present study was performed to test the hypothesis that U.M. patients could be more at risk of treatment failure than patients with lower CDA activities when given gemcitabine.
Patients and methods: Fifty-six adult patients (M35/F21, 63 ± 11 years old) with mostly pancreatic cancers (40 patients) and other digestive localizations (liver, biliary, vesicular cancers) were included in this retrospective study. All patients had been treated with gemcitabine alone (38 patients), associated with oxaliplatin (seven patients), sorafenib (five patients) or with a variety of anticancer drugs. Mean gemcitabine dose was 980 mg/m^2. CDA activity was measured spectrophotometrically from serum samples. Search 79A>C, 435T>C and 208G>A polymorphisms was additionally performed. Treatment efficacy was evaluated at 3 month following the standard RECIST criteria plus CA19-9 monitoring.
Results: CDA activities ranged from 0.8 to 17.4 U/mg (mean = 4.1 U/mg, median = 3.4 U/mg). No correlation between genotype and phenotype was evidenced. However, eight out of 56 patients (i.e. 14%; M4/F4, 58 ± 10 years) displayed CDA activities associated with the U.M. phenotype (min: 6-max 17.4 U/mg). These extensive patients were treated with gemcitabine alone (two patients), or in combination (six patients). A significant difference was observed in efficacy between normal and U.M. patients. Seven out of 8 U.M. patients showed progressive disease (i.e. 88% PD), a score ×5 time higher than patients with normal CDA (PD: 12%, SD + PR: 88%).
Conclusion: Although preliminary, our data suggest that CDA U.M. status is a marker for treatment failure in patients treated with gemcitabine-containing chemotherapy. This study advocates for the development of a CDA-based dose tailoring strategy with gemcitabine.

14-P272
Evidence for a functional genetic polymorphism of the Rho-GTPase Rac1: Implication in azathioprine response?

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Background: Adverse effects of thiopurine drugs occur in 15–28% of patients and the majority is not explained by thiopurine-S-methyltransferase (TPMT) deficiency. Furthermore, around 9% of patients with inflammatory bowel disease (IBD) are resistant to azathioprine (AZA) therapy. Recently, the small guanosine triphosphatase, Rac1, was identified as an important molecular target of 6-thioguanine triphosphate (6-TGTP), one of the active metabolite of thiopurines such as AZA. To date, no functional genetic polymorphism of the human Rac1 gene had been reported.
Methods: We screened for polymorphisms in the Rac1 gene in genomic DNA samples from 92 unrelated Caucasian individuals. Functional consequences of identified polymorphisms were assessed in vitro using transient transfection assays in Jurkat and A549 cell lines. Relationship between polymorphisms of Rac1 and thiopurine response or hematotoxicity was studied in 128 patients under thiopurine treatment.

Results: Three single nucleotide polymorphism (SNP) and one variable number tandem repeat (VNTR) were identified in the promoter region of Rac1 gene. Interestingly, in Jurkat T cells, the c.-289G>C substitution and c.-283_-297[3] VNTR displayed a significantly increased promoter activity ($P < 0.01$) of 150% and 300%, respectively, compared to wild-type sequence. Patients with TPMT mutations presented a significantly increased probability of developing hematotoxicity (OR = 5.68, CI = 1.45–22.23, $P = 0.00625$). Moreover, among the 75 patients who did not develop hematotoxicity, there was a marginally overrepresentation of functional genetic polymorphisms of Rac1 (OR = 0.18, CI = 0.02–1.49, $P = 0.079$).
Conclusion: This study constitutes the first report of a functional genetic polymorphism that could affect Rac1 expression and thus modulate the risk of adverse drug reaction in patients under thiopurine treatment. A larger scale (case-control) study should enable us to confirm or cancel these preliminary results.

14-P273
Anticipating capacitabine-related severe toxicities: when screening the DPYD genetic polymorphism is just not enough
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Background: capecitabine is an oral prodrug of 5-FU. Because it is usually considered as a convenient and safer alternative to 5-FU, capecitabine is widely prescribed in a range of clinical settings, including digestive and breast cancers. As a 5-FU prodrug, known DPD deficiency is a major contraindication with capecitabine. However, beside DPYD genetic porymphism, little is known about the possible role other genetic variations could play in the occurrence of life-threatening toxicities with capecitabine. In particular, the role of cytidine deaminase (CDA), a key activating enzyme of capecitabine that proved to be highly polymorphic, remains questionned.
Observations: our investigations have focused on a couple of patients preliminary screened for DPD deficiency prior to starting capecitabine-based regimen, and who all experienced unexpectedly life-threatening toxicities. Patients were given either capecitabine alone or associated with other drugs (i.e., lapatinib) for various solid tumors. DPD status had been evaluated following the standard U-to-UH2 ratio determination in plasma so as to identify Poor Metabolizer (P.M.) patients, as routinely performed in our institute. Toxicities were monitored following the standard C.T.C grading. Complementary investigations included determination of the CDA metabolic status using a surrogate enzymatic test. Severe toxicities included sepsis, grade-4 stomatis, and grade-4 hand-foot syndrome, and showed soon after capecitabine was administered. Treatment was stopped and capecitabine precluded in all cases. All patients had shown normal DPD status (i.e., no PM profile, U/UH2 < 2) but further investigations on CDA revealed abnormally elevated activities associated with Ultra-Metabolizer (U.M.) status with CDA. Mean CDA activity was 8.5 U/mg in patients with severe toxicities upon capecitabine intake, a value 130% higher than the values usually recorded in patients with cancer.
Conclusions: Our clinical observations strongly suggests that beside the screening for DPYD genetic polymorphism, screening for CDA U.M. status should help to secure the handling of capecitabine, one of the most extensively prescribed oral anticancer drug worldwide.

16-O029
Mechanisms of endothelial dysfunction during essential hypertension
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Background: We aimed to clarify using functional and biological approaches the role of epoxyeicosatrienoic acids (EETs), NO/reactive oxygen species (ROS) balance and endothelin-1 in conduit artery endothelial dysfunction during essential hypertension.
Methods and results: Radial artery diameter and mean wall shear stress were determined in 28 non-treated essential hypertensive patients and 30 normotensive control subjects, during endothelium-dependent flow-mediated dilatation (FMD) induced by hand skin heating. The role of EETs and NO was assessed using the brachial infusion of inhibitors of cytochrome P450 epoxygenases (fluconazole) and NO-synthase (L-NMMA). As compared with controls, hypertensive patients exhibited a decreased FMD in response to post-ischemic hyperemia as well as to heating, as shown by the lesser slope of their diameter-shear stress relationship. In controls, heating-induced FMD was reduced by fluconazole, L-NMMA and, to a larger extent, by L-NMMA+fluconazole. In patients, FMD was not affected by fluconazole and was reduced by L-NMMA and L-NMMA+fluconazole to a lesser extent than in controls. Furthermore, local plasma EETs increased during heating in controls (an effect diminished by fluconazole), but not in patients. Plasma nitrite, an indicator of NO availability, increased during heating in controls (an effect abolished by L-NMMA) and, to a lesser extent, in patients while, inversely, ROS increased more in patients (an effect diminished by L-NMMA). Plasma endothelin-1 decreased during heating in controls but not in patients.
Conclusions: These results show that an impaired role of EETs contributes together with alteration in NO/ROS balance and endothelin-1 pathway to conduit artery endothelial dysfunction in essential hypertension.

16-O030
Aortic stiffness, reflexion wave and arterial hypertension under anti-angiogenic drugs
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Objective: Sorafenib and Sunitinib are anti-angiogenic drugs (AAD) used in an increasing number of cancers. The most common side effect is arterial hypertension. The main pathophysiological hypothesis explaining this arterial hypertension is a impairement of the microcirculation. We hypothesize that AAD also lead to an early damage of large arteries which can be translated by an increase of aortic stiffness determined by the pulse wave velocity measurement (PWV).
Material and method: In a longitudinal study from which we are presenting here the intermediate analysis, 25 patients have been treated for a cancer with Sorafenib or Sunitinib. Subjects have been explored during a visit before the introduction of the treatment and then every 2 weeks for 2 months. Measured parameters are blood pressure, PWV, central pressure, augmentation index (AIx).
Results: 40% of the subjects have developed an early arterial hypertension requiring anti-hypertensive treatement. The initial values of PWV (> or ≤of the median), SBP and PWV adjusted to the MBP were predictive from SBP changes under AAD while AIx was not. Furthermore, in patients who developed hypertension and required treatment, monotherapy with 5–10 mg amlodipine has been effective at decreasing SBP and MBP by −13 [−21; −4] et −8 [−14; −1] mmHg ($P < 0.01$) respectively, and AIx, −10% [−16; −4] ($P < 0.001$).
Conclusion: This study suggests that increased arterial stiffness increases the risk of developing acute hypertension with AAD. It also shows that effective vasodilation could be achieved despite small vessels disruption by AAD.

16-O031
Double blind vs. open design on treatment effect of new oral anticoagulants in atrial fibrillation: a meta-analysis
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Introduction: Because of well-known limitations of vitamin K antagonist (VKA), new oral anticoagulants (NOA) inhibiting directly thrombin or Factor Xa has been developed in atrial fibrillation (AF). Trials used either double-blind (DB) or prospective randomized open, blinded end-point (PROBE) design. We performed a meta-analysis of all randomized AF trials to assess if the PROBE design was associated with an overestimation of treatment effect.
Methods: We searched MEDLINE, EMBASE, and dedicated databases to October 2011 for randomized trials that compared NOA and VKA in AF. Main efficacy and safety outcomes were stroke – systemic embolism (S-SE) and major bleeding (MB), respectively. Other outcomes were ischemic S-SE, hemorrhagic stroke, intracranial hemorrhage, myocardial infarction (MI), all-cause and cardiovascular mortality. Analysis of outcomes was based on the pooling of logarithms of the relative risk (RR) of each study (inverse-variance weighting method) with a fixed-effect model. Interaction was systematically searched for DB and PROBE designs with a threshold at $P < 0.10$. In case of unexplained heterogeneity ($P < 0.1$, $I^2 > 50\%$), a random effect model was used.
Results: Eight studies encompassing 59 238 patients comparing apixaban, edoxaban, dabigatran, AZD0837, ximelagatran and rivaroxaban to warfarin were included. NOA were associated with a reduction of S-SE (RR 0.84; CI 0.76–0.92), MB (RR 0.84; CI 0.74–0.96), hemorrhagic stroke (RR 0.46; CI 0.36–0.59), intracranial hemorrhage (RR 0.48; CI 0.40–0.58), all-cause mortality (RR 0.90; CI 0.84–0.96) as well as cardiovascular mortality (RR 0.90; CI 0.82–0.98)
There was a trend for an interaction ($P = 0.15$) between design and treatment effect for S-SE for PROBE design (five trials, 23 177 patients; RR 0.76, CI 0.65–0.90) compared to DB design (three trails, 36 387 patients; RR 0.88, CI 0.78–0.98). Significant interaction with the design was found for hemorrhagic stroke (RR 0.32; CI 0.20–0.49 vs. RR 0.55; CI 0.41–0.73; $P = 0.05$) and MI (RR 1.39; CI 1.09–1.78 vs. RR 0.82; CI 0.68–0.98; $P < 0.0001$) in PROBE trials compared to DB trials.
Discussion: Interaction between PROBE and DB design may be explain by an increase of concomitant prescription of antiplatelet drugs and VKA in case of knowledge of allocated treatment by physicians in PROBE design.

16-O032
Effect of a treatment strategy based on aldosterone blockade vs. a strategy based on combined renin angiotensin system blockade on left ventricular mass (LVM) in patients with resistant hypertension
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Objective: To compare the effects of aldosterone blockade+diuretic treatment (AB) vs. combined renin angiotensin system blockade (RB) on echocardiographic LVM in patients (pts) with resistant hypertension (RH).
Methods: Pts with daytime ambulatory BP (dABP) ≥ 135/85 mmHg, despite 4 week- treatment with irbesartan 300 mg+HCTZ 12.5 mg+amlodipine 5 mg, were randomised to add-on spironolactone 25 mg (AB group, $n = 85$) or ramipril 5 mg (RB group, $n = 82$) for 12 weeks. Treatment intensity was increased at weeks 4, 8 or 10 if home BP was ≥135/85 mmHg by sequentially adding furosemide 20–40 mg and amiloride 5 mg (AB group), or ramipril 10 mg, bisoprolol 5–10 mg (RB group). The BP results in favour of the AB strategy have been previously reported. Echocardiographic measurements were available at baseline and week 12 in 44 and 39 pts of the AB- and RB group, respectively.
Results: Baseline clinical characteristics did not differ between groups (Age: 55 ± 11 years, Males: 72%, Caucasians: 76%, BMI: 29 ± 4 kg/m^2, baseline dABP: 148 ± 12/92 ± 10 mmHg, LV hypertrophy: 31%). dABP decreased by −19 ± 12/−11 ± 9 mmHg in the AB group and by −9 ± 12/−7 ± 7 mmHg in the RB group ($P = 0.0003/0.03$). LVM, LVMI, LVEDd, LVESd, PWT, E/E' ratio and LA area decreased significantly more in the AB than in the RB group (mean difference between two groups, adjusted on baseline and D 24 h-ABP: −17g*; −8 g/m^2; −1 mm; −2 mm; −0.7 mm*; −1.1*; −2.0 cm^2†, respectively with *$P < 0.05$; †$P < 0.01$; LVMI: LVM index LVEDd: LV end diatolic diameter; LVESd: LV end-systolic diameter; IVST: interventricular septal thickness; PWT: posterior wall thickness; LA: left atrium). Whereas IVST is comparable between two groups

(−0.1 mm). The difference between groups remained significant after adjustment on the decrease in 24-h ABP for LVM, LVMI, PWT, E/E' ratio and LA area.
Conclusion: In pts with RH, a treatment strategy based on aldosterone blockade is more efficient to decrease both BP and LVM than a strategy based on combined renin angiotensin system blockade. The effect of the AB on LVM is independent of its effect on BP and probably related to its effect on volume.

16-P164
Evaluation by trans-thoracic echography-doppler of the response of coronary microvascular function to an increased oxygen demand in type 2 diabetic patients
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Rationale and aims: To assess with a non invasive investigation the relationship between coronary microvascular dysfunction, silent myocardial ischemia (SMI) and silent coronary artery stenose (CS) in type 2 diabetic patients (T2D).
Materials and method: Sixty-six asymptomatic patients with T2D (44 males, 61.6 ± 8.1 years, BMI 28.8 ± 4.7 kg/m^2) and fulfilling the criteria of the French Alfediam-SFC guidelines for SMI screening were prospectively included. A coronary angiography was performed in patients with SMI defined as an abnormal stress myocardial scintigraphy and/or stress echocardiography. The mean inter-ventricular anterior coronary velocity (mCV) was measured by trans-thoracic echo-doppler (7 MHz) before and after a cold pressor test (CPT). The responses to CPT were compared with 11 obese non diabetic subjects (OS) (one male, 43.4 ± 12.2 years, BMI 33.2 ± 3.7 kg/m^2) and 16 control subjects (10 males, 26.3 ± 5.8 years, BMI 22.0 ± 2.0 kg/m^2) who were studied as a reference population.
Results: SMI was found in 35 T2D patients, and 15 of them had CAD. At baseline, the systolic arterial pressure x heart rate product (DP: 89947.4 ± 11971.5, 8527.5 ± 1318.7 and 7094.9 ± 1127.1 mL/min; $P < 0.0001$) and mCV (0.31 ± 0.12, 0.25 ± 0.06 and 0.22 ± 0.05 m/s; $P < 0.01$) were higher in T2D vs. OS and controls. There was correlation at baseline between double product and mean velocity ($r = 0.23$; $P < 0.05$). After CPT, changes in DP 1.43 ± 0.34 vs. 1.36 ± 0.27 vs. 1.32 ± 0.19 and mCV 1.30 ± 0.26 vs. 1.21 ± 0.34 vs. 1.17 ± 0.10 were not significant in T2D, OS and control respectively. Changes in mCV were correlated with those in DP in control ($r = 0.58$, $P < 0.05$), OS ($r = 0.75$, $P < 0.01$), T2D without CAD ($r = 0.56$, $P < 0.0001$) but not in the 15 patients with CAD. In T2D patients, changes in mCV were higher in patients with SMI vs. without SMI (1.37 ± 0.27 vs. 1.21 ± 0.22, $P < 0.001$). In univariate analysis, SMI was associated with male gender ($P < 0.05$), BMI ($P = 0.05$), diabetic nephropathy ($P < 0.05$) and triglycerides ($P = 0.05$) ($P < 0.05$). In multivariate analysis including the significant factors, changes in mCV was associated with SMI (OR: 1.03[1.0–1.1], $P < 0.05$).
Conclusion: In T2D, patients a poor adaptation of the coronary microcirculation to an increase myocardial oxygen demand is associated the presence of CAD and changes in mCV was independently associated with SMI.

16-P165
Genetic deletion of protein tyrosine phosphatase 1B protects against endotoxinic shock and improves endothelial dysfunction
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Sepsis is one of the major causes of mortality in critically ill patients. Endothelial dysfunction (ED) and especially impaired NO production by endothelial NO synthase (eNOS) plays a crucial role in the pathogenesis of sepsis, several studies showed that increased endothelial NO production improves survival in sepsis. Our laboratory has shown that the protein tyrosine phosphatase 1B (PTP1B) negatively modulates NO production and that its inhibition may reduce ED.
The aim of the present study is to assess the effect of genetic deletion of PTP1B (PTP1B$^{-/-}$) on both inflammatory state and endothelial function in mice with endotoxinic shock induced by lipopolysaccharid (LPS) intraperitoneal injection followed by a subcutaneous fluid resuscitation. Vascular function on mesenteric arteries was evaluated in vitro on arteriograph system and plasma was collected to measure the inflammatory cytokine (TNF-a, IL1-b) by Elisa multiplex assay, 4 h (H4) and 8 h (H8) after LPS injection.
Compared to WT, PTP1B$^{-/-}$ mice showed a marked decrease in mortality (30% vs. 100%, $n = 20$). In WT mice, LPS induce a severe ED as shown by a decreased in acetylcholine induced dilatation (WT 92 ± 1% vs. WT H8 42 ± 5%, $P < 0.001$) which was improved in PTP1B$^{-/-}$ mice (56%). Moreover insulin induced dilatation was not altered by sepsis but was higher in PTP1B$^{-/-}$ mice compared to WT mice (PTP1B$^{-/-}$ 45 ± 8% vs. WT 29 ± 3%, $P = 0.039$). The improvment of ED was associated with decreased circulation NO levels and an enhancement of heme oxygenase-1 expression. However the plasma levels of IL1-b and TNF-a were significantly higher in PTP1B$^{-/-}$ mice than WT mice at H4 demonstrating a role of PTP1B in the inflammatory process.
In conclusion, genetic deficiency of PTP1B confers a resistance against LPS induced sepsis despite in increased inflammatory state. The inhibition of PTP1B could be a new treatment of the endothelial dysfunction associated with sepsis.

16-P173
Chronic inhibition of protein tyrosine phosphatase 1B improves diastolic function and limits endothelial dysfunction in insulin resistant/obese mice
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Insulin resistance and obesity induce both endothelial and cardiac dysfunction. Insulin induces endothelial release of NO, and this pathway is altered in insulin resistance. Protein tyrosine phosphatase 1B (PTP1B) modulates insulin action, but we have revealed that it also negatively regulates NO production, and that PTP1B inhibitors improve endothelial function in heart failure. This study assessed the effect of PTP1B inhibition on cardiac and endothelial function in insulin-resistant mice.
Mice were fed with normal or high fat (60%) diet (HFD) for 4 months. HFD mice were either untreated or treated for 3 months with a PTP1B inhibitor, starting after 1 month HFD. Cardiac function was assessed by echography, tissue Doppler imaging (TDI) and left ventricular pressure volume relationships, while endothelial function of coronary and mesenteric arteries was evaluated in vitro in a small vessel myograph.
HFD induced insulin resistance revealed by Homeostatic model assessment (HOMA index) (ctl 0.07 ± 0.01; HFD 0.7 ± 0.1; $n = 11$; $P < 0.001$), and increased body weight (ctl 30 ± 0.6; HFD 42 ± 1 g; $n = 11$; $P < 0.01$). These parameters were improves by chronic PTP1B inhibition (HOMA index: treated 0.34 ± 0.08; $P < 0.01$; body weight: treated 37 ± 1 g)
TDI showed that HFD decreased E/A ratio (ctl 1.08 ± 0.03; HFD 1.23 ± 0.03; $n = 9$; $P < 0.01$) and increased end diastolic pressure-volume relationship (EDPVR: ctl 1.19 ± 0.26; $n = 5$; HFD 4.85 ± 1.17; $n = 7$, $P < 0.05$), demonstrating diastolic dysfunction, in the absence of changes in parameters of systolic function. This diastolic dysfunction was reduced by PTP1B inhibition (Ea/Aa ratio: 1.39 ± 0.06; EDPVR: 1.38 ± 0.31; $n = 6$; $P < 0.05$).
HFD impaired insulin-induced relaxations of mesenteric (control 62 ± 5; $n = 11$; HFD 32 ± 6% $n = 10$; $P < 0.01$) and coronary arteries (ctl 91 ± 1; $n = 8$; HFD 29 ± 10; $n = 11$; $P < 0.01$). This impairment was reduced by PTP1B inhibition (mesenteric: 60 ± 5; $n = 10$; coronary: 91 ± 1; $n = 9$; $P < 0.01$).
Thus, chronic PTP1B inhibition improves diastolic dysfunction and restores endothelial function, suggesting that it may be a treatment of cardiovascular complications in diseases associated with insulin resistance.

Tako-tsubo cardiomyopathy: direct evidences of sympathetic nervous system hyperactivity
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Background: Tako-Tsubo Cardiomyopathy (TTC) is an acute reversible condition that involves left ventricular apical 'ballooning' and mimics acute myocardial infarction with no detectable coronary arterial disease. TTC typically affects aged postmenopausal women and is usually triggered by emotional or physical stress. The exact pathophysiology remains unknown but datas suggest a link between sympathetic hyperactivity (catecholamine plasmatic level, heart rate variability depressed) and TTC. Up to now, no direct evidence of sympathetic hyperactivity has been established. The aim of our study was to determine, by microneurography (direct technique), if patients with TTC present an increased of muscle sympathetic nerve activity (MSNA) in TTC patients in comparison to matched heart failure controls.
Methods and results: We enrolled 13 TTC patients (80.1 ± 2.1 years, all female, Body Mass Index: 22.8 ± 0.9 kg/m^2, Left Ventricular Ejection Fraction: 40 ± 2%) and 13 control patients matched for age, sex, BMI, LVEF, renal function and hemoglobinemia. Between 36 h after admission, all patients underwent a micro-neurography and an arterial baroreflex gain assessment (slope of the relationship between MSNA and diastolic blood pressure). There is no difference between groups on hemodynamics parameters (SBP, DBP, MBP and HR) and oxygen saturation. TTC patients presented a significant increase of sympathetic activity (66.3 ± 2.7 vs. 55.6 ± 2.6 bursts/min; $P = 0.0088$). Arterial baroreflex gain is significantly decreased compared to control patients (1.2 ± 0.3% vs. 2.5 ± 0.4% MSNA/mmHg; $P = 0.005$).
Conclusion: This study showed for the first time with a direct technique, that TTC patients present a sympathetic hyperactivity. The increase of sympathetic nerve activity is associated to a decrease of arterial baroreflex gain. During the acute phase, the benefit of adrenergic antagonist has to be evaluated.

16-P175
Beneficial cardiac effect of ECE inhibition in experimental heart failure
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Introduction: Endothelin (ET) plasma concentrations are of predictive value in terms of clinical outcome and survival in heart failure (HF), but remain increased in humans treated with angiotensin converting enzyme inhibitors. Antagonizing the effects of ET-1 via dual ETA-ETB or selective ETA receptor antagonists abrogate the deleterious effects of ET, i.e. vasoconstriction and adverse left ventricular (LV) remodeling in experimental HF, but only dual ETA-ETB receptor antagonist increase in long-term survival in experimental HF. In contrast, in humans with CHF neither ETA nor dual ETA-ETB receptor antagonists improve, despite beneficial hemodynamic effects, long-term survival. Endothelin converting enzyme (ECE) inhibition might be a therapeutic alternative for ET receptor blockade. However, whether ECE-inhibition exerts beneficial effects HF is unknown. Thus, we sought the effect of ECE inhibition on LV function and structure in CHF.
Method: In Wistar rats with HF (coronary artery ligation), LV hemodynamics and remodeling as well as LV tissue perfusion were assessed by cardiac catheterization (pressure-volume curves), echocardiography and MRI after treatment with the selective ECE inhibitor LF1437213 (10 mg/kg/day for 1-month starting 7 days after ligation) or placebo.
Results: In placebo treated animals, coronary artery ligation induced classical signs of HF, i.e. decreased LV end-systolic pressure (LVESP) and LVESP-volume relation and increased LV end-diastolic pressure (LVEDP) as well as LVEDP-volume relation, associated with increased LV diastolic diameter and reduced cardiac output (CO) and LV tissue perfusion. Compared to placebo treated HF animals, LF1437213 reduced LVESP (−5%, $P < 0.05$), LVEDP (−51%, $P < 0.05$) as well as LVEDP-volume relation (−28%, $P < 0.05$) and increased LVESP-volume relation (+28%, $P < 0.05$), while CO and LV tissue perfusion were increased (+20% and

+20% respectively, $P < 0.05$). Moreover, LF1437213 reduced LV diastolic diameter (−9%, $P < 0.05$) and LV collagen density (−28%, $P < 0.05$) but not LV hypertrophy.
Conclusion: ECE inhibition reduces cardiac pre- and afterload, prevents LV remodeling and improves LV perfusion as well as function, demonstrating the potential interest of ECE inhibition for the treatment of CHF.

16-P176
Increase in sympathetic nervous system activity in patients with spastic angina
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Background: The pathogenesis of vasospastic angina remains incompletely elucidated. Among multiple mechanisms, abnormalities in the autonomic innervation have been underscored. As vagal withdrawal can act as a trigger for spontaneous coronary spasm, changes in sympathetic activity have also been suggested as individual or combined risk factors for vasospastic angina. Previous study based on heart rate variability analysis showed both a reduction and an enhancement of sympathetic nervous activity in patients with variant angina, but direct assessment of sympathetic nerve activity, using Muscle sympathetic nerve activity (MSNA) has never been performed.
Methods and results: We evaluated MSNA, haemodynamic parameters (Blood Pressure, Heart Rate etc.) in 22 patients: 11 having definite vasospastic angina confirmed by ergonovine provocation test during angiography and 11 matched patients (for age, gender, body mass index, distribution of risk factors, treatment) with a negative for provocation test. Parameters were collected during baseline and during a mental stress known to further increase MSNA. At baseline, there were no significant difference between patients with and without spasm for MSNA (56.9 ± 1.78 vs. 52.0 ± 2.78 burst/min; n.s.) and haemodynamic parameters. During mental stress period, patients with vasospastic angina presented a higher sympathetic nerve activity in comparison to control patients (66.45 vs. 59.45 burst/min; $P < 0.05$) without significant difference on heamodynamic parameters.
Conclusion: Our results show for the first time a direct evidence of increased sympathetic activity in patients with vasospastic angina, during mental stress. This propensity to further increase MSNA during stress may play a key role in the pathogenesis and occurrence of coronary spasm.

16-P177
Lack of evidence for a role of BH4/BH2-dependent eNOS uncoupling in hypoxia-induced pulmonary hypertension
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Aims: Tetrahydrobiopterin (BH_4) and dihydrobiopterin (BH_2) levels tightly regulate endothelial NO synthase (eNOS) coupling/uncoupling. Moreover, BH_4 pulmonary availability is a key determinant in the pathogenesis of pulmonary hypertension. Thus, the aim of this study was to investigate the role of eNOS uncoupling in hypoxic pulmonary hypertension.
Material and methods: Mice were exposed to hypobaric hypoxia (0.5 atm for 21 days). BH_4 levels, GTP cyclohydrolase-1 (GTPCH; the limitant step in BH_4 synthesis from GTP), dihydrofolate reductase (DHFR, which recycles BH_2 into BH_4) and eNOS expression were determined in lungs (by Western blotting). The monomer/dimer ratio of eNOS (as an index of eNOS uncoupling), the contractile (phenylephrine) and endothelium-dependent relaxant (acetylcholine) responses were evaluated in intrapulmonary arteries. Histochemistry was applied to lung slices for assessment of remodelling of pulmonary arterioles. The weight ratio right of ventricule to left ventricule plus septum (RV/LV+S) was also determined, as an index of right ventricular hypertrophy.
Results: In lungs, chronic hypoxia increased BH_4 levels (23.4 ± 0.2 vs. 17.5 ± 1.2 pmol/mg protein in hypoxic and control mice, respectively; $P < 0.01$) and eNOS protein expression (1.8-fold; $P < 0.001$), without modifying levels of BH_2 and protein expression of GTPCH or DHFR. In intrapulmonary arteries, the eNOS monomer/dimer ratio did not increase following chronic hypoxia. Sepiapterin (a precursor of BH_4) affected neither hypoxia-induced remodelling, nor alterations of vasomotor responses (hyper-responsiveness to phenylephrine, hypo-responsiveness to acetylcholine) in the pulmonary vasculature. However, sepiapterin (30 mg/kg, three times a week for 3 weeks) partially prevented hypoxia-induced right ventricular hypertrophy (RV/LV+S: 0.329 ± 0.021 vs. 0.440 ± 0.005 in sepiapterin-treated or untreated mice, respectively; $P < 0.001$). In $eNOS^{-/-}$ mice, both hypoxia-induced right ventricular hypertrophy (RV/LV+S: 0.440 ± 0.005 vs. 0.355 ± 0.007 in wild-type and $eNOS^{-/-}$ mice, respectively; $P < 0.001$) and remodelling of pulmonary arterioles, were attenuated.
Conclusions: These results do not provide evidence for a role of BH_4 or BH_2-dependent eNOS uncoupling in hypoxia-induced alterations of pulmonary vasomotor responses and remodelling. The mechanisms by which sepiapterin or $eNOS^{-/-}$ gene deletion protected against hypoxia-induced right ventricular hypertrophy and/or pulmonary vascular remodelling remain to be further investigated.

16-P178
Involvement of the HIF-1 transcription factor and of endothelin-1 in systemic inflammation and vascular remodelling induced by chronic intermittent hypoxia
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Background: Obstructive sleep apnea (OSA) is a public health problem, affecting at least 5% of the general population and characterized by repetitive upper airway collapse during sleep leading to intermittent hypoxia (IH). Both OSA and IH lead to various cardiovascular complications such as hypertension, vascular remodelling and atherosclerosis and are associated with oxidative-stress and systemic inflammation. The aim of this study was to investigate the role of the hypoxia sensitive transcription factor-1 (HIF-1) and one of its target genes, endothelin (ET-1), in the development of the IH-induced systemic inflammation and vascular remodelling.
Methods and results: For this, the response to IH was studied in heterozygous mice partially deficient for the a subunit of HIF-1 (*Hifa* +/−) and in C57BL/6 mice treated with bosentan (a non-selective endothelin receptor antagonist) throughout the period of IH exposure. All animals were exposed for 2 weeks, 8 h/day, to 1-min cycles of IH (30 s at 5% FiO2 followed by 30 s at room air) or to normoxia (similar cycles with room air only). Aortic intima-media thickness and systemic inflammation, assessed through splenocyte activation, were measured in the various experimental groups.
After 2 weeks of IH exposure, splenocyte activation was shown by increased proliferation in response to concanavalin A and migration in response to MCP-1, in hypoxic compared to normoxic mice. Aortic intima-media thickness was also significantly increased by IH. Treatment with bosentan or partial deletion of HIF-1a, suppressed the inflammatory response and the increase in aortic vascular wall.
Conclusion: Both HIF-1 and ET-1 appear to be involved in the IH-induced systemic inflammation and vascular remodelling.

17-O033
High COMT activity is associated with earlier age at onset in Parkinson's Disease
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Objective: In the central nervous system, Catechol-O-methyltranferase (COMT) is one of the main enzymes that metabolize dopamine. A single nucleotide polymorphism (SNP) in the COMT gene causes a trimodal distribution of high ($COMT^{HH}$), low ($COMT^{LL}$) and intermediate ($COMT^{HL}$) enzymatic activity. Little is known about the impact of this SNP (*rs4680*) on motor symptoms in Parkinson Disease (PD). In the present study, we show that COMT activity may modulate the age at onset (AAO) of motor symptoms in PD.
Methods: A two-step association study was performed in which the SNP *rs4680* was genotyped with a Taqman assay in 6574 patients and controls of French and German origin from four independent samples. Survival data methodology was used to analyze AAO.
Results: In the sample used for hypothesis generation, onset occurred 2 years earlier in the high and intermediate activity groups ($COMT^{HH}$/$COMT^{HL}$) than in the low activity group ($COMT^{LL}$), suggesting a dominant effect. Onset tended to be 2 years earlier in the high and intermediate genotypes in the verification sample.
Interpretation: The *rs4680* polymorphism is a genetic modifier of AAO in idiopathic PD patients. This might be explained by an effect on the bioavailability of endogenous dopamine in the striatum of $COMT^{HH}$/$COMT^{HL}$ patients at disease onset; high COMT activity would reduce bioavailability resulting in earlier motor symptoms and *vice versa*.

17-O034
Comparative effects of droperidol, ondansetron and their association on morphological parameters of ventricular repolarization in healthy volunteers
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Introduction: Droperidol (Dro) and Ondansetron (Ond) are the main drugs used for the management of post-operative nausea and vomiting. Both drugs prolong the QTc interval, a marker of proarrhythmic risk. Drug-induced T-wave morphological changes are less frequently used to assess proarrhythmic risk although they are known to be associated with proarrhythmia. We studied the effects of Dro, Ond and their combination on morphological parameters of ventricular repolarization.
Material and methods: Sixteen healthy volunteers were enrolled in this randomized, controlled, double-blind, cross-over study with four periods. Subjects received single iv doses of Dro (1 mg), Ond (4 mg), the association (Dro+Ond) or placebo (PLB). Digitized 12-lead ECH were recorded at: baseline, t0 (=end of injection), 1, 2, 3, 4, 5, 6, 8, 10 and 15 min. Studied parameters were: maximum variation of T wave amplitude (TAmp), T wave area under the curve (AUCTote), Tpeak-Tend interval (TpTe), asymmetry coefficient (CoeffA), kurtosis (flat) and the Morphological Combination Score (MCS) (Graff, J Clin Pharmacol 2009). V2 lead was used. Parameters were compared using ANOVA and HSD Tukey test.
Results: Tamp decreased more with Dro (−21.6 ± 14.4%) than with PLB (−10.7 ± 12.8%; $P = 0.02$) and Ond (−7 ± 10.7%, $P = 0.002$) but was not significantly different with Dro+Ond (−17.5 ± 12.7%, $P = 0.37$). Tamp did not differ with Ond and PLB ($P = 0.41$). AUCToTe decreased more with Dro (−21 ± 13.7%) than with PLB (−8.3 ± 9%, $P = 0.004$) or Ond (−4.4 ± 7.1%, $P = 0.0003$) but no difference was found with Dro+Ond (−17.8 ± 16.3%, $P = 0.45$). AUCToTe was not different between Ond and PLB ($P = 0.37$). TpTe decreased in all groups (Dro: −6.8 ± 6.2; Ond: −0.34 ± 2.18 ($P < 0.001$ and $< 3 \times 10^{-4}$ vs. Dro respectively), Ond+Dro: −4.34 ± 5.32 ($P = 0.15$ vs. Dro), PLB: −1.7 ± 4.4 ms ($P = 0.004$ vs. Dro)). CoeffA, flat and MCS were not modified ($P = 0.18$, 0.67 and 0.8 respectively).
Discussion: Droperidol causes more changes than Ondansetron in the morphology of ventricular repolarization and the combination of both drugs results in changes explained by Droperidol alone. However, recent surrogates of proarrythmogenicity such as CoeffA, flat and MCS were not modified. These results

suggest that the proarrhythmic risk, although very low, may be greater with Droperidol than with Ondansetron. This is consistent with the greater QTc prolongation found with Droperidol than with Ondansetron.

17-O035

Methylphenidate for freezing of gait in parkinsonian patients under subthalamic stimulation (PARKGAIT-II)

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Background: The high benefit of the two pivotal treatments, L-dopa and deep brain stimulation, in Parkinson's disease (PD), is impeded at long term by the appearance of axial disorders with freezing of gait (FOG), which may result from the noradrenaline and dopamine reduction in the striato-frontal loops. Methylphenidate (MPD) enhances synaptic striatal and prefrontal levels of both dopamine and norepinephrine by presynaptic transporter inhibition.

Objective: We aimed to investigate whether MPD would improve freezing of gait and attention impairment in non-demented patients with advanced PD.

Methods: In this double blind, placebo-controlled trial (ClinicalTrials.gov:NCT00914095), patients, from 13 centres in France, who had severe axial disorders with freezing of gait, despite optimized treatment of motor fluctuations by dopatherapy and subthalamic stimulation, were eligible for inclusion. Patients were randomly assigned, in a 1:1 ratio to MPD (1 mg/kg/day) or placebo for 3 months. The primary outcome measure was the change on the number of steps at the Stand Walk Sit Test in standardized conditions without L-dopa between day 0 and day 90 after the start of the study drug.

Results: Eighty subjects were screened and 69 eligible subjects were completed the trial. As compared with placebo, we found, under MPD, a decrease in the number of steps ($F_{1,66} = 6.1$; $P = 0.016$), the time completion ($F_{1,66} = 6.9$; $P = 0.01$), the number of (FOG $F_{1,66} = 6.2$; $P = 0.015$) at the FOG trajectory and the UPDRS motor score ($F_{1,66} = 7.4$; $P = 0.008$) without L-dopa and a significant decrease in the number of FOG ($F_{1,53} = 6.3$; $P = 0.015$) after an acute challenge of L-dopa. Attention performances and sleepiness were also significantly improved under MPD. No significant worsening of dyskinesia, addiction or mood was noted. A significant inhibition of the presynaptic dopamine transporters in the bilateral striatum was observed on the DaTSCAN™. The therapeutic response might be modulated according to the COMT polymorphism (val/met158).

Conclusions: MPD improved freezing of gait and had a positive impact on cognition and behaviour in a selected population of advanced PD population under STN stimulation and moderate daily dose of levodopa.

17-O036

Is brain region-dependent internalization of 5-HT1A receptors relevant to antidepressant therapy?

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Depletion of serotonin (5-HT) is involved in major depression disorders and the serotonin 1A receptor (5-HT_{1A}R) is implicated in the action of selective serotonin reuptake inhibitors (SSRI). The delay in the onset of SSRI therapeutic benefit has been attributed to slowly developing adaptive changes (desensitization) of 5-HT_{1A}R in serotonergic neurons. So far, the mechanism responsible for 5-HT_{1A} autoreceptor desensitization is not known, but evidence has been reported that it might involve receptor internalization. Here, we studied the roles of internalization and recycling to plasma membrane for 5-HT_{1A} receptor function.

Experiments were performed on primary cultures of rat hippocampal and serotonergic raphe neurons infected with a recombinant lentivirus expressing Flag-5-HT_{1A} receptors. The Flag tag was inserted at the extracellular N-terminus of the 5-HT_{1A} receptor, to allow the labeling of neuronal membrane-bound receptors and follow their endocytosis. Neurons were incubated during pharmacological treatments with an antibody directed against the Flag epitope for 1 h at 37°C, and internalization was quantified using ImageJ software.

We observed that 10% of 5-HT_{1A}R were constitutively internalized in somas and dendrites in 5-HT raphe neurons, whereas they were poorly endocytosed in hippocampal neurons. No effect on 5-HT_{1A}R endocytosis was observed in either raphe serotonergic or hippocampal neurons after 1 h 8-OH-DPAT (5-HT_{1A}R agonist) treatment. However, agonist-dependent 5-HT_{1A}R internalization increased up to 30% after a 24 h incubation with 8-OH-DPAT in raphe serotonergic neurons where the 5-HT_{1A}R is an auto-receptor, but not in hippocampal neuron where the 5-HT_{1A}R is a hetero-receptor. This agonist-induced internalization was prevented by the 5-HT_{1A}R antagonist WAY 100635 confirming that internalization depends on 5-HT_{1A} auto-receptor long-term stimulation. Experiments will now be performed to analyse the mechanism of the 5-HT_{1A} agonist-dependent internalization in 5-HT neurons.

This brain region-dependent 5-HT_{1A} receptor internalization after a long term agonist treatment is consistent with electrophysiological results showing a desensitization of 5-HT_{1A} R in raphe neurons but not in hippocampal neurons after chronic treatment with fluoxetine (Le Poul et.al, 1995). Thus, differential regulation of 5-HT_{1A} R depends on cellular adaptive changes occurring specifically in serotonergic neurons after long term treatment and may contribute to the therapeutic effect of SSRI.

17-P183

Cognitive function in patients with postherpetic neuralgia- systemic treatment vs. 5% lidocaine plaster

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Introduction: Neuropathic pain has been shown to be accompanied by cognitive impairment, but the specific impact of post-herpetic neuropathic pain on cognitive processes has not been explored. This study aims at the evaluation of the impact of pain on several domains of cognition in patients with post-herpetic neuralgia (PHN).

Methods: This cohort study (clinicaltrial.gov NCT 00989040) included 84 individuals after signature of the informed consent, 42 patients with PHN and 42 healthy volunteers. They performed a panel of five cognitive tests: reaction time, semantic memory, decision making, visual memory and executive functions (Cantab®, Cambridge). Twenty-one patients had a classical treatment (antidepressants, anticonvulsants, opiates…), 21 had a patch of lidocaïne 5% only.

Results: Patients were aged 72 ± 7 years, 30 men, 54 women and matched by age and gender. Vigilance, decision making and semantic memory were significantly impaired ($P < 0.05$) in patients on classical treatment while no significant changes were noted between the lidocaïne group and matched controls.

Conclusions: This study shows the deleterious effect on several domains of cognition of post-herpetic neuropathic pain. These findings are due to pain itself and also to the side-effects of classical treatments that need to be explored further. Topical treatment when the localisation of pain allows it is a valuable alternative for pain alleviation, and maintains cognitive integrity in this vulnerable population.

17-P184

Improving therapeutic drug monitoring of psycho-pharmacotherapy: impact of clinical audit and guidelines information

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Aim Therapeutic drug monitoring (TDM) is a valid tool to optimize psycho-pharmacotherapy. Despite obvious advantages, the use of TDM in everyday clinical practice is far from optimal[1]. In order to improve clinical care, we aimed (i) to assess professional practices in TDM and biologic monitoring of psychiatric patients treated with the antipsychotic drugs: clozapine, olanzapine, haloperidol and fluphenazine, and (ii) to develop a new index reflecting a good practice.

Methods Managed by a pilot and a co-pilot, 17 psychiatrists participated in the evaluation. They practiced in different clinical departments. Their practice was evaluated during two 6-months periods by an independent multidisciplinary board before and after information about official guidelines[1,2]. One questionnaire had to be filled for each antipsychotic and used for each patient. The questionnaire reported clinical state, medical treatment and monitoring (therapeutic response, biologic monitoring or BM and TDM);

Results One hundred and fifteen questionnaires were analyzed for each period. The first period analysis revealed that the patient monitoring in accordance with the TDM guideline was <20%, whereas BM in accordance with the BM guideline was better with a 50% good practice. The second period analysis showed a significant improvement: on one hand, enhancement of 50% to 80% for TDM practice ($P < 0.00001$); on the other hand, enhancement of 6% to 80% for BM ($P < 0.01$). This improvement was better for TDM of clozapine and haloperidol but the global mean improvement for all drug was equivalent ($P = 0.33$)

Finally, we developed a new index that scores practice from A better monitoring to E deficient monitoring: 80–100% and 0–19% of recommendation respectively. Overall, the global TDM improved from Class E to Class C while the BM improved from Class C to Class B.

Conclusion Our study is the first audit of TDM practice in psychiatric patients. Clinical audit associated with guideline information and index evaluation improves the quality of care.

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17-P342

Long duration omega-3 supplementation may improve spatial memory in mice in parallel to an enhancement of hippocampal neurogenesis

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Introduction: We previously showed that a 6-week duration omega-3 supplementation leads to the development of both antidepressant and anxiolytic effects in parallel to hippocampal neurogenesis (1). We investigated here the effect of omega-3 supplementation on spatial memory in mice.

Methods: Male Swiss mice (20–25 g) received alpha-linolenic acid (ALA, Sigma France, 250 mg/kg, gavage) or placebo during 6 weeks before their behavioral evaluation using the y-maze ($n = 9$ in each group). A paradigm of spatial memory using the Morris water maze (either with visible or hidden platform task) was also used in another subset of mice fed an omega-3 enriched diet (NUMICO research, Germany) or appropriate control diet for 6 weeks ($n = 8$–11 in each group). Finally, to study new born cells in the hippocampus, mice were injected with BrdU (300 mg/kg, i.p.) 24 h before sacrifice. Double-labeling techniques were also used to demonstrate neuronal differentiation.

Results: A 6-week ALA supplementation increased the number of entries in the new arm of the y-maze (Control 22 ± 3; ALA 15 ± 1; $n = 9$ in each group; $P < 0.05$) while the time spent in the new arm was unchanged (Control 85 ± 11 s; ALA 87 ± 10 s). Using the Morris water maze, an improvement of spatial memory was observed in mice fed the omega-3 enriched diet particularly when platform was not hidden (Trial task: Control 1686 ± 306 s, PUFA 1523 ± 252 s; Final task: Control 1374 ± 362 s, PUFA 782 ± 190; $P < 0.004$). In parallel, omega-3 supplementation increased neurogenesis in the hippocampus.

Conclusion: Chronic omega-3 supplementation may improve spatial memory in mice together with an enhancement of hippocampal neurogenesis. Further studies are needed to definitively demonstrate such a beneficial effect of omega-3.

Reference:

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17-P343

Etazolate reduces neuroinflammation, restores sAPPalpha levels and offers persistent neuroprotection after traumatic brain injury in mice

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Objective: Traumatic brain injury (TBI) evokes an intense neuroinflammatory reaction that is reported to further promote neuronal death. Etazolate is a phosphodiesterase-4 inhibitor that also stimulates the production of the neuro-

trophic soluble amyloid precursor protein alpha (sAPPα) [1]. It is therefore supposed to exert anti-inflammatory and neuroprotective properties. The aim of this study was to examine the effects of etazolate on acute and belated post-TBI consequences.
Materials and methods: The mouse model of TBI by mechanical percussion was applied [2]. Injured mice received (i.p.) either the vehicle (PBS 0.01 M) or etazolate at the dose of 1 (E1), 3 (E3) or 10 mg/kg (E10) at 2 h post-TBI. Neurological score, IL-1β and sAPPα levels and lesion size were evaluated by the Irwin-Exit Circle test, ELISA and cresyl violet staining method respectively. Microglial activation was determined by CD11b immunolabeling. Cerebral edema was calculated using the dry-wet weight method. Locomotor activity was assessed by an actimeter and the Novel Object Recognition Test was used to evaluate memory function. Digital images of the whole brains were taken and olfactory bulb lesions were measured using ImageJ software (NIH).
Results: All three doses were able to reduce neurological impairment at 6 and 24 h post-TBI (E1: $P < 0.05$; E3, E10: $P < 0.001$). E10 restored sAPPα levels ($P < 0.01$) and attenuated IL-1β levels ($P < 0.05$), cerebral edema ($P < 0.01$), ventricular enlargement ($P < 0.01$) and CD11b immunolabeling ($P < 0.01$) at 24 h post-TBI. E3 and E10 reduced locomotor hyperactivity in most time-points from 48 h to 12 weeks post-TBI, memory impairment at 5 weeks post-TBI (E3: $P < 0.05$, E10: $P < 0.01$) and olfactory bulb lesions at 12 weeks post-TBI (E3, E10: $P < 0.001$).
Discussion: Etazolate exerts anti-edematous and anti-inflammatory effects post-TBI that are accompanied by lasting neuroprotective effects, namely improvement of locomotor and memory function. These results highlight the therapeutic potential of etazolate in TBI.
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Preclinical imaging in drug development for oncology. Experience of Pharmimage consortium
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Preclinical or small animal imaging has recently been demonstrating its high potential to accelerate drug development in oncology. Several imaging modalities are available for research: nuclear imaging (SPECT and PET), optical imaging (bioluminescence and fluorescence), magnetic resonance imaging (MRI), computed-tomography (CT) and ultrasound (US). Aside from optical imaging, all these imaging modalities are routinely used in clinical settings, making them particularly relevant for translational research, using preclinical animal models. As in the clinic, the trend is to combine imaging modalities with each other: PET/CT, SPECT/CT, Optical/CT, and recently PET/MRI. These hybrid scanners make it possible to gather multiparametric and multitemporal data from the same animal. Preclinical imaging is now considered a powerful tool for pharmacological purposes, giving rise to a discipline coined pharmaco-imaging. These non-invasive technologies allow assessing of: in vivo expression of a target, mechanism of action or biodistribution/pharmacokinetics of a new molecule and therapeutic effects through the monitoring of *ad hoc* biomarkers. However, all these imaging technologies only show their true potential with the use of relevant preclinical models, such as xenografts in immunocompromised rats and mice, which need to be predictive of a given pathology. In this context, small animal imaging allows visualization and quantification of important tumoral features such as: expression modulation of a target, metabolism, proliferation, apoptosis, angiogenesis or hypoxia. Our experience in Dijon, relies on the Pharmimage® consortium, hosting several imaging modalities such as μMRI (anatomy and pharmacodynamic biomarkers of efficacy) and preclinical SPECT/CT platform (specific tumoral targeting through monoclonal antibody radiolabeling) soon to be enhanced with a preclinical PET/MRI (spring 2012). Thus, the aim of our research is to follow a translational path from molecule to patient imaging, in order to strengthen early phases of drug development in oncology and to help fulfill the promises of personalized medicine.

Ultrasound mediated targeted delivery: in vitro evaluation and preclinical validation
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Targeted drug delivery using ultrasound (US) offers new opportunities to improve the therapeutic efficacy of tumor treatment and to reduce toxic exposure to healthy organs and tissues. This approach is based on the combination of ultrasound waves and gaseous microbubbles currently used as an ultrasound contrast agents. Microbubbles under ultrasound activation oscillate and induce a modulation of the permeability of biological barriers favorizing thus the extravasation of drugs into the treated tissue. Hence localized and targeted delivery of the drugs occurs only in the treated region and the release can be controlled and triggered in both time and space under the action of ultrasound focusing. The objective of this study is to investigate the usage of ultrasound, microbubbles, and a co-administered (systemic) antineoplastic small molecule drug in animal cancer model to evaluate the safety and efficacy of this drug delivery method.
As a first step, the efficacy of this drug delivery approach has been evaluated in-vitro using a glioblastoma cell line (U-87-MG). Hence experimental microbubbles (MM1) have been co-injected with doxorubicine and insonified with ultrasound at 1 MHz. The results demonstrated a synergistic effect of doxorubicin when co-administered with microbubbles under ultrasound activation. The cell mortality for the group doxorubicin + microbubble + US was twice higher than the group where the doxorubicin was injected alone.
In order to validate the potential of this new method in-vivo, an animal tumor model was developed in which U-87-MG cells were injected subcutaneously. The drug used in this preclinical study was Irinotecan. This anticancer molecule is an inhibitor of topoisomerase I and conventionally used for the treatment of colo-rectal cancer. However the administration of Irinotecan is associated with severe side effects such as chronic diarrhea and hematological disorders.
The treatment of the tumors was initiated when they reached a volume of 100 mm^3. It consisted of an intravenous co-administration of contrast microbubbles and Irinotecan followed by an ultrasound insonation using a single element transducer operating at 1 MHz. In order to evaluate the efficacy of ultrasound mediated drug delivery in-vivo, four animal groups were defined: (i) control (without treatment), (ii) Irinotecan alone (20 mg/kg), (iii) US and microbubbles and (iv) Irinotecan (20 mg/kg), US and microbubbles (MM1). The therapeutic efficacy was determined by measuring the tumor volume before and after treatment using a caliper and based on ultrasound images made at 18 MHz (Vevo 2100). Our results showed that 2 days after the first treatment, the volume of the tumors treated with Irinotecan alone increased by a factor of 2.3. However tumors treated with Irinotecan in combination with microbubbles and ultrasound remained relatively stable. For all the groups, tumor growth was monitored for 15 days and showed that the application of ultrasound in combination with the microbubbles and Irinotecan engenders a stabilization of the tumor volume and then its regression.
This preliminary preclinical study demonstrates the synergetic effect of the co-administration approach. Hence, ultrasound activation of the microbubbles enhances the permeability of the endothelial barrier and increases by that the bioavailability of the drug inside the tumor tissue. In conclusion, this novel therapeutic approach has promising features since it can be used to reduce the injected chemotherapeutic dose and to achieve a better therapeutic efficacy.

Successes and pitfalls of oncologic imaging in small animals: feedback, findings and consequences.
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Many arguments are in favor of the use of small animal imaging in preclinical studies. Sharing the same imaging technologies than in human beings (eg XRay-CT, SPECT, PET, MRI and US) appears as a simple and efficient way for translational studies and for taking advantages of already acquired knowledge. However, consequences of natural differences in species contribute to blur results and conclusions, especially in oncologic imaging.
First, the size of animals models require the development of specific instruments. Even based on the same physical principles they are designed to meet the necessary higher spatial resolution needed. Some solutions widely accepted distance from clinical instruments: for example small animal MRI systems operate at a main magnetic field value above the value of the majority clinical systems.
In the second place, there are wide anatomical and physiological differences between animals used as model and human beings. However huge differences in uptake of drugs for instance are not expected.
Thirdly, specific acquisition conditions could be used in animals that could not be in human beings for ethical reasons or thanks to animals breeding. Experimental conditions in animal models differ from the development of pathology in human beings just because of cure, immediately applied after diagnosis.
Based on the several modalities available in our unit, we will show some examples of imaging done in oncological models to illustrate the different points listed above:
· subcutaneous xenografts of HT29 cells in nude mice;
· orthotopic human glioblastoma (U87) implanted in nude mice;
· chemical induced coloncarcinoma in wildtype mice;
· spontaneous osteosarcoma in transgenic mice;
· spontaneous hepatocellular carcinoma in wildtype mice.
Doing imaging nowadays in models of cancers require access to a panel of different modalities and to be able to merge them: as in clinic, it is time for multimodality on a large scale.

In vivo imaging of intravenous administered DNA lipid nanocapsules in mice
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Non viral delivery systems can be used to protect DNA from degradation and inactivation in blood, overcome barriers between administration and target site and deliver DNA in the nucleus of cancer cells. In this way, DNA lipid nanocapsules, encapsulating a luciferase coding plasmid, were obtained by a solvent-free phase inversion process [1]. DNA LNCs possess an oily core and a rigid shell mainly composed by PEG (polyethyleneglycol). Encapsulation of a fluorescent probe, DiD, allowed us to follow their biodistribution with in vivo biofluorescence imaging (BFI) after systemic administration in healthy animals and in mice with subcutaneous glioma model (U87MG cells). DNA LNCs exposed a long circulation half-life (up to 7 h) in vivo allowing an interesting tumour accumulation [2].
Afterwards, the biodistribution of DNA LNCs, encapsulating pHSV-tk, was analysed on an orthotopic melanoma mouse model. Luminescent melanoma cells (SK-Mel28), implanted subcutaneously in the flank of the mice, allowed us to follow tumour growth and tumour localisation with in vivo bioluminescence imaging (BLI). The BF-images confirmed a prolonged circulation-time for DNA LNCs as previously observed on the ectotopic model of glioma. Comparison with BL-images evidenced the colocalisation of PEG DNA LNCs and melanoma cells. After these promising results, treatment with DNA LNCs and ganciclovir, using the gene-directed enzyme prodrug therapy approach, was performed and the treatment efficacy measured by BLI. The first results showed tumour growth reduction tendency and so, once optimised, this therapeutic strategy could become a new option for melanoma treatment [3].
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18-P023

Differential implication of kinin B1 and B2 receptors in cardiac functions: an echocardiography Doppler study in knockout mice

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Kinins are vasoactive peptides involved in the therapeutic effect of angiotensin-1 converting enzyme inhibitors prescribed in various cardiovascular diseases. They act through the activation of two receptors denoted as B_2R (constitutive) and B_1R (inducible). The aim of the study was to investigate the impact of B_1R or B_2R gene deletion on cardiac functions.

Echocardiography Doppler was performed in 6-months old male C57BL/6J wild-type (WT), B_1-kinin receptor null (B_1KO) and B_2-kinin receptor null (B_2KO) mice. Left ventricle (LV) wall thickness and internal diameter (LVID) were measured in the parasternal long and short axis views. The fractional shortening (FS) was calculated for the assessment of systolic function. Heart rate (HR) was measured using three consecutive beats and LV mass and cardiac output (CO) were calculated. Transmitral filling was evaluated from the apical four-chamber view and E- and A-waves velocities ratio (E/A) was measured in the mitral annulus for the evaluation of diastolic function.

An upward trend of LVID was noted in B_1KO compared to WT and B_2KO mice. Consequently, FS was significantly decreased in B_1KO mice ($21 \pm 1\%$, $P < 0.05$) compared to WT and B_2KO mice ($26 \pm 2\%$ and $25 \pm 1\%$ respectively). No difference in HR, wall thickness, LV mass and CO was observed. The E/A ratio were similar in all groups.

In conclusion, B_1R gene deletion impairs systolic function whereas lack of B_2R does not affect cardiac function under normal conditions. Further studies in senescence and cardiovascular diseases (diabetic cardiomyopathy) will clarify the beneficial or deleterious role of B_1R and B_2R on cardiac performance.

18-P110

Quasi-simultaneous in-vivo synchrotron imaging of regional ventilation and blood volume distributions after methacholine provocation in rabbit

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Rationale: We previously described a synchrotron imaging technique allowing quantitative imaging of both lung structure and regional specific ventilation [Porra L. et al. J Appl Phys, 2004;96:1899] or blood volume [Suhonen H. et al. Phys Med Biol, 2008;53:775] in rabbit, using inhaled xenon and infused iodine respectively as contrast elements. We are describing a technique to image changes in regional specific (sV) and absolute ventilation (V*) and blood volume (V_B) quasi simultaneously. We used this method to assess the changes in regional V* and V_B after methacholine (MCh) aerosol challenge in rabbits.

Methods: Experiments were performed in anaesthetized and mechanically ventilated healthy New Zealand rabbits ($n = 5$). Regional V* and V_B were imaged using K-edge subtraction imaging before and after MCh aerosol provocation. Ventilated alveolar area (VAA) was estimated as areas where: V*> m-2SD. Heterogeneity was estimated as the coefficient of variation (CV) of both parameters.

Results: Images of regional V* and V_B are shown at baseline and after MCh challenge in one representative rabbit. Mch inhalation produced clustered ventilation defects (VD), and increased the CV of both V* (260%) and V_B (122%) vs. baseline suggesting increased heterogeneity in both parameters. Following MCh challenge, VAA decreased from 98% to 69% total lung area. Significant redistributions in both V* and V_B occurred: V* decreased to 24% within, but remained at 103% outside of VD's compared to corresponding areas at baseline. Mean V_B decreased to 53% within, and remained at 77% in normally ventilated zones compared to corresponding areas at baseline.

Conclusions: Synchrotron CT imaging allowed quasi-simultaneous measurements of both absolute regional ventilation and blood volume. In rabbit, Mch bronchial challenge caused significant redistributions in both V* and V_B.

18-P182

Effect of blood pressure (BP) reduction on myocardial serotonergic receptors expression in relation with cardiac remodeling in spontaneously hypertensive rats (SHRs)

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Chronic high blood pressure is well known to induce cardiac hypertrophy associated with activation of the cardiac extracellular cell matrix. The aim of this work was to evaluate the effect of chronic hypertension and the effect of afterload reduction by the calcium channel blocker, nicardipine, on cardiac remodelling and the expression of the main cardiac serotonergic receptors ($5\text{-}HT_{2a}R$, $5\text{-}HT_{2b}R$ and $5\text{-}HT_4R$).

SHRs were treated by nicardipine (6 mg/kg/day i.p.) (SHR-N) between 37 and 53 weeks of age. They were compared to their normotensive (WKYs) and hypertensive (SHR-C) controls. Cardiac function and anatomy was regularly analyzed by echocardiography and blood pressure was measured by the tail-cuff method. At the end of the treatment period, all animals underwent echocardiography, cardiac and aorta catheterization and the heart was analyzed for the expression of the mRNAs.

SHR-C developed a severe HBP (180 ± 10 vs. 120 ± 8 mmHg in WKY, $P < 0.001$) which caused cardiac hypertrophy associated with mRNA overexpression of collagen I and III, $5\text{-}HT_{2a}R$ (?Cp = 16.1 ± 1.8 vs. ?Cp = 11.43 ± 0.5, $P = 0.0001$),$5\text{-}HT_{2b}R$, $5\text{-}HT_4R$.

Nicardipine markedly reduced BP and decreased cardiac hypertrophy as demonstrated by echocardiography (left ventricular mass: 1.07 ± 0.13 g in SHR-N vs. 1.5 ± 0.16 g in SHR-C, $P < 0.001$), direct cardiac mass measurement (1.95 ± 0.15 g in SHR-N vs. 2.23 ± 0.19 g in SHR-C, $P < 0.05$) and the decrease of the ECG QRS maximal amplitude. BP reduction did not affect dP/dt_{min}, dP/dt_{max} and Tau index. Left ventricular end-diastolic pressure decreased significantly (10.3 ± 1.17 and 16.2 ± 2.6 mmHg in SHR-N and SHR-C respectively, $P < 0.05$). In semi-quantitative RT-PCR (Cp), BP reduction affected mainly the $5\text{-}HT_{2a}R$ (Cp = 12.4 ± 0.07 in SHR-N, Cp = 11.4 ± 0.2 in SHR-C, $P = 0.0016$) and collagens I and III expressions. $5\text{-}HT_{2b}R$, $5\text{-}HT_4R$, ß-MHC, SERCA A2, ANP and BNP mRNA expressions were not affected by BP and cardiac hypertrophy reductions.

In this work, we show for the first time that chronic hypertension and cardiac remodelling trigger the mRNA overexpression of $5\text{-}HT_{2a}R$, $5\text{-}HT_{2b}R$ and $5\text{-}HT_4R$ in SHRs. The afterload reduction by nicardipine reverses $5\text{-}HT_{2a}$ overexpression without affecting the expression of the two others. These data will have to be confirmed at the protein level but they emphasize that serotonergic receptors expression could have different load-dependant patterns of regulation during the natural course of hypertension.

20-O037

Cetuximab infusion reaction: French pharmacovigilance database analysis

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Purpose: To identify factors associated with severity of cetuximab infusion reaction and to compare characteristics of patients exhibiting cetuximab infusion reaction with those of patients exhibiting another adverse reaction related to cetuximab.

Methods: All cases of adverse drug reactions reported with cetuximab were extracted from the French Pharmacovigilance database from 1985 until January 1st 2011. Infusion reactions were identified among these cases and the severity of these reactions was assessed according to the NCI-CTCAE criteria (version 4.0). A multiple logistic regression analysis was performed to identify factors associated with severity in the reports of infusion related reactions.

Results: Among the 602 ADRs reported with cetuximab between 1985 and 2011, 374 infusion reactions were identified. Factor associated with severity of infusion reactions was the rank of the cycle (1st vs. cycle 2 or more: OR = 7.40 CI 95% [2.21–24.71]), after adjustment for age, sex, region of France, quarter of the year, indication, year of occurrence, premedication. When comparing reports of cetuximab infusion reaction with other adverse reactions reported with cetuximab, indication of the cetuximab was more likely head and neck cancer as compared to colorectal cancer (RR = 3.79, CI 95% [2.53–5.68]). We also found a significant difference for the rank of cycle (first cycle vs. the second or more: RR = 10.82, CI 95% [6.96–16.83]) and for the year of occurrence with an increase tendency since 2008 ($P < 0.001$). Among the seven reported death cases following infusion reaction, five patients were treated for head and neck cancer vs. 1 for colorectal (and one unknown).

Conclusion: Analyses of infusion reactions reported with cetuximab to the French Pharmacovigilance database, found that reports of severe reactions were more likely during the first treatment cycle, that reports concerned more often patients treated with cetuximab for squamous cell carcinoma of the head and neck compared to those treated for colorectal cancer and that evolution is in these patients to be more often fatal. Further investigations are needed to assess differences in support care between these two indications and considering the possibility that these reactions are IgE mediated, the interest to identify at risk patients before onset of treatments should be envisioned.

20-O082

Cardiovascular effects of cholinesterase inhibitors in France. Analysis of data reported from 1998 to 2010

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Introduction: Cholinesterase inhibitors (ChI) produce pharmacologic effects that could generate cardiovascular (CV) adverse drug reactions (ADR). Studies on the CV impact of these drugs are conflicting. In the French pharmacovigilance database, the main ADR to be judged serious involve the CV system. The aim of the present study was to describe the CV ADR registered with ChI use in the French pharmacovigilance database between 1998 and 2010.

Methods: All ADR associated with the use of donepezil, rivastigmine or galantamine considered as 'probable' or part of a drug-drug interaction and registered in the French pharmacovigilance database from 1/1/1998 to 31/12/2010 were extracted. A descriptive analysis, global and drug by drug has been carried out in order to describe the CV-ADR, their severity, the drugs involved when known to induce CV effects.

Results: From 1/1/1998 to 31/12/2010, 395 CV-ADR were collected (302 = 76.5% in the cardiac system organ class (SOC), 77 = 19.5% in the vascular SOC, and 16 = 4% in the investigation SOC). An arrhythmia was reported in 74% of the cases, a change in blood pressure in 18%, and another CV effect in

8%. Two hundred and fifty-three (78.1%) of CV-ADR were assessed as severe (63%: hospitalization, 11.7%: death or life threatening). The seriousness of the ADR from the three ChI was similar. CV-ADR were identified in 324 patients (81.4 ± 7.1 years, 62.5% women); a CV background was detected in 41.4%. Among the co-administered drugs the role of which appeared to be probable, 38.5% were for CV disease, 35% for neurological ones.
Discussion: The CV effects of ChI appear to be both frequent and serious. These drugs seem to add an adverse drug reaction in patients with a history of previous CV disease. This is why monitoring the cardiac effects of ChI is important especially in patients with an existing CV disease and in those using concomitant drugs with CV-ADR effects, especially antipsychotics.

20-O039

Hospital readmission for adverse drug reactions (ADRs): experience in Toulouse University Hospital

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Introduction: Adverse drug reactions (ADRs) are a well-known for hospital admission. Two previous study conducted by French PharmacoVigilance Centers have shown a rate of hospitalization due to ADRs around 3.6%. Recent data suggested that old age could be a risk factor to repeated admissions to hospital. The aim of this study was to estimate the hospital readmission rate of patient >65 years old for ADRs.
Methods: Data of hospitalizations were collected from Medical Information Department (DIM) of University hospital of Toulouse, using data system called 'PMSI' (Medicalization Program of Information System). According to CIM-10), all patients >65 years admitted in emergency or geriatric wards with a code corresponding to ADRs were selected during the first half of 2010. All readmissions for this cohort within 6 months after discharge from the index admission were retrospectively reviewed. Data for each patient were completed using medical files.
Results: Among 556 patients, 139 (respectively 60 and 79 in geriatric and emergency wards) (25%) were first admitted for an ADRs between January and June 2010. Their median age was 81.7 years. Among them, 60 patients were readmitted during the next 6 months (sex ratio 0.6, median age 81.4 years). ADRs were identified as the reason of the readmission in six cases (10%). The ADRs for first and second hospitalizations were: a same ADR for both hospitalizations with hyponatremia and confusion induced by olmesartan +hydrochlorothiazide in case 1, azacitidine-induced aplasia/tinzaparin-induced anemia (tinzaparine was introduced during the first admission) in patient 2, neuroleptic-induced psychiatric ADRs/insulin-induced hypoglycemia in case 3, duloxetine-induced dizziness/sulfamethoxazole+trimethoprime-induced allergy in case 4, drug voluntary intoxication with alimemazine/neutropenia with clozapine in case 5 and memantine-induced fall/overdosage of digoxin in case 6.
Discussion: These data show that 4% of aged patients hospitalized after an ADR have a probability to be readmitted in hospital because another ADR. Preventability of cases leading to a second hospitalization must be investigated.

20-O040

Severe adverse events with pyridostigmine bromide (MESTINON®) and human error in magistral preparation

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Introduction: Magistral preparations are sometimes paramount to treat patients with rare illnesses for which either no marketed drug exist, or at an inadequate dose, as for this case.
Observation: We report the case of a 5-year-old child who had been treated since birth for an autoimmune myasthenia, by mean of a magistral preparation (MP) supposedly containing 20 mg of pyridostigmine bromide administered four times a day with a good tolerance until recently. Indeed, during a short regional trip which resulted in a different pharmacy preparing and supplying the magistral preparation (MP), this child suffered for 3 h from severe muscarinic signs of overdose (headaches, palpitation, sweats and vomiting), 2 h after taking a capsule containing 20 mg of pyridostigmine bromide from the new batch. We forwarded both MPs for analysis by HPLC coupled to mass spectrometry (LC/MSMS) in the Dept of Pharmacology.
Results: There was a 36% difference in the concentration of pyridostigmine between the two types of preparations. Hence, if the ill-tolerated one contained the proper titration (20 mg), we were surprised that the up-to then well tolerated ones contained approximately a third less active ingredient. It appeared that the 'well tolerated ones' were made by weighing 20 mg of a crushed processed 'adult' pellet (wrong molecular weight) whereas the other had been prepared from scratch (proper drug powder) and contained the correct molar quantities of pyridostigmine.
Discussion and conclusion: This overdose is connected with MP wrongdoing. Pyridostigmine is a drug with a narrow therapeutic index. To avoid important fluctuations such as the one observed, standardized operating procedures are of utmost importance in preparing MPs. This is valid for pyridostigmine, but also for other drugs with narrow therapeutic index as well as for ailing or frail populations such as elderly patients or children which might benefit of the few remaining MPs.

20-O041

Is there a real outbreak of anaphylactic shocks to suxamethonium in Brest University Hospital ? Using queries on PMSI as an effective way to provide reliable answers

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Neuromuscular blocking agents, especially suxamethonium, are known to be involved in allergic accidents. In France, incidence of them has been estimated about 1 per 6500 anesthesias having used neuromuscular blocking agents, but it could have increased mildly during these last years. In Brest University Hospital, we became aware of this issue when seven notifications of anaphylactic shocks to suxamethonium were spontaneously collected by our Pharmacovigilance Center between October 2010 and August 2011. To respond to questions from anesthesiologists, it was essential to first confirm the increase of anaphylactic reactions to suxamethonium before more research could be undertaken.
We queried the data of Programme de Médicalisation des Systèmes d'Information (PMSI) by matching the name of the drug and a thesaurus of key words over the period January 2004–September 2011. This thesaurus was built by picking up the terms of symptoms reported in our notifications of anaphylactic reactions to suxamethonium. So nine relevant words were kept: 'fibrillation', 'allergy', 'allergic', 'shock', 'anaphylactic', 'hypotension', 'tachycardia', 'bronchospasm', and 'collapse'.
Over the period 2004–2011 the query selected 219 hospitalization's summaries which related to 209 patients. The reading of them let us find all the seven reports of anaphylactic shocks that had been spontaneously reported by the anesthesiologists, confirming the relevance of our query. Besides these notifications we identified four other cases which had never been reported to our Pharmacovigilance Center. By comparing the quantitative use of suxamethonium and the number of reports during the same period, it became obvious that the increase of anaphylactic reactions induced by suxamethonium was real. Indeed the incidence was calculated around one accident per 524 anesthesias for the period October 2010-August 2011, so more than 10 times the incidence noted during the period 2004–2009.
This PMSI query helped us to really confirm the feeling of our anesthesiologists that an outbreak of anaphylactic shocks to suxamethonium, especially the most serious of them, was occuring. Being alerted of this true signal anesthesiologists are now working to explain and understand each accident by studying the individual risk factors and by reviewing their own clinical practices.

20-O042

Event competition bias in signal detection from spontaneous reporting: could what we know hide what we ignore?

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Objective: To explore the effects of event competition bias on safety signals generation from spontaneous reporting database.
Methods: A set of drugs of interest was defined, that included statins, oral anticoagulants, antipsychotics, anti-HIV drugs, and paracetamol. For each of these, a type A reaction was arbitrarily selected, and its potential competitive effect on the generation of other safety signals for the drug was explored. These reactions included rhabdomyolysis/myopathies events for statins, haemorrhages for oral anticoagulants, extra-pyramidal syndrome for antipsychotics, lipodystrophy for anti-HIV drugs, and drug induced liver injuries for paracetamol. Using the case non-case approach in the French Spontaneous Reporting Database for research, that includes data of reporting in France from January 1986 to December 2001, signals were generated at MedDRA HLT level for the drugs of interest before and after removing from the database all reports concerning the events identified as potential competitors (whatever the drug incriminated in the report). The identification of the reports to remove from the database when searching signals for the drugs of interest was performed using the corresponding SMQs (20000002; 20000038; 20000095; 20000177; and 20000006-9, 20000123 for drug induced liver injuries).
Results: The whole database included 207 236 notifications, including 4355 incriminating statins, for which 65 signals were initially generated. The removal or reports of rhabdomyolysis/myopathies identified using the SMQ no. 20000002 concerned 8425 reports among which 867 incriminated statins. After this removal, 14 new signals appeared for statins, which had not been initially generated. For oral anticoagulants, the removal of reports concerning haemorrhages (SMQ no. 20000038) led to generate 20 new signals. Similarly, the predefined reports removal lead to identify 10 new signals for antipsychotics, 11 for anti-HIV drugs, and five for paracetamol.
Discussion: This study confirms that a competition bias can occur between events when performing safety signal generation in spontaneous reporting databases for a given drug or class of drug. The minimisation of this bias could lead to reveal previously ignored signals. As this work was performed on a limited set of drugs, with a limited number of events as potential competitors, this bias will need to be further explored.

20-P381
Preventability of 'serious' adverse drug reactions induced by oral Protein kinase Inhibitors (PKIs)

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Introduction: Results from EMIR study, performed in 2007 in France, showed that incidence of adverse drug reactions (ADRs) who required hospital admission was the highest with vitamin K antagonists (VKA) and then antineoplastic drugs. Currently, several antineoplastic drugs, orally administered, as Protein Kinase Inhibitors (PKI) could be taken at home (ambulatory care). Using data of spontaneous reporting in France, we described in a previous study 'serious' ADRs, specifically cutaneous, with oral PKI. In general, for all drugs, 50% of ADR were 'preventable'. In this study, we aimed to assess preventability of 'serious' ADRs related to oral PKI.

Methods: We used the French Pharmacovigilance Database to select 'serious' ADRs reported from 1st January 2008 to 31st December 2009 with eight oral PKI: sorafenib, imatinib, erlotinib, sunitinib, dasatinib, lapatinib, nilotinib and everolimus. A 'serious' ADR was defined as any untoward medical occurrence that at any dose results in death, requires hospital admission or prolongation of existing hospital stay, results in persistent or significant disability/incapacity or is life threatening. We used the French ADR preventability scale in this study (Thérapie 2005, 60, 39).

Results: This study carried out on 265 notifications: 68 for sorafenib, 64 for imatinib, 44 for erlotinib, 43 for sunitinib, 33 for dasatinib, six for lapatinib, four for nilotinib and three for everolimus. Most of ADRs were 'unpreventable' (65%) because prescription was unavoidable. Thirty one percent were 'unevaluable' (31%) because reports were poorly documented (medical history, dosage, 1st or 2nd intention, concomitant drugs). No ADR was 'preventable' but 10 were 'potentially preventable' (4%): aspergillosis, bleeding, pancreatitis, acute pulmonary edema, hepatic ADR, anal fissure and cutaneous ADR. Few ADRs were 'potentially preventable' because recommendations of SPC (Summary of Product Characteristics) were not respected (drug interactions, indication, age or dosage) and risk factors existed (concomitant pathologies, previous ADRs with a PKI, exposition to radiotherapy, alcohol, tobacco).

Conclusion: The French ADRs Preventability Scale is a useful tool to assess preventability of antineoplastic drug-induced ADRs. However, some items are difficult to score, especially with antineoplastic drugs. Most of ADRs with oral PKI drugs were 'unpreventable'. Respect of SPC could avoid occurrence of some ADRs.

20-P382
Severe hepatitis observed after a bolus administration of methylprednisolone

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Introduction: Although many drugs are implicated in the occurrence of acute hepatitis, it is typically not the case with steroids. We report a case of severe hepatitis observed 6 weeks after a bolus administration of a third cycle of methylprednisolone.

Observation: A 42 years old patient without particular medical history was admitted in the dermatology department for extensive *Alopecia areata* of the scalp in February 2011. In the absence of contraindications, IV boli of methylprednisolone (Solumedrol®) were administered (500 mg/day for 3 days in April, May and June) associated with omeprazole during the cures 7 days thereafter.

Six weeks after the last administration of Solumedrol®, biological monitoring yielded parameters of a severe hepatic cytolysis (ALT 821 U/L, AST 392 U/L). Successive controls showed a progressive increase of the cytolysis in early September with ALT at 1610 U/L (25 × ULN) and AST at 1069 U/L (16.5 × ULN). Bilirubin also increased late August and early September whereas PT decreased to 58% as a sign of liver insufficiency.

Main usual causes of hepatitis were ruled out: no alcohol intake, all viral serologic tests negative, no biliary obstruction of the intra and extra-hepatic ducts, no autoimmune disease, blood copper within normal limits …

Discussion: Methylprednisolone and omeprazole were chronologically suspected. Literature and analysis of cases reported in the Pharmacovigilance French National Database didn't suggest omeprazole as a culprit since its liver toxicity is less severe and in all cases appearing within days of administration.

Regarding methylprednisolone (after excluding cases of viral reactivation), similar cases have been observed after boli administration of methylprednisolone (Weissel 2000, Fernandez et al 2008…). Two cases of severe liver damage have been reported in the Pharmacovigilance French National Database, with mild transaminase increase and a favourable evolution. These cases had in common a delayed occurrence (usually 6 weeks after a bolus cycle) and rather slow improvement of liver function (3–4 months).

20-P396
Improvement of psoriasis, an unexpected reaction during a sunitinib treatment: a first French case report

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Introduction: Sunitinib, a multitargeted tyrosine kinase inhibitor, exhibits antiangiogenic properties. It is marketed for advanced renal cell carcinoma, for second line treatment of gastrointestinal stromal tumor and for certain pancreatic neuroendocrine tumors. Concomitant psoriasis, characterized by excessive growth of epidermal keratinocytes, inflammatory cell accumulation and excessive dermal angiogenesis, seems to benefit from its antiangiogenic activity. We report the first French case, with positive rechallenge.

Case report: A 84 years-old woman was treated in 2005 for clear cell carcinoma of her left kidney, treated with partial nephrectomy and local radiotherapy. Her medical history was notable for hypertension treated for a long time with irbesartan 300 mg/day, for right nephrectomy because of lithiasis in 1981 and for a psoriasis, presented as a guttate psoriasis, on her back and her limbs, emerged 30 years ago and treated with topical betamethasone 0.05% cream.

In July 2010, CT-scan revealed metastases in the pancreas and the hip. Only pain-relieving radiotherapy was performed. Because of disease progression and new lung metastases, sunitinib was introduced in March 2011 without other modification in her treatment. After a month, the patient noticed an improvement of her skin aspect, with complete cure after 3 months. Psoriasis relapsed after about 4 weeks of sunitinib discontinuation. When the drug was restarted, the cutaneous disease improved again.

Discussion: Two case reports have been previously published in the USA, and three in Italy (1–3). Sunitinib is a potent inhibitor of VEGF receptors, FLT3, *c-KIT,* and PDGF receptors. These targets give it direct antitumor and antiangiogenic properties. Some anti angiogenic drugs have been tested for psoriasis but are not yet marketed in this indication. Vascular endothelial growth factor is upregulated in psoriasis and a link between psoriasis and cancer has also been suggested, so we discuss the possible mechanisms of the favourable outcome of psoriasis for these patients.

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20-P397
Photodermatitis from topical ketoprofen with co-sensitisation with octocrylene: study of cases reported in Regional Pharmacovigilance Center of Nantes

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Introduction Ketoprofen is a well-known photoallergen. In spite of all measures being taken, the photoallergies caused by ketoprofen gel have continued to occur. Moreover new allergy appears with cosmetic and personal care products, as was recently the case with octocrylene, a chemical ultraviolet filter. The first cases of contact and photocontact allergy caused by octocrylene were reported in 2003. The number of reports on allergic reactions to octocrylene has increased since then. We have studied this co-sensitisation in CRPV of Nantes.

Observations The interrogation of Nantes cases in the French National Pharmacovigilance database was effected with the terms 'ketum gel, profenid gel, ketoprofen gel, topfena gel'. Over the period of reference from 1 January 1994 to 31 October 2010, 148 cases of adverse effects were reported following use of ketoprofen gel. From these reports, we noted the date of the ADR, patient age and gender, type of clinical symptoms, skin testing results, seriousness and outcome The photo-patch tests were performed in 64 cases, 92% were confirmed by positive photo-patch tests with ketoprofen gel. Among 11 patients who had been photosensitized to ketoprofen, seven patients also presented positive photo-patch test reactions to octocrylene, one positive patch test and three are negative, nine also positive reacted to benzophenone-3.

Discussion The clinical studies show that octocrylene is both a photocontact and a contact allergen. Little is known about the reason for octocrylene's allergenic activity. The use of octocrylene in sunscreen products and cosmetics is increasing and sensitization to octocrylene is therefore expected to increase in the near future. In our study, our photo-patch results are in agreement with earlier findings in the literature. Octocrylene belongs to the cinnamate family. It would appear that in the case of cross-reactions between the molecules of fenofibrate, oxybenzone and ketoprofen, the diphenylketone group, benzophenone, is responsible for this reaction. Up to now, no studies have been reported that have identified the chemical structure responsible to explain this co-sensitisation. Knowledge of allergies is vital to avoid recurrence of cutaneous effects in case of subsequent application of a sunscreen contening oxybenzone or octocrylene.

20-P398
Hallucinations after taking a boldo herb tea: a case report

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Peumus boldus is a tall shrub, which grows mainly in Chile and Peru, but is also cultivated in Europe. The medicinal parts are the leaves, *Boldo folium*. The Boldo is traditionally used as herbal tea in case of difficult digestion, minor hepatobiliary disorders and constipation.

We report here the case of a child who developed hallucinations following Boldo herb tea use.

The patient, a 12-year-old girl, took three following evenings, Boldo herb tea to decrease the digestive disorders from which she suffered. After 2 days of treatment, this girl was admitted to the department of pediatrics in hospital further to the

development of visual hallucinations (zoopsia) with anxiety and agitation, 4 h after the last intake.
She consumed no other medicine and a search for toxics was realized but gave a negative answer for cannabis, cocaine and amphetamine.
The examination of the cranial pairs was without particularity and electroencephalogram was normal.
Thus, the hypothesis of the responsibility of Boldo in these side effects was evoked. The plant treatment was stopped during her hospitalization and neurological disorders declined spontaneously. The patient came back home 2 days later without aftereffect.
So, a plausible chronological score is retained because of the spontaneous regression of the event after stopping Boldo herb tea.
In the literature, the data are extremely poor. The notion of hallucinations is only reported in case of overdose and in old works of herbal medicine (Handbook of medicinal herbs). It could be in accordance with the case of our patient who let infuse during several hours Boldo bags, so a potentially important intake of active principle.
Although unusual, clinicians should keep in mind the possible occurrence of neurologic disorders following Boldo herb tea (and phytotherapy in general).
Reference: 1. Duke JA. Handbook of Medicinal Herbs.

20-P399
Hepatotoxicity following antithymocyte globulin treatment in allogeneic haematopoietic stem cell transplantation: about five cases
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Introduction: Antithymocyte globulin (ATG) is commonly used in acute graft-versus-host disease (GVHD) prophylaxis during allogeneic stem cell transplantation. Many adverse effects have been reported with ATG but acute hepatotoxicity is not well documented. We report five cases of marked elevations of liver enzymes occurring immediately after infusion of ATG.
Observations: Five patients (three females, two males) aged between 58 and 63 years underwent an allogeneic haematopoietic stem cell transplantation (AHSCT). The underlying diseases were chronic lymphoid leukaemia ($N = 2$), multiple myeloma ($N = 1$), myelodysplastic syndrome ($N = 1$) and Hodgkin lymphoma ($N = 1$). Pre-transplant conditioning protocol included 5 days fludarabine between day-6 and day-2 AHSCT and 2 days busulfan between day-5 and day-2 AHSCT. Prophylaxis of acute GVHD included cyclosporine from day-6 AHSCT and rabbit ATG (2.5 mg/kg/day) between day-3 and day-2 AHSCT. Cyclosporine was continued. The liver function tests were normal or subnormal before ATG. The transaminases levels increased suddenly during or immediately following the end of the ATG infusion. Alanine transaminase (ALT) rose from 6 to 80 N and aspartate transaminase (AST) from 6 to 47 N. Prothrombin time was prolonged in three patients. Mild cholestasis was present in four patients. No serious systemic signs were observed. The transaminases levels decreased rapidly to baseline values in few days after the last dose of ATG. The main other causes of liver abnormalities were excluded. All patients had their engraftment as planned.
Discussion: Mechanism of hepatotoxicity is unclear. The marked but transient rise of transaminases is compatible with a hypoxic hepatitis that could be related to impaired hepatic blood flow, while no systemic symptoms were observed. A direct hepatotoxicity via an immune reaction induced by rabbit antibodies cannot be ruled out. This adverse effect should be mentioned in the Summary Product Characteristics of ATG.
Reference:
1. Al-Anazi KA et al, Hepatotoxicity induced by horse ATG and reversed by rabbit ATG: a case report. J Med Case Reports 2007, 1: 35

20-P426
Systemic complications after overdose of lidocaine plus prilocaine exposure. About two case reports and review of the french pharmacovigilance database
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Introduction: Local anesthesia before much of dermatologic and non dermatologic procedures is often achieved with the use of lidocaine. Two drugs containing eutectic mixture of 2.5% lidocaine and 2.5% prilocaine are available in France: Emla° and Anesderm°.
Lidocaïne and prilocaine (amino-amid group), are local anesthetics with a rapid onset and persistent action. Toxicity occurs usually after exceeding maximum recommended dosage. It consists in neurological symptom (usually seizures) linked to lidocaine exposure and methemoglobinemia linked to prilocaine exposure.
Method: Two cases of lidocaine plus prilocaine overdose with systemic complications have been recently reported to the pharmacovigilance center of Marseille.
First case concerns a 3 years old child, 16 kg, who received for curettage of molluscum contagiosum, six tubes (30 g) of EMLA° ointment under occlusive dressing. Two hours later, he presented itself with drowsiness, tonico-clonic seizure and methemoglobinemia. All symptoms disappeared after administration of methylene blue.
Second case concerns a 4 years old child, 23 kg, who received eight tubes of anesderm ointment (40 g) for curettage of molluscum contagiosum under occlusive dressing. Ataxia and drowsiness occurred 1 h after use, all symptoms resolved after 3 h.
Discussion: A research in the French pharmacovigilance database has been performed, during the period between years 1996 and 2011, for all lidocaine +prilocain containing ointment: Thirteen cases were retrieved, including the two cases previously described. Eleven were related to EMLA° and two to Anesderm°, concerning 10 children (between 20 days old and 5 years old) and three adults, with a mean application dose of 30 g. Neurological symptoms were present in seven cases, methemoglobinemia in seven cases and one case of sulfhemoglobinemia without methemoglobinemia. Outcome was favorable in all cases.
Conclusion: Its seems necessary to remind the necessity to respect the maximal dose (specified in the french Summary Characteristic of Products) of two tubes (10 g) of ointment for children between 1 and 6 years old and a maximal time of exposure of 1 h in the 0–3 years age range and 4 h in the other cases.

20-P456
Increased of the frequency of seizures and malnutrition with orlistat in an epileptic patient
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Introduction: Orlistat is a reversible inhibitor of intestinal lipases. In the lumen of the stomach and intestine, it bonds covalently with active serine residues of gastric and pancreatic lipases, making them unavailable to hydrolyze dietary triglycerides into absorbable fatty acids and monoglycerides. Because undigested triglycerides are not absorbed, a calorie deficit may occur, having a positive effect on weight control. At therapeutic doses, orlistat inhibits dietary fat absorption by approximately 30%. One of the foreseeable adverse effects is malabsorption syndrome and so decrease of the absorption of fat-soluble vitamins. Orlistat can also interact with absorption of other drugs. Orlistat is marketed as a prescription or more recently other-the-counter to treat overweight.
Observation: A 50 year-old epileptic woman with a body mass index (BMI) of 22 kg/m^2 took for 2 months orlistat to reduce her weight. She was treated for partial epilepsy with valproate and lacosamide. Since she took orlistat the frequency of seizures has doubled. She also had cutaneous signs of malnutrition.
Epilepsy and malnutrition rapidly improved after dechallenge of orlistat and with vitamins supplementation.
Discussion: Drug interactions with certain antiepileptic drugs are known for orlistat. The problem here is the availability of such drugs without prescription. In this case, Orlistat was used out of the labeled indication (BMI $\geq$ 28 kg/m^2)

22-P295
Sildenafil increases digital skin blood flow during all phases of local cooling in primary Raynaud's phenomenon
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Introduction: Digital skin vasoconstriction on local cooling is exaggerated in primary Raynaud's phenomenon (RP) compared to controls (1). A significant part of such vasoconstriction relies on the nitric oxide (NO) pathway inhibition. We hypothesized that sildenafil, a PDE5 inhibitor potentiating the effect of NO, may reverse the exaggerated microvascular response to local cooling in primary RP. We assessed the effect of a single oral dose of sildenafil on digital resting skin blood flow and during local cooling. We also assessed the effect of sildenafil on the perfusion gradient between the different phalanges and the hand.
Methods: We recruited 15 patients with primary RP, performing a 30-min local cooling without sildenafil (day 1), after a single 50 mg oral dose (day 2), and 100 mg (day 3). Skin blood flow, skin temperature and arterial pressure were continuously recorded, and data were expressed as cutaneous vascular conductance (CVC). Skin perfusion of the dorsal surface of the hand was recorded with laser Doppler imaging to assess the perfusion gradient.
Results: Sildenafil at 100 mg, but not 50 mg, significantly lessened the cooling-induced decrease in CV. It also increased resting CVC and resting skin temperature. The difference in skin perfusion between 100 mg sildenafil, but not 50 mg, and control is significant for each phalange and for the hand.
Conclusion: These data show that 100 mg sildenafil improves digital skin blood flow to local cooling in primary RP. This pilot pharmacology study suggests that a single oral dose of sildenafil could be used 'as required' before exposure to cold in primary RP. This should be confirmed in a randomized double-blind controlled trial.
Reference: 1. Roustit M, Blaise S, Millet C, Cracowski JL. Impaired transient vasodilation and increased vasoconstriction to digital local cooling in primary Raynaud's phenomenon. Am J Physiol Heart Circ Physiol 2011; 301: H324–30.

22-P296
Treatment of Polycythemia Vera with hydroxyurea and pipobroman: long term results of a randomized trial
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Purpose: Hydroxyurea (HU) and pipobroman are the two drugs approved in France for the treatment of patients with polycythemia vera (PV). However, the overall impact of these cytotoxic treatments on the long-term outcome of patients with PV (including survival, hematological evolution to myelofibrosis, to acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS)) has not been assessed in randomized studies. In addition, the leukemogenic risk associated with these agents is debated. We report final analyses from the French Polycythemia Study Group (FPSG) study, which randomized HU vs. pipobroman as first-line therapy in 285 patients younger than 65 years.
Patients and methods: Full methodology of this multicenter, randomized phase 3 study has been described previously (Najean et al., Blood 1997; 9:3370–77). FPSG results were updated with a median follow-up of 16.3 years. Statistical analysis was performed using competing risks on the intention to treat population, and according to main treatment received.
Results: Median survival was 17, 20.3 and 15.4 years for the whole cohort, HU and pipobroman arms, respectively ($P = 0.008$), and differed significantly from the general population. At 10, 15 and 20 years, cumulative incidence of evolution to AML/MDS was 6.6%, 16.5%, 24% in the HU arm and 13%, 34%, 52% in the

pipobroman arm ($P = 0.004$). Cumulative myelofibrosis incidence at 10, 15 and 20 years according to main treatment received was 15%, 24% and 32% with HU vs. 5%, 10% and 21% with pipobroman ($P = 0.02$).
Conclusion: Data from this unique randomized trial comparing HU with another cytoreductive drug in PV showed that: (i) survival of patients with PV treated with conventional agents differed from the general population; (ii) evolution to AML/MDS is the first cause of death; (iii) pipobroman is leukemogenic and is unsuitable for first-line therapy; (iv) incidence of evolution to AML/MDS with HU is higher than previously reported, although consideration should be given to the natural evolution of PV.

22-P297

The tricky use of placebo in investigator-based clinical trials

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Objectives: In investigator-based clinical trials, the use of placebo is often justified as it increases the probability from the peers' expertise of 1/gaining a public grant; 2/publishing results in higher-rank journals.
Methods and results: Among the 139 randomized clinical trials (RCT) evaluating drugs and currently managed by the Paris Hospitals, 68 are placebo-controlled. Aim is to analyze the hurdles in obtaining the placebo and to check whether the placebo is really needed.
For half of the studies there were difficulties in obtaining the placebo. In rare cases, the study was simply not feasible. When the placebo concerns a new drug, the company may accept to provide the drug and its placebo, at the eventual expense for the institutional sponsor to provide all the data without any further compensation. It may be considered as a disguised industrial sponsorship, the institutional sponsor while taking the responsibility of the study, being relegated to a role of a CRO. Obtaining a placebo of an old drug is trickier since the princeps company may not sell anymore its product and companies selling generics are not able and/or interested to manufacture the placebo. The request of a manufacturer can be so expensive (up to 200 000 Euros) that is exceeds by far the price of the verum, and of the grant. Indeed, the rationale for using a placebo as comparator is to ensure a double-blind. However, when the drug administration is single or short (e.g. emergency setting), or when the endpoint is 'hard' (i.e. mortality, imaging, biology), it is unlikely that any placebo effect from subjects and/or investigators may impact the endpoint assessment. In such situations, the comparator may be 'no treatment' with whenever possible a blind assessment.
Conclusion: Placebo-controlled RCT are challenging for institutional sponsors. It happens that despite both the lack of rationale for a placebo and the hurdles for obtaining it, investigators are not willing to change the study design. Investigators and methodologists when writing a protocol and peers' expertise of a grant or a publication submission may consider more carefully the necessity and the feasibility of a placebo in a RCT.

22-P306

Is clinically relevant non-major bleeding a good surrogate endpoint for major bleeding? Validation in meta-analyses of randomized clinical trials in major orthopedic surgery, venous thromboembolic treatment and atrial fibrillation

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Background: New recent anticoagulants are assessed in terms of efficacy and safety. Due to the low incidence of major bleeding (MB), a new endpoint has been proposed to estimate the safety profile: the clinically relevant non-major bleeding (CRNMB). However the validity of this endpoint as a surrogate for MB has never been investigated.
Methods: We identified all randomized trials assessing new anticoagulant compared to the reference treatment in the prevention of venous thromboembolism (VTE) after major orthopedic surgery, in the treatment of acute VTE or in atrial fibrillation (AF) patients. Surrogacy of CRNMB for MB was assessed through the association between the treatment effects on these endpoints expressed as the logarithm of relative risks (logRR). Squared correlation coefficients (R^2) were estimated along with their 95% confidence intervals (95%CI).
Results: Overall, 37 studies were available, yielding a total of 105 667 patients. After major orthopedic surgery, 13 studies (32 416 patients) were available (three dose-ranging studies). The coefficient of correlation between treatment effects R^2 was 0.49 (95%CI 0.29–0.88). The linear regression equation was logRR (MB) = $-0.04 + 0.32 \times$ logRR (CRNMB). Nine studies (16 773 patients) in acute VTE were included (two dose-ranging studies). The coefficient R^2 was 0.13 (95%CI 0.00–0.42). The linear regression equation was logRR (MB) = -0.42 to $0.27 \times$ logRR (CRNMB). For the last indication, 10 studies were conducted in 44 360 AF patients (small small dose-ranging studies). The coefficient R^2 was 0.77 (95%CI 0.61–1.00). The linear regression equation was logRR (MB) = $-0.05 + 0.64 \times$ logRR (CRNMB). These analyses are quite similar whether treatment effects are estimated by the pharmacological class, the kind of comparator, the population of analysis or the design.
Discussion: These analyses provide only modest support for considering CRNMB an acceptable surrogate for MB neither in prevention of VTE after major orthopedic surgery, nor in the treatment of acute VTE. It could be acceptable in AF patients, but further investigations are needed with analyses by strata.
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22-P307

Central blood pressure early predicts bevacizumab induced hypertension

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Antiangiogenic therapy (AAT) is frequently associated with the occurrence of arterial hypertension, however to date no bioclinical parameters are able to predict those patients at risk for hypertension. The aim of our study was to assess if parameters evaluation of central blood pressure (CBP) and pulse wave velocity (PWV) were able to identifiy patient at risk for AAT induced-hypertension.
We performed a prospective study involving 37 patients without history of arterial hypertension and who were exposed for the first time to bevacizumab treatment. Data were collected before AAT was started and before every chemotherapy session. Blood pressure was assessed during clinic visit and at home using an OMRON device. CBP and PWV were recorded at every visit using theSphygmoCor© device. During the follow-up, diagnosis of hypertension was determined with home blood pressure self-measurement.
After a follow up of 18 weeks, 16 patients (43.2%) were diagnosed with AAT induced hypertension (HTN+). Hypertension occurred on average 7.3 ± 1.0 weeks after the first administration of the AA. At visit V0, before AAT exposition, HTN+ patients presented an increase of Aortic Systolic Pressure (AoSP: 128.0 ± 2.7 vs. 122.7 ± 2.3; $P < 0.0001$), Aortic Pulse Pressure (AoPP: 44.9 ± 3.0 vs. 33.0 ± 2.3; $P < 0.001$) and Pulse wave velocity (PWV: 9.0 ± 0.5 vs. 7.6 ± 0.4; $P < 0.05$) in comparison to patients without AAT induced hypertension. At visit V1, 3 weeks after the first AAT exposition, HTN+ patients presented an increase of AoSP (143.9 ± 2.7 vs. 114.0 ± 2.3; $P < 0.00001$), AoPP (49.0 ± 3.2 vs. 36.9 ± 4.0, $P < 0.0001$) and Aortic augmentation pressure (AoAP: 14.3 ± 1.6 vs. 8.9 ± 1.3; $P < 0.05$). For visite 1, ROC curves determined a cut-off value of 129 mmHg for AoSP able to identify patients at risk for hypertension (ROC AUC: 0.988 ± 0.011, $P < 0.0001$; specificity: 100%; sensitivity: 87.5%).
AoSP is able to early identify patients with risk for AAT induced hypertension

22-P308

Gluco- and mineralocorticoid biological effects of a 7-day treatment with low doses of hydrocortisone and fludrocortisone in septic shock: secondary analysis of a placebo-controlled, randomized, double blind trial

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Objective: The benefits of low-dose steroids in septic shock remain controversial. We investigated if these low doses were able to induce their expected hormonal effects by analyzing the biological modifications observed during the study which first demonstrated the benefit of low-dose steroids on survival.
Methods: This was a multicenter, placebo-controlled, randomized, double-blind study in which 299 septic shock patients received a 7-day treatment with a combination of hydrocortisone (50 mg intravenously four times daily) and fludrocortisone (50 µg orally once daily) or matching placebos. Gluco- and mineralocorticoid biological effects observed during the 7 days of treatment were compared between groups.
Results: Steroids significantly decreased eosinophil counts from day-2 to day-7. Steroids significantly increased plasma glucose from day-2 (compared to placebos: +0.8 mM) to day-7 (+1.8 mM) and cholesterol from day-3 (+0.54 mM) to day-7 (+0.39 mM). Steroids significantly increased plasma sodium from day-3 (+2 mM) to day-7 (+5 mM) and significantly decreased plasma potassium on day-7 (−0.2 mM). Steroids significantly decreased urinary sodium/potassium ratio from day-2 (−47%) to day-7 (−57%) and sodium fractional excretion from day-3 (−25%) to day-7 (−66%). Steroids significantly increased urine output on day-4 and 5 and osmolar clearance from day-4 to day-7, and decreased free-water clearance from day-4 to day-7, this effect being significant on day-4 and 6.
Conclusions: In septic shock, low-dose steroids induced both gluco- and mineralocorticoid biological effects and seemed to improve renal function. Most of these effects appeared after 2–3 days of treatment and lasted at least until the end of treatment.

22-P371

Population pharmacodynamic analysis of effect of hydrocortisone and fludrocortisone on phenylephrine-mean arterial pressure dose-response relationship in healthy volunteers

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Introduction: Low doses of hydrocortisone (HC) and fludrocortisone (FC) have been shown to decrease mortality in septic shock patients [1]. A single administration of HC has also been shown to enhance pressor-responses to norepinephrine in these patients [2] and to phenylephrine (PE) in healthy volunteers (HV) [3]. We recently reported the biological and hemodynamic effects of HC and FC in 12 HV with saline-induced hypoaldosteronism [4]. We herein report the results observed, during this study, on PE-mean arterial pressure (MAP) dose-response relationship.
Methods: The study was performed according to a placebo-controlled, randomized, double-blind, crossover, 2×2-factorial design. Drugs doses and routes of administration were those used in septic shock (HC: 50 mg intravenously, FC: 50 µg orally). At 1 h 30 after treatment administration, incremental doses of PE were infused (0.01–3 µg/kg/min), each dose being maintained 5 min. A nonlinear mixed-effects model with inter-occasion variability was used. A sigmoid model was chosen to describe the PE-MAP dose-response relationship: $E = E0 + [Emax.D^{\gamma}/(ED50^{\gamma}+D^{\gamma})]$ (E: effect on MAP, D: dose of PE, E0: MAP without drug, Emax:

maximum theoretical effect, ED50: dose of PE which induces Emax/2, γ: Hill coefficient). The parameters of the model were estimated by the SAEM algorithm in Monolix [5].
Results: In univariate analyses, HC was found to influence Emax and ED50 (P = 0.02 and 0.091, respectively), FC was found to influence Emax, ED50 and γ (P = 0.23, 0.00083 and 0.013, respectively), and the interaction was found significant for Emax, ED50 and γ (P = 0.024, 0.0021 and 0.092, respectively) with a threshold value of 0.25. In the final model, HC significantly decreased Emax (−17%, P = 0.013) and FC significantly increased ED50 (+28%, P = 0.0013). There was no significant interaction for any of the parameters.
Conclusion: In HV with hypoaldosteronism, both HC and FC decreased PE-MAP dose-response relationship. There was no evidence of interaction between the two drugs.
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22-P318

Arterial and endothelial function assessment among patients with resistant hypertension treated with two different pharmacological intensification strategies
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Background: We have previously shown that combined renin angiotensin system (RAS) blockade was less efficacious than anti-aldosterone based (AB) diuretic treatment for controlling BP in patients with resistant hypertension (RH). Whether this is explained by improvement in arterial properties and endothelial function (EF) is unknown.
Aim: To compare the effects of AB treatment vs. combined RAS blockade on arterial parameters and EF in pts with RH.
Method: Pts with daytime ambulatory SBP/DBP (dASBP/dADBP) ≥135/85 mmHg, despite 4 week-treatment with irbesartan 300 mg+HCTZ 12.5 mg+amlodipine 5 mg, were randomised to diuretics (spironolactone 25 mg, then furosemide 20–40 mg and amiloride 5 mg, AB group, n = 85) or RAS targeted treatments (ramipril 5–10 mg, then bisoprolol 5–10 mg, RB group, n = 82) for 12 weeks. High-resolution echotracking system (Walltrack®) was used for the determination of diameter (D), wall thickness (WT) at the site of the brachial artery (BA) and common carotid artery (CCA). Central pulse pressure (CPP) and carotid-femoral pulse wave velocity (PWV) was measured by aplanation tonometry. EF of BA was assessed by ischemia-induced flow-mediated- and GTN-induced vasodilatation. All parameters were measured at baseline and week 12 in 50 and 45 pts of the AB- and RB group, respectively.
Results: Baseline clinical characteristics did not differ between groups. dASBP decreased more in the AB (−19 ± 13 mmHg) than in the RB group (−9 ± 13 mmHg, P < 0.001). Stroke change in D and external D of CCA decreased more in the AB- than in the RB group: the difference between groups was −68 mm ([IC95%: −120; −16], P = 0.01) and −0.18 mm ([IC95%: −0.33; −0.02], P = 0.02), respectively. In contrast, WT of the CCA did not significantly differ between two groups. CPP decreased more with AB than RB (difference between groups: −11 mmHg [IC95%: −17; −4], P < 0.001). CCA stiffness and PWV decreased similarly in both groups. None of the EF parameter significantly differed between two groups.
Conclusion: In pts with RH, a strategy based on AB treatment is more efficient to decrease BP, diameter and strain of elastic arteries. However, neither stiffness nor EF was improved differentially. Longer duration of treatment might be necessary to observe differential effect of drugs on arterial stiffness and EF.

PEPCK: nutritional and pharmacological target in pathophysiology
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The cytosolic isoform of phosphenolpyruvate carboxykinase (PEPCK-C) is the key enzyme in hepatic and renal gluconeogenesis. It is expressed at high levels in four other tissues that are not gluconeogenic: adipose tissue (AT), lactating mammary gland, small intestine and colon. In these other tissues, PEPCK-C plays a glyceroneogenic role in favoring the synthesis of glycerol-3P from non-carbohydrates precursors like lactate, pyruvate or certain amino acids. To give substrates to gluconeogenesis and glyceroneogenesis, PEPCK-C allows the removal of Krebs cycle intermediates in a pathway named cataplerosis. In liver and in AT, fasting, lipid-rich or protein-rich diet, beta-agonists and retinoic acids induce the expression of this enzyme through the activation of its gene (*pck1*) whereas glucocorticoids stimulate *pck1* in the liver but inhibit PEPCK-C expression in AT. The strategic position of this enzyme in glucose and lipid metabolism, its tissue-selective expression and its cell- and tissue-specific differential regulation of expression, all point to PEPCK-C and its gene as major targets in several pathophysiological situations. Selectively in AT, hypolipidemic thiazolidinediones induce *pck1* expression. The resulting increase in glyceroneogenesis and fatty acid (FA) reesterification reduces FA output and provokes an improvement of the insulin-resistant state in diabetic animals. In AT and in cells of the colon, polyunsaturated FAs and more particularly docosahexaenoic acid (DHA) stimulate pck1 expression. DHA effect on PEPCK-C expression in AT seems to be one of the mechanisms through which DHA exerts a beneficial action on metabolic syndrome. In addition, DHA inhibits the proliferation of tumor cells from the colon and induces *pck1*. These data combined with the observation that *pck1* expression is drastically reduced in human tumors of the colon point to this gene as a new negative marker for colonic tumors and a target for the anti-tumorigenic action of omega-3 polyunsaturated FAs.

Peripheral effects of the endocannabinoid system and obesity
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It is well established that activation of central endocannabinoid system (ECS) through cannabinoid receptors 1 (CB1R) promotes food intake and weight gain. Accordingly, pharmacological strategies have been developed to antagonize CB1R. Rimonabant (SR141716) was the first CB1R antagonist to be marketed and prescribed as an anti-obesity agent and its efficiency for weight reduction was supported by a series of major reports. CB1R antagonism has also been shown to improve several pathological features associated with obesity including hyperinsulinemia, insulin resistance, hyperglycemia and dyslipidemia in obese rodents and humans. Nevertheless Rimonabant has been withdrawn from the market after compelling evidence indicates that it is associated with severe adverse psychiatric events related to the blockade of central CB1R.
However, even if the reduction in food intake may be the main initial cause of the beneficial effects of Rimonabant, several data collected from animal and human studies indicated that peripheral CB1R may also directly control lipid metabolism. In this context, we showed that treating obese mice with Rimonabant normalizes lipid parameters and liver steatosis improving both liver and visceral adipose tissue metabolism. The role of peripheral ECS was suggested by the reduced expression of CB1R in these tissues and further demonstrated using tissue explants. Indeed SR141716 stimulated liver ß-oxidation activity and the involvement of CB1R in the regulation of this pathway was particularly emphasized when ECS was hyperactivated in the presence of anandamide and in ob/ob tissue. SR141716 also improved carbohydrate and lipid metabolism blunting the anandamide-induced increase in gene expression of proteins related to lipogenesis. Consistent with other studies performed with peripherally restricted CB1R antagonists, our results support the evidence that CB1R blockade improves energy expenditure, lipid and carbohydrate metabolism independently of central effects affecting food intake. In conclusion, the development of low brain penetrance CB1R antagonists should be considered as a promising strategy for the treatment of obesity and its metabolic complications.

24-O043

Stim1 is essential for the regulation of orai1/3 calcium channels, and fat preference in mice
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Introduction: In addition to the basic taste modalities, sweet, sour, bitter, salty and *umami*, recent convincing evidences raise the possibility for an additional sixth taste modality devoted to the perception of lipids. A number of studies have suggested that lingual CD36, a glycoprotein, mainly expressed by circumvallate papillae (CVP) on the tongue, might be implicated in oro-gustatory of dietary lipids in rodents.
Aim of the study: We highlight the cell activation mechanisms, responsible for the downstream cell signaling which might help understand the lipid-mediated regulation of feeding behavior, critically involved in the development of several diseases like obesity and other metabolic disorders.
Materials and methods: CD36-positive cells were isolated from C57BL/6J mice according to the published procedure [*JBC*, 2008; 283:12949–59]. The presence of PLA_2 isoforms was detected by confocal microscopy; and the enzymes activity was determined as protocol furnished with *Cayman* kit. The PLA_2 activation was measured by the release of [^{3}H]-arachidonic acid. The presence of Orai1/3 and Stim1 mRNA was sought by Real Time- PCR in CD36-positive, cells calcium signaling was measured with a designed Calcium free/calcium reintroduction (CFCR) protocol. Experiments on the spontaneous preference for lipid-enriched solutions were investigated in wild type or $Stim1^{-/-}$ mice by means of the two-bottle preference test [*FASEB J.* 2008; 22:1458–68].
Results/discussion: We demonstrate that linoleic acid (LA), a long-chain fatty acid (LCFA), by activating different isoforms of $sPLA_2$, $cPLA_2$ and $iPLA_2$ via CD36, produced free arachidonic acid (AA) and lyso-phosphatidylcholine (Lyso-PC) which triggered calcium influx in CD36-positive cells. The LCFA induced the production of calcium-influx factor (CIF). CIF, AA and Lyso-PC exerted different actions on the opening of store operated calcium (SOC) channels, constituted of Orai proteins and regulated by Stim1, a sensor of calcium depletion in the endoplasmic reticulum. Wild-type mice exhibited a stronger preference than $Stim1^{-/-}$ animals for an oil-enriched solution, suggesting that Stim1 plays a key role in the oral detection of lipids.

24-O044

Insulin control of lipolysis in subcutaneous adipose tissue during diet-induced weight loss in obese women
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Low calorie diets have been shown to improve liver and skeletal muscle insulin sensitivity but it is still unclear whether adipose tissue insulin sensitivity is affected by weight loss. The aim of this study was to investigate insulin-mediated antilipolytic effect in subcutaneous adipose tissue (SCAT) of obese subjects during different phases of a dietary weight loss program.
Eight obese women underwent a 3-months dietary intervention consisting of 1 month of very low calorie diet (VLCD), followed by 2 months of low calorie diet (LCD). At each phase of the dietary intervention, microdialysis of SCAT was performed at rest and during a 3-h hyperinsulinemic euglycemic clamp. Lipolysis, measured by the responses of dialysate glycerol concentration, was

determined at baseline and during local infusion of epinephrine combined with phentolamine (beta-adrenergic stimulation), before and during the last 30 min of the clamp.
The dietary intervention induced a significant weight and fat mass loss (−10% and −26% respectively, $P < 0.05$), and improved whole-body insulin sensitivity measured by clamp (Glucose disposal rate: +46%, $P < 0.05$). Basal lipolysis decreased by 2-fold after weight loss. During the clamp, insulin inhibited basal lipolysis similarly before and after weight loss by 40% ($P < 0.05$). Interestingly, insulin suppression of epinephrine-induced lipolysis was similar between pre-diet condition, VLCD and LCD.
Together, these data highlight a dissociation between weight-loss induced improvement in whole-body insulin sensitivity and SCAT sensitivity to the antilipolytic effect of insulin in obese individuals.

24-O045

Treatment by GLP-1 agonist modulates hedonic response to food and taste sensitivity in type 2 diabetes

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Context: Besides their potential action in the treatment of type 2 diabetes mellitus (T2DM), GLP-1 analogues have been shown to decrease satiety and food intake. However, little is known about their effects on food hedonic and taste perceptions.
Objective: The objective of the study was to investigate the impact of GLP-1 analogue Liraglutide on the liking and wanting component of the food reward system as well as on taste sensitivity in T2DM patients.
Research design and methods: Thirty T2DM patients were studied before and after 3 months of daily Liraglutide treatment (1.2 mg). In each trial, blood samples were collected and body mass composition was analyzed by dual-energy X-ray absorptiometry. Liking, recalled liking and wanting for lipids, proteins and carbohydrates were assessed and detection thresholds for salt, sweet and bitter tastes were measured.
Results: After 3 months of daily treatment with Liraglutide, T2DM patients had a significant decrease in hunger sensation ($P < 0.01$), food intake ($P < 0.01$) and weight ($P < 0.01$) but not in the pleasure in eating. A reduction in plasma leptin ($P < 0.01$) and ghrelin ($P < 0.01$) levels was also observed. Liking ($P = 0.04$), recalled liking ($P = 0.05$) and wanting ($P < 0.01$) for fatty foods were lowered by the treatment. Sensitivity to sweet taste was improved ($P = 0.04$) whereas it remained unchanged for salty and bitter tastes.
Conclusions: This is the first study to show a decrease in hedonic response for fatty foods and an increase in sweet taste sensitivity induced by GLP-1 analogues. These results are of interest in the understanding of weight loss mechanisms and cardiovascular risk improvement induced by GLP-1 analogues.

24-O046

Effect of apple vinegar cider in the expression of aromatase enzyme and estrogen receptors in gonadal tissues of male rats

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Objectif: Diabetes mellitus (DM) is associated with increased risk of reproductive problems. Aromatase catalyzes the conversion of androgens to estrogens and is expressed in a variety of tissues. Estrogens effects are mediated by classical receptors, ESR1 and ESR2. The benefic effects of vinegar have been known for more a century and have been demonstrated in animal as well as human studies. The purpose of the present study was to investigate the effects of apple cider vinegar on the expression of testicular aromatase and estrogen receptors (ER) in diabetic rats.
Materiel et methodes: Male albino rats were used for the present investigations. The animals were fasted overnight and diabetes mellitus (DM) was induced by a single intraperitoneal injection of freshly prepared streptozotocin (STZ) (65 mg/kg) in citrate buffer. Vinegar cider was orally administrated to the diabetic rats. Control rats were injected with citrate buffer and the treatment was continued for 4 weeks.
Resultats: Our results demontrated a decrease of the aromatase and of ER (1 and 2) gene expression of diabetic rats and a return to near control values of the vinegar diabetic group.
Discussion: These results indicate that apple vinegar cider is beneficial for spermatogenesis and likely improves the protection of the cells via an increase of aromatase/ER gene expression.

24-P047

Defect of intestinal pressure-induced vasodilation in diabetic rats: New insights into gastrointestinal dysfunction

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Background and aims: The intestinal wall is subjected to strains and changes in blood flow during digestion. In case of diabetes gastrointestinal dysfunction may occur in presence of neuropathy with impaired motility and nutrient absorption. We hypothesized a link between pressure and vasodilation on the intestine similar to the one we discovered in the skin and that a defect of this link could be largely involved in gastrointestinal dysfunction during diabetes. The objective of the study was to assess the pressure-induced vasodilation (PIV) on the intestine during diabetes.
Methods: Intestinal microvascular properties were assessed in control, 4- and 8-week diabetic rats (STZ4 and STZ8) using laser Doppler flowmetry to test PIV, endothelium-dependent and independent vasodilation to acetylcholine (ACh) and to sodium nitroprusside (SNP), respectively.
Results: Intestinal PIV exists in healthy rats. Four-week diabetic rats had no changes in intestinal PIV, ACh and SNP responses compared to control rats. In contrast 8-week diabetic rats exhibited a reduction of 80% of PIV and 60% of ACh responses compared to control rats. PGP 9.5 pan-neuronal marker was reduced in the mucosal layer in STZ4 diabetic rats and further decreased in STZ8 diabetic rats. However, CGRP-positive axons were only clearly decreased in STZ8 diabetic rats associated with an increase in neutral endopeptidase that is responsible for CGRP degradation.
Conclusions: Intestinal impairment occurred during late-diabetes with a defect in PIV along the intestine due to the reduction in intestinal CGRP neuropeptide. We speculate that this defect could be detrimental for both digestion and products absorption leading to gastrointestinal dysfunction.

24-P048

Hunger sensation, liking, wanting and food consumption in patients with renal failure under haemodialysis

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In uremic-patients taste and smell alterations play an important role in malnutrition. Indeed, it has been observed that a decrease in taste sensitivity and odour perception are predictive factors announcing subsequent alterations of the nutritional state. A change in food preferences has also been observed in uremic-patients. However, 'olfactory liking' for foods, 'wanting' (desire to eat a particular food) and sensory specific satiety (SSS) have never been studied in these patients. The present study aimed to access these parameters.
Sixty subjects were divided into three groups: patients with renal failure under haemodialysis (age: 61 ± 13 years; IMC: 26 ± 7 kg/m^2); patients with renal failure without dialysis (age: 65 ± 12 years; IMC: 25 ± 4 kg/m^2); healthy subjects (age: 64 ± 7 years; IMC: 24 ± 3 kg/m^2). Patients with haemodialysis participated in two experimental sessions: immediately after their dialysis; 24 h before or after the dialysis (random order). The other subjects had only one experimental session. In each experimental session (performed at 11:30 a.m.), hunger (H), 'olfactory liking' (OL) for 10 food items, 'wanting' (W) for 16 food items and pleasantness (P) for jam on buttered toasts were evaluated. The same parameters were reassessed immediately after the ad libitum intake of these toasts.
Results showed no difference in H and P before collation. H after collation was higher in uremic patients (under and without haemodialysis) compared to healthy subjects ($P < 0.05$). After intake, P for the toasts was lower in uremic-patients under haemodialysis the day of the dialysis ($P < 0.05$) and in healthy subjects ($P < 0.05$) than in patients under haemodialysis the day of no dialysis and in uremic patients without dialysis. SSS was abolished in uremic patients under haemodialysis the day of no dialysis and for patients without dialysis. Preliminary results also indicate a higher W for protein-rich foods in uremic patients after their dialysis ($P < 0.05$) than in healthy subjects.
In conclusion, the feeling of hunger is altered in uremic-patients but satiation mechanisms are restored after haemodialysis. Furthermore, in order to improve the nutritional state of the patients under haemodialysis, results suggest supplying protein intake immediately after their dialysis.

24-P049

Effect of green tea decoction on intestine glucose uptake, Na+-dependent glucose transporter (SGLT-1) and carbachol

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Objective: The effect of green tea decoction (GTD) cooked at 20 min in water on intestinal glucose, Na^+ uptake by its specific transporter SGLT-1 and carbachol is unknown. The aim of this study was to examine the effect of GTD on glucose transporter-1 (SGLT-1) and carbachol system in rat proximal jejunum. The SGLT-1 activity was evaluated by a glucose-induced short-circuit current (Isc) by Ussing chambers.
Materials: Our study was performed on 16 male Wistar rats. After an adjustment period of 1 week, rats were weighed and randomly assigned into four groups with comparable body weights. During the experimental period (6 weeks), the rats were given a normal diet (ND) or high fat diet (HFD) with or without GTD. The group 1 received a ND + distilled water (ND), group 2 received the ND + green tea decoction (NDGTD) obtained from 50 g/L, the group 3 received HFD + distilled water (HFD) and group 4 received HFD+ green tea decoction (HFDGTD). Foods and tea consumption during the experimental period were daily measured. At the end of the experience, animals were anesthetized and the proximal jejunum was dissected out and rinsed in cold saline solution. Four adjacent proximal jejunum samples were mounted in Ussing chambers. Electrogenic ion transport was monitored continuously as short-circuit current (Isc) using the Easy Mount System apparatus. Results were expressed as the difference (Δ Isc) between the peak Isc after glucose challenge (maximum measured after approximately 3 min) and the basal Isc (measured just before the addition of glucose).
Results: The initial body weights did not differ among groups, however, at the end of the experimental period, rats consumed tea had lower weight gains. The glucose uptake, Na^+ conductance and the carbachol were significantly decreased in NDGTD and HFDGTD groups than those of the control groups ($P < 0.05$).
Conclusion: This new research showed that GTD could inhibit the intestinal border brush uptake of glucose by reducing the sodium-dependent glucose transporter SGLT-1 activity.

24-P050
Effect of chickpea proteins combined with sardine oil or a mixture of vegetable oils on oxidative stress markers in rats fed a cholesterol-enriched diet

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Objective: To investigate the effects of chickpea proteins combined with sardine oil or a mixture vegetable oils on some markers of oxidative stress in rats fed a cholesterol-enriched diet.

Materials and methods: Male Wistar rats initially weighing approximately 200 g were fed a 20% chickpea proteins diet combined with of a mixture of vegetable oils (olive 3.9% + nut 1% + sunflower 0.1%) (CPv) or 5% sardine oil (CPs) and 1% cholesterol for 28 days. Serum total cholesterol (TC), triacylglycerols (TG), albumin and uric acid (UA) were determined by colorimetric methods. Serum, lipoproteins and tissues lipid peroxidation was measured by thiobarbituric acid reactive substances (TBARS) analysis (Quintanilha *et al.*, 1982). Oxidized proteins were estimated by carbonyls content according to the method of Levine *et al.* (1990).

Results: CT in serum was 2.6-fold higher in CPs vs. CPv group. The lipid source induced similar effect on serum TG, UA and albumin. TBARS were 1.2-, 10-fold higher in CPv vs. CPs in serum and VLDL fraction, respectively. Sardine oil combined with chickpea proteins increase TBARS in LDL-HDL_1 (twofold). In contrast, TBARS in HDL_2 and HDL_3 were not influenced by the oil origin. In tissues, TBARS were higher in muscle (1.6-fold) and aorta (6.7-fold) in CPv vs. CPs group but lower in heart (1.5-fold). The carbonyls in serum were 1.5-fold higher in CPv vs. CPs group. Chickpea proteins combined with vegetable oils increase the levels of carbonyls in liver (2.8-fold), muscle (1.5-fold) and aorta (30-fold) otherwise, sardine oil increases at the heart (1.6-fold).

Discussion: Sardine oil combined with chickpea proteins provoked higher level of total cholesterol than vegetable oils diet but seems to protect the serum and some tissues against the cytotoxic action and oxidative stress of cholesterol supplementation. In conclusion, the effect of dietary chickpea proteins may be modulated by the nature of lipids present in the diet.

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24-P051
Calcium influx factor (CIF) plays a crucial role in linoleic acid induced calcium signaling in mouse CD36+ lipid gustatory cells

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Introduction: Recently, we described that linoleic acid, a long chain fatty acid (LCFA), induced increases in free intracellular calcium concentrations, $[Ca^{2+}]i$, in CD36+ cells isolated from mouse lingual circumvallate papillae. This increase in $[Ca^{2+}]i$ was contributed by an initial increase from endoplasmic reticulum (ER), followed by calcium influx *via* opening of store operated calcium (SOC) channels. The molecular mechanism of the store-operated Ca^{2+}-entry (SOC) pathway remains one of the most intriguing and long lasting mysteries of Ca^{2+} signaling. The elusive calcium influx factor (CIF) that is produced upon depletion of Ca^{2+} stores has attracted growing attention, triggered by new discoveries that filled the gap in the chain of reactions leading to activation of store-operated channels and Ca^{+} entry. Here we undertook the present study to elucidate role of CIF in linoleic acid induced calcium signaling in mouse CD36+ lipid gustatory cells.

Methods: We used the probe Fura-2/AM to monitor intracellular calcium signaling by single cell and patch-clamp studies.

Results: Our data report that LCFA evoked the release of calcium-influx factor (CIF). CIF extracted from CD36+ cells isolated from mouse lingual circumvallate papillae and stimulated by linoleic acid activated Ca^{2+} influx not only in these cells but also in Jurkat T-cell and HEK cells. Ca^{2+}-independent phospholipase A2 emerged as a target of CIF, and a major determinant of the SOCE mechanism. The $iPLA_2$ was responsible for the production of lyso-phosphatidylcholine (Lyso-PC). Indeed, CIF and Lyso-PC did not exert additive responses on calcium influx, indicating that both the agents open the same calcium channels.

Conclusion: These data show that CIF produced by CD36+ cells isolated from mouse lingual circumvallate papillae can be involved in the perception of the taste of fat.

24-P052
Effect of caloric deficit on inflammatory mediators and on anaerobic exercise

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In order to test the hypothesis that dietary restriction may have a negative influence on physiological and immunilogical adaptation . We examined the effects of weight loss induced by restricting energy and fluid intake on the physical performance and inflammatory mediators of football players.

Twelve football players (mean ± SD: age 21.2 ± 1.2 years, height 1.7 ± 0.1 m, body mass 73.5 ± 1.7 kg) restricted their caloric intake for 7 days, to determine the acute effect of caloric deficiency on aerobic and anaerobic exercise performance as well Interleukin-6 and TNF-? levels. The subjects were tested while on a normal caloric diet (NC) and at the end of the dietary restriction (CR). The responses to the Wingate Anaerobic Test, peak power (P[peak]), mean power (P[mean]), fatigue index (FI; % of decrease in power output throughout the 30 s) were significantly higher ($P < 0.05$) in the NC than CR. Plasma concentrations on IL-6 were measured before, immediately after exercise and 60 min after the exercise The caloric restriction, regardless of dietary composition, increased the plasma concentration of IL-6 and TNF-? response more for the NC than the CR ($P < 0.05$). These results indicate that caloric restriction alter anaerobic performance. Furthermore, the caloric restriction appears to have an effect on inflamatory mediators.

24-P053
The activity of honeys produced in Algeria to some pathogenic bacteria responsible for gastrointestinal infections

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The infections gastrointestinal are the most known worldwide. Microbial resistance to antibiotics is on the rise, because of inappropriate use of antibiotics in human medicine. Honey is an ancient remedy for the treatment of infected wounds, which has recently been rediscovered by the medical profession, particularly where conventional modern therapeutic agents are failing. The present study was carried to determine the antibacterial activity of honey produced in Algeria on several pathogenic bacteria (*Staphylococcus aureus*, *Bacillus cereus*, *Helicobacter pylori*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus sp.* and *Citrobacter freundii*). The first part was to find out whether there are correlations between the antibacterial activity of honeys and their physicochemical parameters (conductivity, pH, HMF and content of water). The second part deals with the evaluation of the antibacterial activity of different varieties of honey (1). Our results showed that the samples of honey have different physicochemical properties and all pathogenic bacteria have been affected by the different honeys examined, the degree of inhibition varied with honey and the bacterial strain tested, with a zone of inhibition up to 28.0 ± 0.12 mm. The antibacterial effect of honey depends on several factors: water content, acid pH, H_2O_2 and phenolic compounds (2, 3). These findings suggest that honey could be used to protect humans against the bacteria that cause gastrointestinal diseases.

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24-P054
Metabolic syndrome: alarm bell in Algeria

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Objectives: Put the light on a pathology that has spread alarmingly throughout the world including Algeria and that assessing the nutritional, metabolic and physical links;Highlight the importance of an adapted and varied diet combined with regular physical activity and a healthy lifestyle in the management of MS;

Patients and methods: A cross-sectional survey was conducted in the western Algerian region (city of Sidi Bel Abbes) between (02/2009–03/2010) on a sample of 204 patients (sex ratio = 1). Our population was recruited at the Endocrinology Service. Metabolic Syndrome was assessed according the criteria of the (NCEP ATP III, 2002).

Results and discussion: The results show that the frequency of the MS is 86.27% whose predominance women which (92.15% women vs. 80.39% men). The age group most affected by the MS among women is (54–66), while among men (67–79) years. The main risk factors found are; diabetes type 2 and/or hyperglycemia with 89.77% which (81.91% women vs. 98.78% men); abdominal obesity which 78.97% (95.74% women vs. 59.75% men); hypertension with 87.50% which (82.97% women vs. 92.68% men) and dyslipidemia with 72.15% (69.14% women vs. 75.60% men). Moreover, the whole is underlined by a low physical activity with only 21.56% who practice it regularly which (15.95% women vs. 28.04% men). The estimate of food intake shows that: the daily energy intake is higher than the recommendations of the Mediterranean diet at women with MS; the food intake is characterized by a qualitative imbalance: proteins intake is characterized principally by a vegetables proteins which (83.72% women vs. 72.85% men) that exceed the recommended intake by the MD (60%);lipids characterized by an important contribution in saturated fatty acids (37.24% women vs. 36.10% men) compared to MD (25%) and a low intake of mono-unsaturated fatty acids which (39.44% women vs. 40.24% men) compared to MD (50%);lower concentration in calcium and magnesium; lower concentration en fibers; very important concentration of sodium and a very insufficient contribution of water. Concerning the biological results, the desorders infected the function of the glycoregulation and the lipidic statue.

25-O047
AMPK activation stimultes autophagy and ameliorates muscular dystrophy in MDX mice

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Duchenne muscular dystrophy (DMD) is characterized by myofiber death from apoptosis or necrosis, leading to fatal respiratory muscle weakness. Among other pathological features, DMD muscles show severely deranged metabolic gene regulation and mitochondrial dysfunction. Defective mitochondria are not only energetically deficient but also play a role in cell death via opening of the mitochondrial permeability transition pore (PTP). Autophagy is a bulk degradative mechanism which favors energy production and the elimination of defective mitochondria. Here we hypothesized that pharmacological activation of AMP-activated protein kinase (AMPK), a metabolic sensor and on-switch for the autophagy pathway, would be beneficial in the mdx mouse model of DMD. Treatment of mdx mice for 4 weeks with an AMPK agonist, AICAR (5-aminoimidazole-4-carboxamide-1-b-D-ribonucleoside), potently triggered autophagy in the diaphragm without inducing muscle fiber atrophy. Indices of mitochondrial PTP function were significantly improved, along with dystrophic diaphragm histopathology and force-generating capacity.

25-O048
Contribution to the study of central mechanisms involved in the respiratory depression induced by hypoxia in newborn rodent

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Acute hypoxia elicits in newborn a biphasic respiratory response: a transient hyperventilation followed by a severe decrease. Ponto-medullary O_2-activated areas are known to contribute to respiratory depression induced by hypoxia, although precise involvement of cell populations remains to be determined. Having a thorough knowledge of these populations is of relevance because perturbations in the respiratory response to hypoxia may participate in respiratory diseases in newborns. In such a context, we aimed to analyze the hypoxic respiratory response and the ponto-medullary change in cell activity in newborn rat and mice (wild type and *kreisler* mutants).

Hypoxic respiratory response was appreciated in vivo by plethysmography and ex vivo on central nervous system preparations. In both the cases, analysis of respiratory parameters was completed by the study of *c-fos* expression. Additionally, ex vivo experiments were made under reduced synaptic transmission in order to identify intrinsically hypoxia-activated cells and their Phox2b immunoreactivity.

In newborn mice in vivo, hypoxia resulted in an early increase in ventilation followed by a secondary decline. This response was accompanied by an increase in *c-fos* expression in several ponto-medullary areas involved in the respiratory control.

In ex vivo preparations, after an increase in respiratory frequency (fR) encountered in mice but not in rat, the main response to low O_2 level was a decrease in fR. Only the ventral medullary surface (VMS) showed an increase in *c-fos* expression that persisted under reduced synaptic conditions. Less than 40% of intrinsically hypoxia-activated cells of this area, corresponding to the retrotrapezoid nucleus and parafacial respiratory group, were also Phox2b-positive. Absence of these hypoxia-activated cells in *kreisler* mice or their destruction in wild type rodents reinforced the decrease in fR.

Present data highlighted ponto-medullary changes in cell activity induced by low O_2-level in newborn rodents. One of the main results was that VMS contains intrinsically hypoxia-activated cells that participate in central mechanisms causing the respiratory depression. Some pathological dysfunctions of these cells could participate in respiratory disorders by reducing the ability to conserve O_2 stores by depressing the respiratory muscle activity during hypoxia, a major component of the newborn's strategy for surviving hypoxia.

25-O049
Nicotinic receptors regulate production of M1 cytokines by human lung macrophages

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Introduction: Numerous studies have provided evidences for the role of the 'cholinergic anti-inflammatory pathway' in the regulation of inflammation, and it has been shown in monocyte-derived macrophages that acetylcholine was able to attenuate the production of pro-inflammatory M1 cytokines whereas the markers of the regulatory M2 subset were not affected (Borovikova *et al*, 2000).

Aim: The current study was performed to investigate the expression and the function of cholinergic receptors on the production of M1- and M2-type cytokines by human lung macrophages (LM).

Methods: LM were isolated from lungs of patients undergoing surgery for carcinoma, challenged with 10 ng/mL LPS (to obtain classically activated M1 LM) or 50 ng/mL IL-13 (to obtain alternatively activated M2 LM), and exposed to cholinergic agonists or antagonists for 24 h. Transcript expression of cholinergic receptors and cytokines was assessed with RT-qPCR. M1-type (TNF-α, CCL2, CCL3, CXCL8) and M2-type (CCL18, CCL22) cytokines were quantified in supernatants with ELISA.

Results: Expression of nicotinic α7 and muscarinic M2 and M3 receptors was found in LM. The stimulation of these receptors with cholinergic agonists did not alter the production of M1- or M2-type cytokines, either unstimulated or after challenge with LPS or IL-13. However, when the LM were cultured in basal conditions and in the presence of the α7-nicotinic receptor antagonist α-bungarotoxin, the expression of M1-type cytokines was increased, whereas M2-type cytokines remain unaffected. Transcript expression increased by 157-fold for IL-6, 48-fold for CCL4 and CCL20, 24-fold for CXCL8, 15-fold for CCL3 and fivefold for TNF-α. At the protein level, TNF-α production was increased by about 46-fold, and CXCL8, CCL2 and CCL3 were increased by 2.5, 4 and eightfold respectively. In the same conditions, the muscarinic antagonists tiotropium and 4-DAMP were devoid of effect.

Conclusion: The blockade of α7-nicotinic receptors induces an increase in the production of M1-type cytokines by human LM without affecting M2-type cytokines. Our results, in line with previously described anti-inflammatory effects of cholinergic agonists (acetylcholine or nicotine), suggest that endogenously produced acetylcholine exerts a negative regulatory loop on the production of M1 cytokines by unstimulated lung macrophages which is unmasked by the α7-receptor antagonist.

25-O050
Roflumilast regulates the release of M1- but not M2-related cytokines by human lung macrophages

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Introduction: Lung macrophages (LM) play a critical role in polarized innate and adaptive responses, and in the pathophysiology of chronic obstructive pulmonary disease (COPD). When exposed to specific microenvironments, macrophages acquire either M1 or M2 polarization states that refer to the two extremes of a spectrum of possible forms of macrophage activation. M1-related cytokines are involved in the inflammatory reaction mainly to pathogens and M2-related cytokines in tissue remodeling and immunoregulation.

Aims: To characterize the patterns of M1- and M2- related cytokines induced by lipopolysaccharide (LPS) and IL-13, respectively, and to evaluate whether Roflumilast (R), a PDE4 inhibitor approved for severe COPD, affects the production of these cytokines from human LM.

Methods: LM were isolated from resected lungs (from eight patients undergoing surgery for carcinoma) and stimulated with LPS from Escherichia coli (10 ng/mL) or IL-13 (50 ng/mL) in the absence or presence of R (provided by Nycomed) at 10^{-9} and 10^{-7} M. Transcript expression of 91 inflammatory/immune-related genes was determined by RT-qPCR (TaqMan®) after a 6 h LPS or IL-13 exposure. After 24 h, M1-related cytokines (TNF-α, CCL2, CCL4, CXCL1, CXCL8, and CXCL10) and M2-related cytokines (CCL13, CCL17 and CCL22) were determined in supernatants by ELISA.

Results: LPS caused an increase in mRNA expression of 24 cytokines/chemokines that encompasses TNF-α, CCL4 and CXCL10 by 16-fold, CCL2 and CXCL8 by fourfold and CXCL1 by 64-fold. IL-13 induced the expression of four chemokines (not induced by LPS): CCL13, CCL22 and CCL26 by fourfold and CCL17 by eightfold. R at 10^{-9}M (plasmatic level after repeated dosing with the recommended dose of 500 μg) inhibited CCL4 by about 20%, TNF-α by about 30% and CCL2 and CXCL10 by about 40%. At 10^{-7}M R, the inhibitions reached maximal values of 30% for CCL4, 40% for TNF-α and 50–60% for CXCL10 and CCL2. However, R did not alter the LPS-induced production of M1 chemokines involved in neutrophil recruitment (CXCL1 and CXCL8). M2 cytokines induced by IL-13 were not affected by R.

Conclusion: At therapeutic relevant concentrations, R exerts anti-inflammatory effects on LPS-induced M1 response on human LM whereas IL13-induced M2 cytokines were not altered.

25-P092
Hypoxia-induced central apneas and periodic breathing in humans do not require a triggering fall in PaCO2

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Introduction: Hypoxia can induce cluster-type periodic breathing (PB) and/or central apneas (CA) during sleep in healthy subjects. The precise mechanisms of this phenomenon remain unclear. A fall of PaCO2 is claimed to be necessary for some types of PB (e.g. in congestive heart failure). This study reports preliminary data on the role of the level of CO_2 in hypoxia-induced PB in healthy humans. It is part of a larger trial aiming at evaluating the effect of baclofen on hypoxia-induced PB.

Subjects and methods: Forty healthy men performed a diurnal nap following 24H of sleep deprivation, into a hypoxic tent (FiO2 = 12.5–13.5%), with a slightly hypercapnic atmosphere (approximately 7 mmHg).

CA was defined as expiration longer than the mean expiration duration + 3 SD. PB was defined as at least three clusters of breaths separated by a CA. The apneic threshold (AT) was defined as the end-tidal partial pressure of CO2 ($P_{ET}CO_2$) value of the last exhalation that immediately preceded the first apnea of PB/CA. For each subject, we calculated the mean difference between the apneic threshold and the average of the 20 preceding values of $PETCO_2$ ($DeltaP_{ET}CO_2$).

Results: PB/CA occurred in 16 subjects over 40 (40%) but, for technical reasons, the $P_{ET}CO_2$ of only 15 of them could be analyzed. The value of the AT was higher than the mean of the 20 preceding values of $P_{ET}CO_2$ in 138 episodes of PB/CA among a total of 180 episodes observed in 15 subjects (77%). $DeltaP_{ET}CO_2$ was positive in 13 among 15 subjects and it was, on average, significantly different from zero ($P = 0.0012$). The mean AT value was 42.18 mmHg whereas the mean of the 20 preceding PetCO2 values was 40.80 mmHg.

Discussion: hypoxia-induced periodic breathing/central apnea does not dependent critically on a decrease in $P_{ET}CO_2$. Therefore, other possible mechanisms, like a reconfiguration of the neural oscillators, possibly toward an ancestral pattern, should be envisioned.

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25-P093

Rapid onset of diaphragm dysfunction in a mice model of mechanical ventilation

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The use of controlled mechanical ventilation (MV) is associated with impaired diaphragmatic contractility in both animal models and Humans, a condition termed Ventilator-Induced Diaphragmatic Dysfunction (VIDD). In order to gain a better understanding of the molecular mechanisms underlying VIDD, one would like to take full advantage of various transgenic and gene knockout models which only exist in the mouse. However, no mouse model for VIDD exists. Therefore, The primary aim of this study was to ascertain the feasibility of developing a murine model of VIDD. In addition, we wished to determine whether atrophy, sarcolemmal injury and the main calpain proteolysis systems are activated under these conditions.

Methods: Healthy adult male C57/BL6 mice were assigned to three groups: (i) MV with endexpiratory positive pressure of 2–4 cm H2O for 6 h ($n = 6$); (ii) Spontaneous breathing with continuous positive airway pressure (CPAP) of 2–4 cm H2O for 6 h ($n = 6$) and (iii) Controls with no specific intervention ($n = 6$). Isometric contractile properties of the diaphragm and extensor digitorum longus (limb muscle) were evaluated. Histology and immunoblotting for total and cleaved (activated) forms of calpain 1 and 2 and caspase-3 were also performed.

Results: Diaphragmatic force production at all frequencies of stimulation and during fatiguing contractions declined in the MV group (maximal force decreased by approximately 40%) compared to the control and CPAP groups. However, no differences in diaphragmatic function were found between the control and CPAP groups. No histological difference was found between groups. In opposition with the calpain proteases, caspase-3 was activated in the MV group. The amounts of cleaved calpain- 1 and calpain-2 in the diaphragm were similar among the three experimental groups.

Conclusion: Controlled MV for as brief a period as 6 h in the mouse is associated with significant diaphragmatic but not limb muscle weakness without atrophy or sarcolemmal injury but activates proteolysis. The existence of a murine model of VIDD provides new opportunities to design mechanistic studies using genetically modified mice, which should greatly advance our understanding of the pathophysiology of VIDD and aid in developing specific therapies.

25-P094

The lung oscillator is sufficient for ventilatory complexity in the tadpole isolated brainstem

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Introduction: Human ventilatory flow at rest follows a nonlinear complex trajectory with mathematical chaos-like features. Determining the source of this behaviour may help understanding its physiological meaning. The neural respiratory output of brainstems, isolated in vitro from post-metamorphic tadpoles, shares the same properties. The latter experimental model displays two ventilatory motor patterns: the buccal and the lung rhythms. They depend at least on two distinct but interacting oscillators, possibly homologous to the respiratory oscillators in mammals. Previous data suggested that the lung oscillator was necessary to the complex dynamics. Our goal was to assess whether it was also sufficient.

Methods: The neural respiratory output of isolated brainstems of five post-metamorphic tadpoles (Rana – Pelophylax – esculenta) was recorded while superfused with artificial cerebrospinal fluid in control condition and with reduced chloride concentration. We analyzed the whole trajectory of the Root Mean Square (RMS, moving window of 100 ms) of the raw signal. Nonlinear complex dynamics was ascertain with the noise titration technique and quantified with the noise limit (NL) value. The sensitivity to the initial conditions was assessed through the largest Lyapunov exponent (LLE).

Results: Reduced concentration of chloride into the artificial cerebrospinal fluid significantly decreased the buccal frequency (Paired t test with Benjamini-Hochberg correction, $P = 0.011$) without changing the pulmonary frequency ($P = 0.24$). The NL value ($P = 0.011$) and the LLE ($P = 0.027$) increased significantly.

Conclusion: Chloride concentration reduction did not affect the lung activity and reduced the buccal one. Chaos like complexity persisted and increased when the buccal rhythm was lessened. Thus, the lung oscillator is sufficient to the nonlinear complexity of the ventilatory dynamics. The buccal oscillator seems to reduce its intensity. The probable homology between the lung oscillator and the pre-Bötzinger complex suggests that the latter may also be a sufficient source of chaos-like ventilatory complexity in mammals.

25-P095

Effect of moderate PEEP on regional ventilation distribution during severe bronchoconstriction in rabbit studied by K-edge subtraction synchrotron imaging

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Introduction: In patients with severe bronchoconstriction and acute respiratory failure who require ventilatory support, the clinical benefit of applying positive end-expiratory pressure (PEEP) remains controversial since PEEP can potentially worsen hyperinflation of open alveolar units. The aim of this study was to assess the effects of moderate PEEP on regional ventilation distribution and prenchymal density in normal lung and following histamine-induced bronchoconstriction.

Methods: Experiments were performed in anesthetized, tracheostomized, paralyzed and mechanically ventilated New-Zealand rabbits (2.5 ± 0.1 kg, $n = 6$). Synchrotron CT images of tissue density (D) and specific ventilation (sV) were acquired using K-edge subtraction imaging with inhaled stable xenon gas, in middle and caudal thoracic levels at 0 and 5 cm H_2O PEEP, at baseline and twice after histamine inhalation. The area of well-ventilated lung regions (VAA) and ventilation heterogeneity (CV of sV) were calculated from the ventilation images.

Results: Histamine provocation resulted in a significant drop in VAA (60.6 ± 17.8 vs. 92.3 ± 2.8% total area, $P < 0.0001$) and increased CV of sV (43.8 ± 14.5 vs. 14.6 ± 4.3%) compared to baseline. PEEP5 significantly but partially improved the VAA (84.3 ± 15.6, <0.0001) and reduced the CV of sV (23.5 ± 6.0%, $P < 0.0001$). Limited but significant increases in the area of hyperinflated lung zones were observed at PEEP 5 in constricted lung (11.0 ± 6.0% total area vs. 4.3 ± 2.1 at baseline, $P < 0.001$).

Conclusions: The findings of this study suggest that in mechanically ventilated rabbit with severely heterogeneous bronchoconstriction, a PEEP of 5 cm H_2O significantly improves regional ventilation homogeneity, through dilation of flow-limited airways and recruitment of closed airways. This improvement went together with a significant albeit limited increase in the area of hyperinflated lung zones.

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25-P096

Ageing and ventilatory response to hypoxia

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Context: Ventilatory response to hypoxia is an important physiological characteristic of the individual capability of adaptation to physiological (altitude) or pathological (lung disease) hypoxic conditions. The variation of this parameter with ageing remains to be explored and its evaluation might be of importance in a context of overall ageing of the population.

Objectives: Explore the influence of age on respiratory and cardiac responses to hypoxia at rest and exercise.

Methods: A cohort of 4012 healthy active subjects (aged from 10 to 84 years old) performed a hypoxic (FIO2 = 0.115) submaximal (30% VO_2max) exercise test. Basal cardiac and ventilatory parameters have been measured at rest and exercise in normoxia and hypoxia. Ventilatory and cardiac responses to hypoxia have been calculated (Richalet et al. AJRCCM, 2011). The effect of age on each parameter has been evaluated by a linear regression analysis.

Results: Ventilatory response to hypoxia at rest ($P < 0.001$) and exercise ($P < 0.001$) increase with age. Cardiac response to hypoxia at rest ($P < 0.001$) and exercise ($P < 0.001$) decrease with age. Hypoxia-induced desaturation at rest ($P < 0.001$) and exercise ($P < 0.001$) decrease with age. Resting arterial saturation in normoxia slightly decreases with age ($P < 0.05$) while resting and exercise arterial saturation in hypoxia increases with age ($P < 0.001$). Ventilation increases while heart rate decreases with age in all conditions ($P < 0.001$).

Conclusions: Ventilatory response to hypoxia is preserved with ageing in healthy active subjects. Arterial saturation is maintained during exercise, while cardiac response is blunted. Chemoreceptors seem to be protected with ageing while cardiac beta-receptors are down-regulated. The overall ability to adapt to hypoxic conditions is maintained.

Reference:

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25-P097

Role of the medullar pathway of NO production in the ventilatory response to hypoxia in chronic anemic mice (Epo-TAgh)

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Objectives: NMDA receptor stimulation by glutamate induces the formation of nitric oxide (NO), via neuronal NO synthase (nNOS). NO is a putative neurotransmitter with a role in the hypoxic chemotransduction pathway and is known to be involved in ventilatory acclimatization to hypoxia in Wild Type (WT) mice. Erythropoietin-deficient mice (Epo-TAg^H) have a good survival rate in chronic hypoxia despite severe anemia. This seems to be due to their hyperventilation and a better chemosensitivity to hypoxia. In such a context, we aimed to examine the role of NMDA *via* nNOS activation in the ventilatory response to acute hypoxia after acclimatization to chronic hypoxia (*cHx*) in Epo-TAg^h mice.

Methods: Breathing parameters of unrestrained non-anaestheized WT and Epo-TAg^h mice at 8 weeks were measured by plethysmography. Hypoxic ventilatory response (HVR – O_2 8%, 5 min) after acclimatization to hypobaric *cHx* (14 days, 0.42 bar) was analyzed following a single intra peritoneal injection of MK801 (NMDA antagonist, 3 mg/kg) or SMTC (nNOS inhibitor, 10 mg/kg). Moreover, the medulla was collected for proteins and mRNA analysis of NMDA-Receptors (NR1) and nNOS. The measurement of NO metabolites was performed using a colorimetric method.

Results: In normoxia, MK801 increased ventilation in WT and Epo-TAg^h mice before and after acclimatization to *cHx*, whereas SMTC increased ventilation only in non-acclimatized WT. HVR was abolished following MK801 injection in non-acclimatized WT and Epo-TAg^h mice and in acclimatized WT mice. HVR following SMTC injection was reduced in WT mice before and after *cHx* and in acclimatized Epo-TAg^h mice. Biochemical analysis showed that mRNA and proteins expression of NR1 and nNOS was increased by chronic anemia while NO metabolites were unchanged. Moreover, NO metabolites and mRNA and proteins expression of NR1, nNOS were enhanced after *cHx* in WT mice. In Epo-TAg^h mice NR1 expression was unchanged whereas nNOS protein tended to rise and NO metabolites was increased after *cHx*.

Discussion: Present results highlight the role of NR1 and nNOS activation in ventilatory adaptation to chronic anemia in Epo-TAg^{h} mice. However, NO response to acute hypoxia after acclimatization in Epo-TAg^{h} mice seems to be independent of the NR1 pathway and could imply others neuromediators.

25-P098
Combination of cigarette smoke extract (cse) and lipopolysaccharide (lps) induced cytokine release from epithelial cells by jak/stat and jnk signaling pathways

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Cigarette smoke is a major cause chronic obstructive pulmonary disease (COPD). Airway inflammation is a hallmark of COPD and is currently associated with a migration of inflammatory cells, oxidative stress production, parenchymal destruction or recurrent infection which play a crucial role in the progression of the disease. Nevertheless, the early mechanism of activation of epithelial cells by CSE associed with LPS is unclear. We investigated the effect of combination of CSE and very low concentrations of LPS in A549 epithelial cells activation.

CSE was prepared from Kentucky 2R1 cigarette smoke in contact with 20 mL RPMI medium. Alveolar epithelial type II cells A549 were treated with CSE (2% and 4%) alone or with LPS (0.1 μg/mL). Production of interleukin (IL)-8/CXCL-8, CCL2/MCP-1 and CXCL1/GRO-α were determined by ELISA. Activation of JNK and JAK/STAT pathways were determined by protein phosphorylation by Western blotting and Phospho-kinase array.

CSE did not induce production of IL-8/CXCL-8 (78 ± 12 pg/mL vs. control 62 ± 8 pg/mL) and VEGF (131 ± 11 pg/mL vs. control 45 ± 3 pg/mL) from A549 cells, but increased production of CCL2/MCP-1 (1417 ± 33 pg/mL vs. control 498 ± 83 pg/mL) and CXCL-1/GROα (445 ± 42 pg/mL vs. control 332 ± 62 pg/mL). LPS (0.1 μg/mL) was not able to induce the production of cytokines. However the combination of LPS (0.1 μg/mL) with CSE induced an important production of IL-8/CXCL-8 (242 ± 22 pg/mL), CCL2/MCP-1 (1666 ± 328 pg/mL), CXCL-1/GROα (820 ± 204 pg/mL) and VEGF (185 ± 4 pg/mL). This combination also induced activation of JAK/STAT signaling pathway and transient peak activation of JNK after 5 min.

Combination of CSE with LPS resulted in strong production of several cytokines. We also showed that the increased production of cytokines, involved JAK/STAT JNK signaling pathway. This also demonstrates the important role of combined activation of LPS during recurrent infections with cigarette smoke in the inflammatory process in COPD.

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The role of the basal ganglia in behavioral disorders: experimental studies in monkey

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There are growing evidences supporting the implication of the Basal Ganglia in a large spectrum of behavioral disorders; like obsessive-compulsive disorders (OCD), attention deficit/hyperactivity disorder (AD/HD), anxiety disorders, depression and apathy. However, experimental results from animal models and specifically on monkey are required to determine the involvement of specific territories within the Basal Ganglia. As it was demonstrated that movement disorders could be obtained by microinjections of bicuculline in the sensorimotor territory of the external Globus Pallidus (GPe), the purpose of our study was to assess the consequences of such injections in the associative and limbic GPe. Spontaneous behavior and performances in a simple-choice task were monitored. Effectively, two types of behavioral disorders were obtained, dependant of the injection site into the GPe. These behavioral effects shared similar features with AD/HD and compulsive disorders observed in human. Anatomical study of input and output projections related to the sites which induced the most striking effects was done. The production of AD/HD was linked to the associative territories of the basal ganglia and the production of compulsive behavior was linked to the limbic territories. In a last study, we showed that the same approach, applied to the associative and limbic territories of the striatum, induced a larger spectrum of behavioral disorders, including impulsive disorder, anxiety disorder and apathy. In conclusion, our results support the idea that the associative and limbic territories of the basal ganglia are involved in large spectrum of behavioral disorders and could be the site for new therapeutic approaches like deep brain stimulation in some psychiatric disorders.

26-O051
Early effects of high-fat diet on hypothalamic cell proliferation

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The hypothalamus is one of the main brain structure involved in the control of energy homeostasis. Recent reports indicate that hypothalamus exhibits neuroproliferative potency in adult. Moreover, it has been found that hypothalamic cell proliferation could be modulated by numerous intrinsic factors such as CNTF, IGF-1, bFGF and EGF, and by external and internal conditions such as dehydration, variation in ambient temperature and during ovarian cycle. Interestingly, experimental manipulation of neurogenesis can affect body weight. However, whether nutritional conditions could influence hypothalamic cell proliferation and thereby modify energy homeostasis is still unknown. To address this question, mice were subjected to a high fat diet (HFD) for 1 week and hypothalamic cell renewal was assessed through central chronic infusion of Bromodeoxy-Uridine (BrdU). Using this approach, we report that hypothalamus constitutively exhibits ≈2000 BrdU-positive neo-formed cells per day. This proliferative rate was significantly higher in hypothalamus 3 days after the onset of HFD (+60%) but decreased by 50% on day 5. To determine whether these HFD-induced modifications of cell renewal were linked to change in cell proliferation, we counted Ki67 immunoreactive cells. We found 1937 Ki67 immunoreactive cells in hypothalamus from mice fed with standard chow. However, the number of such cell was significantly higher in mice fed with HFD for 1 and 3 days (+30% and 50%, respectively), and returned to basal value after 5 days. In order to evaluate the role of newborn cells, HFD fed mice were treated with the anti-mitotic arabinoside cytosine (AraC) for 30 days. Results show that AraC treatment increased HFD-induced body weight gain, suggesting that newborn HFD-induced cells produce anorectic function. Altogether these data demonstrate that a change in diet induces cell proliferation in the hypothalamus which might be involved in the control of long-term energy homeostasis.

26-O052
Weight gain following deep brain stimulation: a pet study

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Objective: Several hypotheses have been proposed to explain weight gain (WG) following deep brain stimulation (DBS) in Parkinson's disease (PD) but none fully succeed to clarify this common adverse effect that may have prejudicial health consequences. The aim of this study was to analyze the correlation between changes in cerebral metabolism and WG following bilateral DBS in patients with PD.

Methods: Body Mass Index (BMI) was calculated and cerebral activity prospectively measured using FDG (2-deoxy-2[18F]fluoro-D-glucose) 3 months before and 3 months after subthalamic (STN) DBS in 23 PD patients and pallidal (GPi) DBS in 19 PD patients.

Results: Mean [SD] increase in BMI was similar in both groups: +0.8 [1.5] in the STN group and +0.6 [1.7] in the GPi group. In contrast, group comparison revealed a dramatically different pattern of correlation between WG and changes in cerebral metabolism. Positive correlations were clearly marked in limbic and associative areas in the STN group, including both lateral and medial parts of the temporal lobe, right Brodmann Area (BA) 20, left BA21, BA22 and BA37, and the dorsal anterior cingulate cortex (BA32). In the GPi group, positive correlations were observed in sensori-motor areas, mainly in the prefrontal cortex (left and right BA6, left BA8, right BA9 and right BA46) and the right parietal cortex (BA7).

Discussion: These findings confirm a previous clinical study from our group suggesting that WG following DBS in PD is linked to improvement in motor dysfunction following GPi DBS and to change in limbic processes following STN DBS. This study also confirms that the STN is a key structure of the basal ganglia in limbic circuitry.

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26-O053
Severity disease biomarkers in patients with Spinocerebellar ataxia type 7 (SCA7)

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SCA7 is an autosomal dominant neurodegenerative disease that belongs to polyglutamine disorders . Patients present progressive cerebellar ataxia and the disease is usually fatal after 10–30 years of evolution. Age at onset and clinical severity are highly inconstant between patients and are not predictable. Because the causal gene *SCA7* code ataxin-7 that is a transcriptional factor, we hypothesized that ataxin-7 interacts with transcriptional machinery and that transcriptomic alterations may partly explain this variability.

Objective: To identify biomarkers of disease severity in SCA7.

Methods: Twenty-four SCA7 patients and 23 healthy controls matched for age and gender were recruited at the Pitié-Salpêtrière Hospital. A trained neurologist performed clinical assessments using a specific severity scale and blood samples were collected at the same time. RNA from lymphocytes was extracted, amplified and hybridized on Illumina HT-12 arrays. Expression profiles of 48 804 probes were generated, and we performed statistical analysis using BRB-array Tools. Significant genes were confirmed by real time RT PCR.

Results: We found 1498 genes differentially expressed between patients and controls ($P < 0.01$, FDR<0.05), thus revealing a molecular signature of SCA7 disease (DNA catabolic process ($P = 0.009$, FE = 2.2), leucocyte chemotaxis ($P = 0.01$, FE = 2.8), regulation of defense response to virus ($P = 0.01$, FE = 3.0), biogenic amine metabolic process ($P = 0.01$, FE = 2.1), neuron apoptosis ($P = 0.02$, FE = 3.5), regulation of cell shape ($P = 0.05$, FE = 2.27), proteoglycan metabolic process ($P = 0.05$, FE = 2.8)). Among those deregulated genes, the expression of *ATG12*, a regulator gene of autophagy, was the most correlated with the severity of the disease (correlation coefficient = 0.64, $P = 0.0009$). We then looked at the autophagic pathway and discovered that 14 genes of this pathway (17%) were deregulated among SCA7 patients ($P < 0.01$, FDR<0.10).

Conclusion: The expression of *ATG12* in blood cells of SCA7 patients is correlated with disease severity. Other genes involved in the autophagic pathway are also deregulated in accordance with previous study showing elevated autophagy markers in mouse cerebellum. Although to be confirmed in independent studies, our results suggest new therapeutic strategies in this rare disease and provide a potential biomarker to follow disease course and treatment effects.

26-O054

TRPV1 in brain is involved in paracetamol-induced antinociception

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Background: In 1948, Brodie and Axelrod demonstrated that paracetamol is the major active metabolite of acetanilide in man. Since then, paracetamol has become one of the most popular over-the-counter analgesic and antipyretic agents, consumed by millions of people daily. However, its mechanism of action is still a matter of debate. We have previously shown that paracetamol is further metabolized to *N*-(4-hydroxyphenyl)-(5Z,8Z,11Z,14Z)-icosatetra-5,8,11,14-enamide (AM404) by fatty acid amide hydrolase (FAAH) in the brain and that this metabolite is a potent activator of transient receptor potential vanilloid 1 ($TRPV_1$) in vitro. Pharmacological activation of $TRPV_1$ in the midbrain periaqueductal gray elicits antinociception in rats.

Aim: It is therefore possible that activation of $TRPV_1$ in brain contributes to the analgesic effect of paracetamol.

Materials and methods: Thermal, mechanical and chemical pain tests in rat and mice were used to assessed the analgesic effet of drugs (n = 6–8 animals *per* group). Pharmacological and genetical (knock-out mice) approches were used to study the involvement of $TRPV_1$ receptor. Prostanoids and endocannabinoids were assessed by a liquid chromatography system with an autosampler coupled to a tandem mass spectrometer (LC-MS-MS).

Results: Here we show that the antinociceptive effect of paracetamol at an oral dose lacking hypolocomotor activity, is absent in FAAH and $TRPV_1$ knockout mice in different pain tests. This dose of paracetamol did not affect the global brain contents of prostaglandin E_2 (PGE_2) and endocannabinoids. Intracerebroventricular injection of AM404 produced a $TRPV_1$-mediated antinociceptive effect in the mouse formalin test. Pharmacological inhibition of $TRPV_1$ in the brain by intracerebroventricular capsazepine injection abolished the antinociceptive effect of oral paracetamol in the same test.

Discussion: This study shows that TRPV1 in brain is involved in the antinociceptive action of paracetamol and provides a strategy for developing central nervous system active oral analgesics based on the coexpression of FAAH and TRPV1 in the brain.

26-P119

Human Merkel cell in culture express thermosensitive ion channels

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Skin is the body organ of touch and thermo-sensation. Slowly adapting type I receptors in vertebrate are identified with Merkel cells connected to Ab nerve endings. It has been suggested that Merkel cells are mechano- and thermotransducers that signal to adjacent nerve. Nevertheless, the molecular identity of the Merkel cell ion channels and the functioning of these complexes remain poorly understood. Our objective was to develop a method for culturing Human Merkel cells to characterize ion channels sensitive to thermal stimuli. We used the expression of surface protein to sort on affinity column Merkel cells from other cells of the epidermis. Cell extract contained 80% of Merkel cells immuno-positive for cytokeratine 20, 18 and 8. Using patch-clamp and calcium imaging, we determined the response of Merkel cells to mechanical and thermal stimulation. Merkel cell swelling, hot and cold temperature provoked calcium entry. TRPM8 and TRPV3 mRNAs were identified by PCR and channel activities were induced by the agonist menthol, eucalyptol and 2-APB. These results demonstrate that human Merkel cells express thermo-TRP channels that may act as transducer of skin sensation.

26-P120

Functional rehabilitation of social communication in young children with autism: clinical and neurobiological correlates

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Background: The exchange and development therapy (EDT) is applied in very young children with autism. The EDT consists in the early and harmonious re-education of the basic neurophysiologic functions involved in communication abilities. Previous studies have confirmed the efficiency of the EDT in autism but the cerebral correlates that underlie these improvements have never been studied.

Objectives: The aim of the current study was to investigate the evolution of clinical and neurophysiological markers (ocular exploration, electrophysiological responses to face) before and after the EDT.

Methods: Twenty-nine children, aged 2–8 years, with severe autism and moderate to severe mental retardation were recruited. Changes in autistic symptomatology were evaluated with the BSE scale (Behavioural Summarized Evaluation scalerevised) at the beginning of treatment and 10 months later. Neurophysiological evaluations were assessed for six of these children: before the beginning T0, 1 year after T1 and 2 years after T2. Using an eye-tracking method, the visual exploration of face was investigated and visual ERPs (P1, N170) were recorded during an implicit emotional task. A group of typically developing children (TD) matched by chronological age also participated to the eye-tracking and ERPs recordings.

Results: ASDs displayed strongly abnormal pattern of face exploration (children with ASDs looked less at the eye region compared to TD children) and visual ERPs in response to face (P1 and N170 were delayed and smaller in ASD) confirming that these processes, involved in social adaptation, were affected in our sample at T0. One year after the beginning of the therapy, improvements were observed in both socio emotional and cognitive area. During the firts year the time spent on the eyes increased. Electrophysiological indices appeared to be affected by the EDT only during the second year. The amplitude of P1 and N170 increased and the topographical analysis revealed a normalization of the N170.

Conclusion: Both clinical and neurophysiological markers appeared to be affected by the EDT. Although these data are only preliminary, they are very encouraging and suggest an effect of therapy on brain development in agreement with the principles of the EDT.

26-P121

Involvement of Cx43 in the hypothalamic glucose-sensing

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Introduction: The hypothalamus is implicated in the nervous regulation of the glucose homeostasis. Detection of increased blood glucose level by specific hypothalamic glucose sensitive neurons triggers physiologic responses such as increased insulin secretion and decreased food intake. Astrocytes are suspected to be involved in the brain glucose-sensing. They present a network organization formed by numerous connexin gap-junctions (GJ). This allows the transfer of glucose from bloodstream to neurons. The major connexin expressed in astrocytes is the connexin 43 (Cx43). We hypothesized that the Cx43-dependent astrocyte networks plays a critical role in hypothalamic glucose-sensing.

Materials and methods: Expression of Cx43 was studied by immunochemistry in rat ventro-median hypothalamus (VMH). Decrease in VMH Cx43 expression was assessed, in vivo, by stereotaxic injection of siRNA against Cx43. Evaluation of hypothalamic glucose-sensing was assessed by monitoring insulin secretion or refeeding of 20 h-fasted animals in response to an intracarotid injection of a glucose bolus towards the brain.

Results: Cx43 is highly expressed in the VMH at the astrocytic end-feet, around blood vessels. Inhibition of Cx43 (about 30%) leads, 72 h after the injection, to a decrease of food intake whitout modification of weigth, blood glucose or insulin levels. Intracarotid glucose injection-induced insulin secretion is significantly decreased in siCx43 rats. Similarly, the satietogenic effect of glucose is significantly attenuated in 20 h-fasted siCx43 rats during the refeeding.

Conclusion: These results show that Cx43-dependant astrocyte networks are critical for hypothalamic glucose sensitivity.

26-P122

The neuroretina is a novel mineralocorticoid target organ: aldosterone up-regulates ion and water channels in Müller glial cells

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Retinal Müller glial cells (RMG) are key elements for the control of retina hydration and homeostasis of potassium, as they establish an anatomical and functional connection between the retinal neurons and the retinal blood vessels on one hand, and with the vitreous and the subretinal space on the other hand. Any alteration in the retinal dehydration processes leads to retinal edema. Glucocorticoids (G) reduce diabetic macular edema, but the mechanisms underlying G effects are imperfectly elucidated. G may bind to glucocorticoid (GR) and also mineralocorticoid (MR) receptors. We hypothesize that MR activation may influence retinal hydration. The effect of the MR agonist aldosterone (24 h) on ion/water channels expression (real-time PCR, western blot, immunofluorescence) was investigated on cultured retinal Müller glial cells (RMG, that contribute to fluid homeostasis in the retina), in Lewis rat retinal explants and in rat retinas following intra-vitreous aldosterone injection. We evidenced cell-specific expression of MR, GR and 11-beta hydroxysteroid dehydrogenase type II. Aldosterone significantly enhances expression of sodium and potassium channels ENaC- α (6.5-fold) and Kir4.1 (1.9-fold) through MR and GR occupancy, while aquaporin 4 (AQP4, 2.9-fold up-regulation) is MR-selective. Aldosterone intravitreous injection induces retinal swelling (24% increase compared to sham-injected eyes) and activation of RMG. It promotes additional localization of Kir4.1 and AQP4 towards apical microvilli of RMG. Our results highlight the mineralocorticoid-sensitivity of the neuroretina and show that aldosterone controls hydration of the healthy retina through regulation of ion/water channels expression in RMG. These results provide a rationale for future investigations of abnormal MR signalling in the pathological retina.

26-P123

Evaluation of an educational Program in Parkinson's Disease: A medical and economic study

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Rational: Parkinson's disease (PD) has considerable impact on motor, psychological and social activities and significantly affects the quality of life of patients and their families. To improve the medical care of PD patients, we have developed an educational program specific to PD.

Aim: The principal aim of this study was to evaluate our educational Program, comparing the quality of life of PD patients with or without the educational program after 1 year follow-up.

The secondary aims were to evaluate the evolution of motor and psychological states in these two groups of patients and to compare the medical costs.
Patients and methods: This was a monocentric, comparative, prospective randomised study. We evaluated 120 PD patients, 60 patients benefiting of the educational program and 60 patients with a traditional medical care. Quality of life of PD patients was evaluated using a specific scale (PDQ39) and a generalist scale (SF36) at 6 and 12 months. Motor and psychological states were assessed with UPDRS and HAD Scales. The educational program consisted of both individual and collective educational consultations. Medical costs (radiological and laboratory tests, physician fees, drug therapy and ancillary care) were recorded over 12 months.
Results: After 1 year follow-up, we observed a significant improvement in several dimensions of PDQ39 in patients benefiting of the educational program compared to the other group of patients (mobility 22.9 ± 19 vs. 31.2 ± 21.9, $P = 0.03$; activity of daily living 22.2 ± 15.3 vs. 29.1 ± 19.3, $P = 0.03$; emotional well being 26.4 ± 19.5 vs. 34.3 ± 21.6, $P = 0.04$; cognition 25.95 ± 13.7 vs. 36.1 ± 17.4, $P = 0.0003$; communication 16.8 ± 19.2 vs. 23.9 ± 17.9, $P = 0.03$; bodily discomfort 35.7 ± 18.6 vs. 44.9 ± 22, $P = 0.01$, respectively). We also observed a significant difference between the two groups for motor and psychological states. Furthermore, our educational program didn't induce a supplementary medical cost (€7301 ± 8446 in patients receiving educational programme vs. €7365 ± 8026 in patients who did not ($P = 0.27$)).
Discussion: This is the first educational program for PD patients in France. This study showed that this program associated to the traditional medical care improves the quality of life of PD patients without increasing the medical costs.

26-P124
Memantine, a *N*-methyl-D-Aspartate (NMDA) receptor antagonist, as a promising drug in preclinical neuropathic pain development
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Introduction: The *N*-Methyl-D-Aspartate receptor (NMDAR) is involved in many pathophysiological processes such as memory and neurological disorders. In the last few years, attention has focused on NMDA receptor antagonists as potential therapies for neuropathic pain states. Severe side-effects at therapeutic doses may limit their clinical use. The aim of this study is to evaluate the antinociceptive and cognitive effects of memantine and also the molecular events associated with the NMDAR, like phosphorylation of the NR2B subunit at residue 1472, when administered at different period (pre-emptive and post-operative) in a neuropathic pain model, the L5 spinal nerve ligation (SNL).
Methods: Experiments were carried out on SNL and sham-operated animals which received memantine (20 mg/kg/rat/day, intraperitoneal route) or saline. For the pre-emptive protocol, treatment started 4 days before surgery and was administered daily for 7 days. For the post-operative protocol, treatment started the day of surgery and was administered during 7 days. After treatment, antinociceptive effect was evaluated by nociceptive tests (von Frey/Randall & Selitto) and cognitive function using a spatial memory test, the Y-maze. Expression of $pTyr^{1472}$NR2B was evaluated by western-blot analysis.
Results: In SNL rats, memantine administered preventively totally reversed mechanical hypersensitivity and tactile allodynia, and was also efficient in restoring spatial memory. In these same rats, western-blot analysis showed a significant decrease of spinal $pTyr^{1472}$NR2B compared to saline-treated animals. However, after a post-operative treatment, memantine had no effect on mechanical hypersensitivity, tactile allodynia and spatial memory in SNL rats. Furthermore, no variation of spinal $pTyr^{1472}$NR2B was observed between SNL rats that received saline or memantine.
Conclusions: In view of these results, a pre-emptive protocol is interesting to improve painful symptoms induced by neuropathic pain. This promising strategy would be interesting in clinical practice. A placebo-controlled, randomized study is planed in oncology, where memantine will be administered in pre and post-mastectomy to confirm these pre-clinical results.

26-P125
Involvement of small sensory nerve fibers in the skin protection against a pressure-induced ulcer
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Objectives: We have recently shown that erythropoietin can restore C-fibers functions and cutaneous nerve density, and also prevent pressure ulcer development in a mouse model of diabetes mellitus. The aim of this study was to determine whether there is a direct link between presence of functional sensory nerve fibers in the skin and protection against pressure-induced ulcer.
Material and methods: Small-fiber sensory neuropathy was induced in mice by a single intraperitoneal injection (50 µg/kg) of an analogue of capsaicin, resiniferatoxin (RTX). Seven days after injection of RTX, the nerve fiber dysfunction was assessed by measuring thermal (hot-plate) and mechanical (Randall-Selitto) nociception. Pressure-induced vasodilation (PIV), a cutaneous physiological neurovascular (C-fiber/endothelium) mechanism, was assessed using laser Doppler flowmetry. Skin pressure ulcers were created on mouse back using magnets. The skin was gently pulled up and placed between two round ceramic magnetic plates. The stage 2 ulcers (assessed visually and histologically) were evaluated during the days following the pressure.
Results: Seven days after RTX injection, mice showed thermal and mechanical nociception alterations associated with a PIV alteration. Stage 2 ulcer areas were larger in RTX mice than in control mice for the same duration of ischemic pressure application.
Conclusion: Our results demonstrate that the presence of functional small nerve fibers in the skin has a protective effect against pressure-induced ulcer. These results suggest that neuroprotective drugs (such as erythropoietin) could be useful to regenerate skin nerve fibers and prevent pressure-induced ulcers in patients with small-fiber neuropathy of various origin.

26-P126
The two-pore domain potassium channel TREK2 is involved in mechanical nociception and thermal perception
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Recent results from our team have highlighted the involvement of TREK1 and TRAAK channels, two members of the two-pore potassium channels family (K2P), in polymodal pain perception [1] [2]. The aim of this work was to study the role of TREK2, the third channel of the TREK/TRAAK subclass of K2P channels, in nociception and inflammatory pain using knock-out mice. We also assessed the impact of these channels deletion on nociception and inflammatory pain using triple TREK1, TREK2 and TRAAK knock-out mice.
All experiments were performed on 20–24 g mice with C57BL/6J genetic background. Nociceptive thresholds were assessed using the von Frey test for mechanical sensitivity, and thermal sensitivity was assessed by tail immersion test (hot and cold temperatures), dynamic cold plate, place preference and hot plate tests. Neurons electrophysiological response to nociceptive stimuli was measured with skin-nerve preparations.
The TREK2 knock-out mice showed hypersensitivity to all mechanical and thermal stimuli. This hypersensitivity was increased in triple TREK1, TREK2 and TRAAK knock-out mice. Contrary to TREK1 and TRAAK channels, TREK2 were involved in the perception of cool (between 20 and 25°C) and warm (between 40 and 46°C) temperatures, highlighting a specific role of this protein in the innocuous thermosensitivity. In an inflammatory context, mice lacking TREK2 produced a lesser hypersensitivity than wild-type animals. We found a similar reduction of hypersensitivity in triple knock-out mice.
Although, TREK2 channels share many properties with TREK1 channels, we found that their involvement in pain physiology and pathophysiology are different. Further work is needed to assess the interest of the modulation of these channels for the management of different pain symptoms and/or of pain syndromes of various etiology.
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Antioxidant supplementation: more harm than good for exercise performance?
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This review seeks to introduce the main concepts and remaining controversies concerning the roles and needs in antioxidants for exercise training and performance. It aims to give scientists, athletes and coaches the current recommendations in dietary antioxidants intakes during exercise training.
Since 1969 when McCord and Fridovich (1) demonstrate, for the first time, the existence of the fundamental antioxidant enzyme, called superoxide dismutase (SOD), intensive researches have been conducted to clarify the roles of oxidative stress and antioxidants in the human body. Oxidative stress was first defined by H. Sies in 1985 (2), as an 'imbalance between the production of reactive oxygen species (ROS) and the occurrence of cell antioxidant defenses'. Almost at the same time (1982), K. Davies (3) demonstrates that exhaustive exercise increases ROS production. Since then, research in this area has grown spectacularly, showing that high levels of ROS produced during intense exercise result in oxidative stress and are associated with muscle damage and impaired muscle function.
So the idea of boosting the antioxidant reserves with additional oral doses of antioxidants to offset the perceived negative effects emerged as a suitable strategy to prevent or reduce oxidative stress, decrease muscle damage and improve exercise performance. Over 150 articles have been published on this topic. A meta-analysis of all these literature data has just been published by Peternelj and Coombes (4) and their conclusions are disappointing. Although several reports have concluded that the administration of various antioxidants decreases the markers of oxidation in skeletal muscle and other tissues (5), the positive effects of dietary antioxidants on exercise performance, contraction-induced muscle damage and muscular fatigue are not commonly observed. Much recent evidence suggests that taking large doses of antioxidants could suppress some adaptive responses to contractions in muscle (6) and this finding has been supported by experiments using cell cultures approaches as well as animal and human studies.
Now it is clear that animals and humans that engage in long-term heavy exercise are more resistant to oxidative stress. Evidence is growing that the continued presence of a small stimulus, such as low concentrations of ROS, can induce the expression of antioxidant enzymes and other defense mechanisms. This phenomenon is based on the concept of hormesis, which refers to a particular kind of dose-response in which a low dose of a substance is stimulatory, and a high dose is inhibitory. The hormesis theory has recently been extended to the ROS-generating effects of exercise (7). Thus, within a concentration range, ROS are 'friends' rather than 'foes'. Redox-sensitive signaling pathways are the most important link between ROS and the hormetic effects and exercise stimulates the cellular events leading to useful gene products that enhance muscle antioxidant defense, growth, differentiation and performance. As a consequence, antioxidant supplementation may be harmful by blunting the positive effects of exercise training and interfering with important ROS-mediated physiological processes. While selective nutritional supplementation has been shown to offer some kinds of protection against various chronic or age-related diseases, there is a lack of evidence-based guidelines regarding the use of antioxidant supplementation during exercise training. In the present state of knowledge, an adequate intake of vitamins and minerals through a

varied and balanced diet remains the best approach to maintain the optimal antioxidant status in exercising individuals.

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Free leucine dietary supplementation to improve muscle mass: a friend or a foe?

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In the 1970s, a number of laboratories first demonstrated in vitro that among amino acids, branched chain amino acids (BCAA) and especially leucine have the potential to regulate muscle protein metabolism by stimulating protein synthesis and inhibiting protein degradation. Later, it has been clearly confirmed in vivo that leucine was also able to stimulate muscle protein synthesis to the same extent as a complete meal and in a dose-dependent manner. The attenuation of in vivo skeletal muscle proteolysis by leucine has been also described but is, however less documented than for protein synthesis. Leucine serves not only as a substrate for protein synthesis but is also recognized as a potent nutrient signal that regulates protein turnover through activation of cell signaling pathways common to insulin including the mammalian target of rapamycin complex (mTOR) which, in turn, activates two key regulatory proteins involved in the regulation of translation initiation such as S6K1 and 4EBP1. Based on these observations, dietary leucine supplementation has been predicted to represent a useful nutritional tool for increasing muscle mass (in association or not with exercise) or maintaining muscle protein content in pathological states which induced muscle wasting. However, despite numerous studies have demonstrated the positive effect of BCAA and/or leucine on muscle anabolism, it is still not obvious that leucine alone is able to improve muscle mass in long-term supplementation. The purpose of this presentation is to examine if long-term dietary leucine or leucine-rich protein supplementation had a beneficial effect on skeletal muscle in selected physiological situations such as aging, exercise, immobilization and to point out the potential deleterious effects of such supplementations on glucose homeostasis and insulin resistance development.

27-O055

Specific muscle functional, morphological and angiogenic responses to exercise training in copd patients

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Introduction: The skeletal muscle dysfunction in chronic obstructive pulmonary disease (COPD) patients is a major factor of their incapacity and morbid-mortality, and the contribution of physical inactivity remains debated. Indeed, the alterations in muscle fiber type and level of capillarization observed in COPD mimic severe disuse.

Objective: As muscle disuse is reversed by training, we compared the functional, morphological and angiogenic muscle responses to similar training in COPD patients and sedentary healthy subjects (SHS) matched for physical inactivity.

Methods: Twenty-one COPD patients and 23 SHS completed a 6-week rehabilitation program based on individualized moderate-intensity endurance and resistance training. Histomorphological muscle analysis and measurements of pro-angiogenic vascular endothelial factor-A (VEGF-A) and antiangiogenic thrombospondin-1 (TSP-1) were conducted before and after training.

Results: Both COPD patients and SHS improved their peak oxygen consumption (VO_2p) (respectively, +0.96 ± 2.4 and +2.9 ± 2.6 mL/kg/min, $P < 0.001$) and muscle endurance (respectively, +65% and +108%, $P < 0.001$), although improvements were lower in COPD patients (time-group interaction: $P < 0.05$ and $P = 0.06$, respectively). Whereas the proportion of type I fibers significantly increased in SHS muscles (+8.4 ± 11.6%, $P < 0.01$), no change occurred in COPD patients. In contrast, both the capillary-to-fiber ratio (C/F) and the angio-adaptive VEGF-A/TSP-1 ratio increased in COPD patients and SHS (C/F:respectively, +16% and +37% in COPD and SHS, $P < 0.01$; VEGF-A/TSP-1 ratio: respectively, +35% and +65%, $P < 0.05$).

Conclusion: Our study shows that the muscle dysfunction in COPD patients is more complex than a simple consequence of deconditioning, and it opens perspectives for interventions targeting the fiber type shift or enhancing the angio-adaptive capacity in COPD patients.

27-O056

Effects of lactate on skeletal muscle sodium channel: modulating the current opinion

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Introduction: Muscle lactic acid production and efflux increase during exercise. While lactic acid dissociates into H^+ and lactate at physiological pH, much attention has been directed at eluciding the consequences of acidity on muscle excitability. It has been shown that H^+ decreases chloride conductance, leading to increase sarcolemma excitability. Conversely, few attempts were made to look for possible effects of lactate ion on muscle excitability. Voltage-gated sodium channels (Na_v) initiate and convey the action potential on muscle fibre membrane. Thus, the electrophysiological properties of Na_v determine the excitability and the contractile feature of muscle. We hypothesized that lactate ion modulates the electrophysiological properties of muscle Na_v, regarding its transmembrane location and the subsequent exposition to lactic acid.

Methods: The electrophysiological properties of muscle Na_v were studied in the absence and in the presence of lactate (10 mM) by using the macro-patch-clamp method in dissociated fibres from rat *Peroneus Longus*.

Results and discussion: Lactate increases the maximal sodium current, while the voltage-dependence of activation is shifted toward the hyperpolarized potentials. This indicates a more rapid depolarization, allowing an earlier recruitment of the muscle fibre. The voltage-dependence of Na_v fast inactivation is shifted by lactate toward the hyperpolarized potentials. This implies a more rapid membrane repolarisation which is crucial for the elicitation of a novel action potential. Lactate induces a leftward shift in the relationship between the kinetic parameters and the imposed potentials, resulting in an acceleration of Na_v activation. The slow inactivation process is decreased by lactate, corresponding to an enhancement in the number of excitable Na_v.

In an additional series of experiments, lactate was only added in the Petri dish, while the pipette remained sealed on the membrane area. In this approach, the electrophysiological properties of Na_v were unaffected by lactate compared with control condition, suggesting an extracellular pathway.

Conclusion: Altogether, these data indicate a direct effect of lactate ion on skeletal muscle Na_v and hence, on muscle excitability. This leads to preserve force production by reducing the muscle fatigability related to membrane excitability failure. Our results bring new insights concerning the role of lactic acid in muscle physiology.

27-O057

Pharmacological regulation of Akt/mTOR signaling pathway in skeletal muscle in response to formoterol and 007-AM treatments

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Traditionally used as bronchodilator for the asthma treatment, β_2-agonists, such as formoterol, are effective in inducing muscle hypertrophy. It has been shown that β_2-agonists positively regulated protein synthesis by activating the Akt/mTOR signaling pathway. Nevertheless, the molecular links between the cAMP-dependant pathway triggered by β_2-agonists and Akt/mTOR pathway remain unknown. Recently, a cAMP analogue, the '007-AM', which is a potent and specific agonist of an exchange protein directly activated by cAMP (Epac), has been described to be an alternative to the use of β_2-agonists. It stimulates the Akt/mTOR pathway by acting downstream from the β_2-receptors on Epac. To determine the mechanisms by which β_2-agonists trigger Akt/mTOR pathway activation, we *A)* investigated the dose-response and the time-course response of Akt/mTOR pathway in rat skeletal muscle after a formoterol treatment and *B)* after studying the 007-AM detection by HPLC/MS/MS, we analysed the effects of 007-AM on C2C12 myotubes.

1 Formoterol was administrated *i.p* [0.02 mg/kg ($n = 8$), 0.2 mg/kg ($n = 8$) and 2 mg/kg ($n = 10$)] in male Wistar rats (224 ± 19 g). A 10-day treatment induced a marked hypertrophy of *Tibialis Anterior* muscle in a dose-dependent manner (12%, 15% and 25% for 0.02, 0.2 and 2 mg/kg, respectively). When considering the highest formoterol dose, fiber cross-sectional area was increased by 20% ($P < 0.05$). Surprisingly, analysis of the Akt/mTOR signaling pathway in the three groups showed a significant dephosphorylation of Akt (Thr^{308} and Ser^{473}) and S6 ($Ser^{235/236}$). By contrast, one and 3-day formoterol treatment (2 mg/kg) induced a significant increase in Akt and S6 phosphorylation.

2 007-AM administration [0.1, 1 and 10 μM ($n = 4$/group)] for 2 h in C2C12 myotubes increased S6 phosphorylation in a dose-response manner. In agreement with this observation, our preliminary analysis of Akt/mTOR pathway suggested an increase in translation initiation.

One and 3-day formoterol treatment stimulates the Akt/mTOR pathway, which may increase protein synthesis. The paradoxical inhibition of the Akt/mTOR pathway in response to the 10-day formoterol treatment may be explained by a desensitization of β_2-adrenoceptors and/or a negative feedback of p70S6k on the Akt/mTOR pathway. The potential anabolic role of 007-AM in stimulating the Akt/mTOR pathway needs to be further investigated.

27-O058

Autophagy-lysosomal pathway in response to fasting/refeeding in C2C12 myotubes

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The balance between protein synthesis and protein degradation is regulated by the Akt/mTOR pathway. Accelerated breakdown of protein have been largely attributed to the activation of ubiquitine-proteasome pathway. However, the role of autophagy-lysosomal pathway is now becoming increasingly important particularly during fasting. Our preliminary data showed that Akt/mTOR pathway was significantly inhibited in C2C12 myotubes during a 4-h-fasting protocol, while

refeeding of myotubes markedly reactivated Akt/mTOR pathway. This regulation suggested that the autophagy-lysosomal pathway would be coordinately regulated in response to fasting/refeeding in C2C12 myotubes. Thus, the histone deacetylase SIRT1, can play a role in this pathway, since SIRT1 regulates several cellular processes, such as the maintenance of energy homeostasis and cellular survival, which are also the main functions of autophagic degradation. The purpose of this study was, therefore, to characterize *in vitro* the kinetic response of autophagy-lysosomal pathway during fasting and refeeding in C2C12 myotubes.
C2C12 myotubes were metabolically challenged by 4 h of fasting (Dulbecco's Phosphate Buffered Saline, 2% horse serum) followed by 4 h of refeeding (Dulbecco's modified Eagle's Medium, 2% horse serum). Cells were harvested at 0, 30, 60, 120 and 240 min of fasting and refeeding (n = 4–6 dishes/group).
We showed that 4 h of fasting induced a significant increase in ULK1 (215% at 120 min), Atg13 (200% at 120 min), Atg5 (203% at 120 min) and Vps34 (191% at 120 min) protein level. ULK1 and Atg13 protein level increased earlier (as soon as 30 min of fasting) than Vps34 and Atg5 protein level (60 min of fasting). Proteins level were also differentially regulated during refeeding: while ULK1 and Atg13 protein levels remained elevated, Atg5 and Vps34 protein levels regularly decreased. Then, protein level of Sirt1 increase during fasting (120 min of fasting) suggesting the possibility of a role of this histone in our model. Conversely, ARNms levels of Atg genes and SIRT1 were not significatively modify during fasting and refeedind. These results suggest that autophagy could be regulated by posttranscriptional modifications. This study will be extended to the analysis of Atg genes level during starvation and refeeding, after inhibition or overexpression of SIRT1 activity.

27-P138
Vascular BDNF: Impact of physical exercise
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Aim: Physical exercise (EX) is recognized to modify vascular endothelial phenotype in part through changes in shear stress. Moreover, EX has been consistently shown to enhance brain health and neuroplasticity by increasing the cerebral production of BDNF (Brain Derived-Neurotrophic Factor). The aim of the present study was to investigate for the first time the effect of EX on vascular BDNF.
Methods: BDNF levels (Western blotting analysis) and cellular localization (immunohistochemical analysis) were assessed in aorta from sedentary rats (SED, n = 7) and rats subjected to EX (EX, n = 8). EX consisted in a 30-min daily treadmill walking (30 cm/s) for seven consecutive days. BDNF levels were also measured in HUVECs subjected to low (2 dynes/cm^2) or high (14 dynes/cm^2) shear stress (n = 4 each). Analyses of BDNF were performed the day following the last session of EX in rats or 24 h after the induction of shear stress in HUVECs.
Results: In aorta from SED, BDNF was expressed in endothelial cells and smooth muscle cells. EX increased by 70% the vascular expression of BDNF, an effect that was associated with a dramatical increase in BDNF staining in endothelial cells. The expression of BDNF was 4-fold higher in HUVECs exposed to high shear stress than those exposed to low shear stress.
Discussion: Our results reveal that physical exercise increases the production of BDNF by vascular endothelial cells through a shear stress-dependent mechanism. The role of vascular BDNF remains to be explored.

27-P139
Effects of pulmonary rehabilitation program on oxidative stress biomarker and antioxidant enzymes activities in chronic obstructive pulmonary disease patients
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This study designed to evaluate the effects of 8 weeks pulmonary rehabilitation program on oxidative stress response and antioxidant activity in patients with chronic obstructive pulmonary disease (COPD) compared to healthy subjects.
Twenty six patients and 12 matched healthy subjects participated in the study. Trained groups performed an individualized exercise training enrolled to an 8 weeks pulmonary rehabilitation program three times a week. Prior to and after the programme, exercise testing and pulmonary function were evaluated. Red blood cell antioxidant enzyme activities –superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) were measured by spectrophotometry and spectrofluorimetry was used for plasma levels of thiobarbituric acid substances (TBARS).
Exercise capacity improved significantly in trained groups after the program. At baseline, plasma TBARS was increased in patients with COPD (trained COPD, 2.37 ± 0.52 μM and untrained COPD, 2.2 ± 0.57 μM) compared to the healthy subjects (1.51 ± 0.46 μM). In the same way, our study showed that SOD and CAT activities were significantly lower in patients with COPD (P < 0.01). After the programme, plasma TBARS remain unchanged. However, significant differences were found in SOD and GPx activities. SOD activity increased in both trained groups (32.67 ± 12.15–42.98 ± 16.06 U/g Hb in healthy subjects vs. 17.49 ± 16.24–37.08 ± 7.53 U/g Hb in patients with COPD) and GPx activity increased significantly only in trained patients (37 ± 20.38 vs. 46.36 ± 18.33 U/g Hb).
At baseline the results obtained showed a decrease in antioxidant enzyme activities and an increase in lipid peroxidation confirming the existence of oxidative stress in patients with COPD. After pulmonary rehabilitation program SOD activity increase as a first line in antioxidant defence in patients with COPD and in healthy subjects. In addition GPx increased only in patients with COPD which appears to be related to the muscle wasting commonly observed in these patients. However, CAT response to regular training remains a topic of debate. We also point out to the possibility that change in aerobic capacity with moderate intensity is important factor in inducing changes in antioxidant activity able to regulate oxidative stress damage. Pulmonary rehabilitation can improve oxidant and antioxidant status in patients with COPD.

27-P140
High frequency power of heart rate variability predicts individual performance in 400-m front crawl swimming
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Introduction: The purpose of this study was to assess the potential use of High Frequency (HF) indice of HRV as a modeling tool to predict performance in maximal aerobic power for 400-m front crawl swimming (FCS) on a full training season.
Methods: Ten swimmers, regional to national level (six male aged 17.3 ± 2.8 years, and four female aged 15.8 ± 1.9 years) were included in the study. They had a history of 5.6 ± 2.0 years of practice and were swimming 10.7 ± 2.9 h per week during the ongoing season. Swimmers were monitored during 30 consecutive weeks of training. Each week, Autonomic Nervous System activity was measured through HRV, once, during the night before the performance. Training load was quantified weekly. Performance, measured once a week, during a standardized session, was the time achieved on 400 m FCS.
Results: Relative individual evolution of HF component of ANS activity and of performance. When HF individual data were plotted against individual performance data, a strongly significant relationship appeared for each of the 10 subjects between HF and performance (all P < 0.001) with R^2 values ranging from 0.55 to 0.80. The relationship fits a logarithmic shape. Modelisation of performance: measured and predicted values. The two-components systems model, initially proposed by Banister (1975), presented a strong statistical adequacy for the description of the training effects on performance and on ANS activity.
Conclusion: The statistical significance fit of both, the performance and HF spectral component of HRV, confirms the relevance of the modeling approach in the study of individual response to training. Computer simulations based on mathematical modeling, associated with HRV as a control tool, could offer new perspectives for innovative tapering strategies and performance optimization.

27-P141
Physiological responses to submaximal exercise in hypoxia are intrinsic characteristics of chemosensitivity
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Aim: The individual chemosensitivity to hypoxia is usually evaluated in standard conditions of simulated altitude and exercise intensity (4800 m – 30% of normoxic Maximal Aerobic Power (MAP)). Three main parameters are calculated during this test: the decrease in arterial oxygen saturation induced by hypoxia at exercise (ΔSa_e) and the ventilatory (HVR_e) and cardiac (HCR_e) responses to hypoxia at exercise. The aim of this study was to observe the modifications of ΔSa_e, HVR_e and HCR_e values when the test is performed in other altitude and exercise intensity conditions.
Subjects and methods: Nine subjects underwent hypoxic exercise tests at three simulated altitudes (3000, 4000, and 4800 m) and three exercise intensities (20%, 30% and 40% MAP).
Results: ΔSa_e increased with altitude and was higher for 40% MAP than for 20% or 30% (P < 0.05). For a constant heart rate, the loss in power output induced by hypoxia, relative to ΔSa_e, was independent of altitude (4000–4800 m) and of exercise intensity. HVR_e and HCR_e were independent of altitude (3000–4800 m) and exercise intensity (20–40% MAP). Moreover, the intra-individual variability of responses to hypoxia was lower during moderate exercise than at rest (P < 0.05 to P < 0.001).
Discussion: These results suggest that HVR_e and HCR_e are invariant parameters and can be considered as intrinsic physiological characteristics of chemosensitivity to hypoxia. During an outpatient mountain medicine consultation, we recommend therefore to use an approximate exercise intensity based on the heart rate reserve (between 40% and 50% of heart rate reserve) instead of an exact percentage of MAP.

27-P142
Validation of a prediction model of physical activity-related energy expenditure with Dynaport Minimod in obese subjects: preliminary study
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Objective: The aims of this study were to evaluate the impact of obesity on the validity of the prediction model of Energy Expenditure (EE) with tri-axial seismic accelerometer (Dynaport Minimod©). This model is algorithm based on the detection of activity type and movement intensity.
Subjects and method: Eight obese subjects were included in this preliminary study (age: 51.3 ± 13.6 years; height: 168 ± 14 cm; Body Mass Index: 33.0 ± 3.0). They performed the following standardised activities in chronological order: lying on a bed, sitting in a chair, standing, walking at 2.4 km/h, walking at 4.8 km/h and climbing up and down stairs with a tempo of 90 bpm. During these standardised activities, EE was measured and estimated by two systems: indirect portable calorimetry (K4b2; Cosmed, Rome, Italy) as the reference system and the triaxial seismic accelerometer (DynaPort MiniMod©, The Hague, the Netherlands).

Results: EE predicted by the accelerometer model was higher than by K4b2 for lying down (6.67 ± 1.28 vs. 5.29 ± 1.61 Kj/min, $P < 0.05$), sitting (7.34 ± 1.02 vs. 5.85 ± 1.87 Kj/min, $P < 0.05$), in fast walking (38.16 ± 15.46 vs. 26.45 ± 7.52 Kj/min, $P < 0.05$). In the climbing up and down the stairs task, EE predicted by the accelerometer model was lower than EE measured by the K4b2 (32.63 ± 8.33 vs. 38.89 ± 13.23 Kj/min, $P < 0.05$). The relative duration detected by algorithm in each physical activities standardized were: in lying position 99.2%, in sitting 61.11%, standing 43.43%, slow walking 66.35% and fast walking 86.46%.
Discussion: The Bland and Altman plots confirmed the reliability of the prediction model of physical activity-related EE with K4b2. In order to provide better estimations, inaccuracies in detection of type PA must be further corrected. Then, the prediction model needs to be further developed in large groups of participants and for each type of activity individually.

27-P143
Influence of a combined program of physical activity and diet on atherogenic subfractions of ldl cholesterol in metabolic syndrome treatment

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Introduction: The main risk of morbi-mortality in subjects suffering from metabolic syndrome (MS) is cardiovascular. It is mainly associated with elevated LDL-cholesterol (LDL-c). Electrophoresis of LDL-c screens subfractions numbered 1–7. Among them, the subfractions 3–7 are specifically atherogenic. Diet and exercise improve this atherogenic profile. The relationship between the exercise mode (resistance vs. endurance) and the improvement of atherogenic profile has not been well characterized. Thus, the aim of this study was to compare the efficiency of these two different modes of exercise on the subfractions of LDL-c.
Subjects and methods: Hundred subjects (43 M, 57 F, age 59.4 ± 5.0 years, BMI 33.4 ± 4.1 kg/m^2) suffering from MS completed a 3 weeks residential program controlled for nutrition (negative energy balance: −500 kcal per day) and physical activity (3.5 h per day with intensity monitored by a heart-rate recorder). They were randomly assigned to three groups of physical activity: Re (Resistance at 70% of 10 maximal repetitions – endurance 30% VO_2max), rE (resistance 30% – Endurance 70%) and re (both at 30%). A control group of healthy subjects (26M, 24F, age 58.0 ± 4.7 years, BMI 22.4 ± 6.5 kg/m^2) served as reference. At baseline and at the end of the study (D21), LDL-c subfractions profile was assessed using Lipoprint® electrophoresis by summing the subfractions 3–7.
Results: At baseline, 3–7 LDL-c subfractions corresponded to a healthy profile in control (1.36 ± 2.77 mg/dL). They were significantly higher in MS groups than in controls: 3.55 ± 5.49, 2.06 ± 3.00 and 2.35 ± 3.13, for Re, rE and re groups, respectively. At D21, these values dropped significantly to 1.29 ± 1.77, 0.97 ± 1.28 and 0.85 ± 1.50 corresponding to a decrease of 63%, 53% and 71%, respectively, and were no more significantly different from controls. These results failed to demonstrate a difference in efficiency irrespective the mode of applied exercise.
Discussion: The use of Liporpint® device allows discriminating the specific effect of LDL-c subfractions. Contrary to previous results showing a better effectiveness of Re on visceral fat loss, the improvement of atherogenic profile of LDL-c after treatment of MS by diet and physical activity is independent of the mode of applied exercise.

33-O059
Etifoxine-induced acute hepatitis: a case series

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Introduction: Etifoxine chlorhydrate, a benzoxazine derivative, was approved in 1979 for the treatment of psychosomatic manifestations of anxiety. A single case of etifoxine acute hepatitis has been published so far, but other cases have been registered in the French pharmacovigilance database (FPD). A recent and convincing case of etifoxine hepatotoxicity prompted us to review all relevant cases retrieved in the FPD.
Methods: All cases of liver disorders involving etifoxine coded as a suspected drug and reported between November 1995 and September 2011 were extracted from the FPD. After careful review, only cases with suggestive chronological events, no other drug or non-drug other causes, and sufficient information, were included.
Results: Of the 30 selected cases, 18 were retained for further analysis (16 women and two men; mean age: 48.3 ± 17 years). The median daily dose of etifoxine ($n = 13$) was 150 mg/day and the median delay between the initiation of etifoxine and the onset of symptoms was 18 days (range: 11–61 days). Twelve patients experienced clinical symptoms such as asthenia, abdominal pain, pruritus and jaundice. Results of liver tests evidenced cytolytic hepatitis in 15 cases and mixed type hepatitis in 3. One patient exposed to etifoxine during 16 days and also to lisinopril/hydrochlorothiazide during 13 months developed a fulminant hepatitis that required liver transplantation 6 weeks after the onset of symptoms. Six other patients had biological signs of severity with jaundice or marked hyperbilirubinemia. Two patients were positive for antinuclear antibody (1/320 and 1/640), but negative for other specific liver autoantibodies. Except for the transplanted patient, 15 patients fully recovered within 3 months and two significantly improved (further outcome unknown) after etifoxine withdrawal.
Discussion: The only previously reported case described severe acute cytolytic hepatitis occurring in a 76-year-old woman after 11 days of etifoxine treatment. This case and our series confirm that etifoxine can cause acute cytolytic and/or mixed hepatitis with possibly severe outcome. This potentially severe adverse effect not mentioned in the summary of the product characteristics should be weighed against the limited efficacy of etifoxine.

33-O060
Azathioprine or 6-mercaptopurine associated with coagulation disorders

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Introduction: Azathioprine and its metabolite 6-mercaptopurine (6-MP) are purine analogs used in organ transplantation, acute leukemias and several chronic inflammatory diseases. As a decrease in factor V activity associated with both drugs was recently published, our aim was to review all possible cases reported in France.
Methods: Cases of decrease in prothrombin time or factor V activity involving purine analogs were extracted from the French pharmacovigilance database. Reports with evidence of disseminated intravascular coagulation, acute hepatocellular insufficiency, liver cirrhosis or concomitant vitamin K antagonist treatment were excluded.
Results: Twenty-four cases (azathioprine: 13 and 6-MP: 11; median dose: 125 mg/day) were retained (18 women and six men; mean age: 40.2 ± 19.6 years). Indications were inflammatory bowel diseases in 11 patients, acute leukemias in 8, and other autoimmune diseases in 5. Prothrombin time before treatment was normal in all nine tested patients. All patients were asymptomatic when coagulation disorders were detected, i.e. after a median treatment duration of 12 weeks (range: 2–728). The lowest values for the prothrombin time and factor V activity ranged from 40% to 64% (median: 51.5%) and 21% to 57% (median: 36%), respectively. Other coagulation factors measured in some patients were inconsistently decreased (decreased factor II in 3/11, factor VII in 8/10, factor X in 2/6). Partial activated thromboplastin time was increased in all eight tested patients. A slight increase in liver enzyme levels (≤3 N) was found in 7, and four had concomitant pancytopenia or severe neutropenia. Except for two patients who continued treatment and had persistent coagulation disorders, all others recovered within 3–60 days after purine analogs discontinuation (outcome unknown in 1). In four patients, drug rechallenge was associated with recurring coagulation disorders.
Discussion: Although the mechanism of purine analogs-induced coagulation disorders remains to be elucidated, our series suggests their causative role with a clear causal relationship in four patients. Interestingly, all patients were asymptomatic and the two patients who continued treatment despite coagulation abnormalities experienced no further decrease in prothrombin time of factor V activity.

33-O061
Patient reporting of adverse drug reactions: experience of Toulouse regional pharmacovigilance center

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Introduction: Patient Reporting of Adverse Drug Reactions (ADRs) was legalized in France in June 2011. The aim of this study was to analyze the patient reporting of ADRs to Toulouse Regional PharmacoVigilance Center (RPVC) before and after the legalization.
Methods: We analyzed ADRs reported by patients to Toulouse RPVC between October 1st, 2009 and October 31th, 2011. We excluded 22 notifications corresponding to benfluorex and H1N1 vaccines, since specific procedures were implemented by Regulatory Authorities (RA) for these drugs.
Results: A total of 21 patients' ADRs reports were registered during this period. There was a significant increase of patients' ADRs reports after June 2011 (3.3 vs. 0.4 ADRs reports per month; $P = 8.9.10^{-4}$). Most of the reports were sent by mailing (38%) or e-mail (29%), by patient himself (76%) or his family (24%). Most of the observations (86%) were well informed. The mean age was 52 years (SD = 21; min = 13, max = 80) and the sex ratio was 0.6 (H/F). Most of the ADRs were 'serious' (81%), medically confirmed (86%), and 'expected' with the suspected drug (76%). ADRs mainly concerned musculoskeletal system (24%) and principally involved 'Nervous system' (32%), 'Antiinfectives' (28%) and 'Respiratory system' (12%) drugs. In 48% of cases, the suspected drug was associated with warnings of the RA or was included in the list of 'drugs enhanced surveillance' edited by the RA.
Conclusion: This study underlines the compliance of patients to ADRs reporting. Most of the reported ADRs were 'serious', with a good intrinsic quality. Patients' ADRs reports could contribute to a better evaluation of drugs safety profile.

33-O062
Notifications of adverse drug reactions reported by pharmacist students in pharmacy in Loire-Atlantique and Vendée departments: differences between student and physician reports

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Introduction: Since 10 years, we have set up an adverse drug reactions (ADRs) report collecting system thanks to student pharmacists by educational intervention. Fourty-three pharmacist students were involved during their internship in pharmacies from Loire Atlantique and Vendée. Over the period of reference from November 2010 to May 2011, each notification was classified by Anatomical Therapeutic Chemical (ATC) and the System Organ Class (SOC). The aim of this study is to analyze these reports and to compare them to physicians reports during the study period.
Observation: A total of 97 notifications were recorded with 192 ADR associated with 129 drugs of which 54 generic drugs (41.86%). Eight cases of serious adverse effects (SAE) (8.25%) were identified. The most reported drugs were antibacterial agents (12%), analgesics (12%), followed by NSAIDs (10%) and psycholeptics (8%). The most reported ADR were gastrointestinal (18%), ear and labyrinth (13%), psychiatric (13%) and skin and subcutaneous tissue disorders (13%).
A total of 48 notifications were recorded by physicians with 60 ADR with 48 drugs of which seven generic drugs (14.58%). More frequently SAE (16% vs. 8%) and more skin disorders (24% vs. 13%) have been reported by physicians but less gastrointestinal disorders (10% vs. 18%) in comparison to student notifications.

Generic drugs have not been frequently noted in these reports (14.58% vs. 41.86%). Vaccines and drug used in diabetes were involved in 25% of ADR (75% SAE), but had not been notified by students.
Discussion: ADR reports originating from students have their own characteristics: less serious effects, particular classes of drugs and more reports of generic products. The surveillance of generic products is an integral part of their professional duties. Differences between students and physicians reports appeared important perhaps due to that patient's relationship is not the same.
Since June 2011, patients can report ADR and the French competent authority will promote specific programs to involve the patient in the reporting system. This decision could contribute to the identification of new drug safety signals. In a near future it will be interesting to compare the different types of ADR reported by health professionals and patients.

33-O063
Methods of preparation of solid oral forms and medication errors in elderly nursing homes in Poitou-Charentes
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Objective: The study aimed to identify the main issues related to the methods of preparation of solid oral forms in elderly nursing homes in Poitou-Charentes region, and to approach the nursing staff actions when confronted with a risk of error, near-error or medication error.
The objective was to propose recommendations to minimize such medication errors.
Methods: Two questionnaires were sent to the managers of each elderly nursing home in the Poitou-Charentes, i.e. 640 questionnaires to 320 elderly nursing homes. The managers were requested to release the questionnaires to the nursing staff exclusion made of physicians and pharmacists. Answers to the Poitiers' University hospital were anonymous.
Outcomes included information on the quality of the respondent, the methods of preparation and administration of solid oral forms, the approach of nursing staff faced with risk of medication error, including the risk of confusion between solid oral forms (administration of drugs, consequences for patient, reporting of the errors, actions taken) and suggestions to prevent medication errors.
Results: The response rate was 48.6%; 60.8% were nurses. Forty-five percent of participants faced a situation of a mix-up between solid oral forms and 62.1% of them reported that the drugs were administered to patients. Most of these errors were asymptomatic. Half of respondents confronted with a confusion reported the case to their manager, or to a healthcare professional (doctor, pharmacist, pharmacovigilance regional center, etc.).
Conclusions: The methods of preparation of solid oral forms are heterogeneous and may be a direct cause of medication errors. Preventive systems and precautionary measures such as improving the quality of drug-use-process, making easier identification of solid oral forms and facilitating the reporting of medication errors should be put in place to reduce drug administration errors. Besides, the new recommendations for drugs care quality management in health institutions are setting up preventive measures such as local reporting and management of medication errors.

33-O064
Adverse drug reactions to proton pump inhibitors: a review of the French pharmacovigilance database
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In 2009, the French Health Technology Assessment Agency (HAS) have reassessed proton pump inhibitors (PPIs) and published a guideline for their safe and effective use. PPIs are, indeed, among the most widely prescribed medications, often without appropriate indications and long-term use, sometimes lifetime, is becoming increasingly frequent. After many years of widespread use, PPIs have been demonstrated to be relatively safe, the most often reported adverse drug reactions (ADRs) being headache, abdominal pain, diarrhea and skin rashes. However, recent publications have raised concerns about their potential toxicity while some medications of this group are now over-the-counter medicines.
Aim: The aim of this survey was to characterize the safety profile of five PPIs available in France, using data reported through the French Pharmacovigilance system.
Methods: All cases of ADRs (with at least 'possible' according to the French causality assessment score) associated with esomeprazole, lansoprazole, omeprazole, pantoprazole, and rabeprazole registered in the French Pharmacovigilance database from January 1, 2010 to December 31, 2010, were reviewed. We focused on ADRs reported in the following system-organ classes: hematologic disorders, cutaneous and neuropsychiatric events. For each of these reported ADRs, information about patient (age, gender, medical history), drug exposure (long-term use of PPIs, suspected and concomitantly used drugs), characteristics of ADRs (imputability score, time of onset, seriousness, outcome) were collected.
Results: A total of 826 adverse effects were identified. Esomeprazole was associated with the highest cumulative rate of ADRs (49%), followed by omeprazole and pantoprazole (19% each), lansoprazole (9%) and rabeprazole (3%). The most frequently reported ADRs were hematological effects (25%), including thrombocytopenia (40%) and agranulocytosis (15%), followed by cutaneous effects (20%), including bullous dermatosis (21%), and by neuropsychiatric disorders (10%).
Discussion and conclusion: We observed an unequal distribution of hematologic ADRs among the class of PPIs with numerous effects involving esomeprazole. This descriptive analysis highlights an over-representation of unexpected ADRs, like hematologic and cutaneous complications. These observations have to be further investigated to overcome spontaneous notification limitations but, now, physicians should be aware of the possible occurrence of some serious ADRs when using PPIs.

33-O065
Medicine-related deaths: use of the hospital database in a teaching hospital
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Objective: To identify medicine-related deaths (MRDs) from the hospital database and to compare these data to those reported to our pharmacovigilance centre.
Methods: A retrospective study was conducted over a 35-month period. Preselected ICD-10 codes evoking medicines or adverse effects were used to extract hospital stays with fatal issue from the hospital database. Corresponding hospitalisations discharge summaries were extracted and sorted out after double reading, in possible-MRDs or other cause of death. A validation committee confirmed MRDs. A description of the population, type of adverse effects and type of medicines involved was performed.
These data were compared to those reported to the pharmacovigilance centre.
Results: Of the 8800 deaths that occurred during the study period, 671 corresponded to our selection criteria. The electronic hospital discharge summary corresponding to the fatal stay was not available for 184 cases (27.4%), which were not included. Among the other 487 cases, 101 were considered as possible MRDs. The mean age of patients was 71 years. Medicines the most often involved were oral anticoagulants, followed by anticancer drugs, antiplatelet agents and non-steroidal anti-inflammatory drugs. Haemorrhages (n = 55) represented the most frequent cause of death (mostly due to oral anticoagulants), before infectious causes (n = 31) (mostly due to anticancer drugs). More than half deaths (55%) occurred in an intensive care unit.
Twenty-four cases had been reported and identified in the hospital database of whom 5 belonged to the 101 cases validated.56 other MRDs had been reported but not identified.
Discussion: These results confirm the magnitude of fatal adverse effects of oral anticoagulants and anticancer agents, already pointed by other studies in France or elsewhere. Use of the hospital database to identify MRDs and improve reporting is possible but time-consuming. This pilot study shows that more precise coding of hospital stays by medical units could facilitate identification of cases; there is also a need to further improve the method for extracting potential cases of the database. A further study is ongoing, which could be performed on a regular basis to obtain indicators on trends of MRDs, at least for the more identifiable cases: haemorrhage from anticoagulants and anticancer agent-related deaths.

33-O066
Auto-immune diseases as sequelae of drug-induced hypersensitivity syndrome
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DRESS (Drug Rash with Eosinophilia and Systemic Symptoms) is a delayed systemic disorder mediated by T lymphocytes and macrophages. After drug withdrawal, and corticotherapy (CC) for severe organ failure, the recovery is usually complete.
Objective: To describe two cases of auto-immunity and review literature data on auto-immune thyroid, pancreas, skin and liver diseases, following DRESS.
Methodology: Data from two patients with DRESS were recorded. Delayed complications following DRESS were searched on Pub med. Cases with diagnosis of autoimmune diseases were retained. Fulminant type 1 diabetes, without autoantibody, was excluded.
Results: *Case 1*: A 5 years old girl was diagnosed with severe DRESS 27 days after initiation of oxcarbazepin for epilepsia. She had multiple organ involvement with nephritis, hepatitis, eosinophilia, hemolysis. Oxcarbazepin was stopped and CC at 1 mg/kg/day introduced. Her clinical condition dramatically improved. CC was slowly tapered with only some eosinophil flares. Ten months later, when CC dose reached 3 mg/day, hyperthyroidism with positive anti-TSH receptor and antithyroglubulin antibodies appeared, revealing Graves disease. She is currently treated by carbimazole.
Case 2: A 56 years old man, HIV-infected, had manifestations of DRESS (fever, rash, hepatitis) 1 week after the exposure to amoxicilline/clavulanic acid. Autoantibodies were negative. CC at 1 mg/kg/day was introduced, then slowly tapered for 6 months. Hepatic enzymes deteriorated 6 months later, reaching 5–7 fold normal values. All hepatitis virus were negative. Antinuclear and antiactin antibodies became positive. Liver biopsy showed lymphoplasmocytic infiltration with fibrosis. Type I autoimmune hepatitis was diagnosed, treated by immunosuppressive drugs, with favorable evolution.
In literature, several cases of auto-immune diseases triggered by DRESS were reported: thyroiditis (Graves disease, hypothyroidism), diabetes, (with anti-GAD and anti-IA2), hepatitis, bullous pemphigoid, connectivitis. Median age was 39 years (5–69). Generally, autoimmune symptoms appeared at CC withdrawal or rapid tapering.
Discussion: This data show that DRESS can be complicated by autoimmune diseases. Some authors suggested the role of viral reactivation associated with DRESS. An alternative hypothesis is the modification of self-proteins by reactive metabolites derived from the culprit drug. Patients who experienced severe DRESS should be followed for clinical and biological signs of auto-immunity, especially at CC withdrawal.

33-P441
Hypertriglyceridemia during anti-TNF therapy
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Introduction: Anti-TNF biotherapies are now largely prescribed in the treatment of autoimmune inflammatory rheumatic or bowel diseases. In some of these pathologies, inflammation appears to affect lipid levels. Thus, anti-TNF therapy

could play a protective role in cardiovascular system by the inhibition of the pro-inflammatory effect of TNF cytokine. However, some recent studies reported contradictory results such as increased lipids. We report five additional cases of hypertriglyceridemia during anti-TNF therapy.
Cases: Hypertriglyceridemia was observed in five patients (4M/1F, mean age 45 years [28–56]) treated with anti-TNF (infliximab $n = 4$; adalimumab, $n = 1$) for ankylosing spondylitis ($n = 3$, including one case with Crohn's disease), severe rheumatoid arthritis ($n = 1$) and psoriatic arthritis ($n = 1$).
The triglycerides peak level ranged from 4.26 to 35.80 g/L. The delay of onset of hypertriglyceridemia was between 2 weeks and 9 months after the start of treatment. Only two patients had a concomitant increased cholesterol level (3.62 and 3.67 g/L).
Three patients had history of dyslipidemia, controlled by fenofibrate in one case. Before the beginning of anti-TNF therapy, basal TG levels ranged from 2.59 to 4.42 g/L. The patient with the highest hypertriglyceridemia (35.80 g/L) had to discontinue anti-TNF therapy and to start fenofibrate. Within 2 months, decreased TG values (7.54 g/L) were observed. In two patients, anti-TNF therapy was maintained with addition of statin or fibrate therapy, resulting in return to initial TG values. In one patient, anti-TNF was continued without modification of TG values (4–4.5 g/L). In the last patient, the discontinuation of the treatment and the evolution were not mentioned.
Discussion: After initiation of anti-TNF biotherapy, five patients experienced hypertriglyceridemia (or worsening of their dyslipidemia). Two cases were particularly severe. Three cases of marked hypertriglyceridemia during anti-TNF therapy have been published [1]. This side effect could be related with the modulation of activity of enzymes implied in lipids metabolism like lipoprotein lipase or 7α-hydroxylase.
It appears that lipids monitoring should be performed in patient treated by anti-TNF therapy, especially those with history of vascular disease or dyslipidemia.
Reference:
1. Haroon M, Devlin J. Marked hypertriglyceridemia upon treatment with etanercept. Joint Bone Spine 2009; 76:570–1.

33-P457
Metformin: from pharmacovigilance to medical practices improvement ! A morbi-mortality review (MMR)
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Contexte: Despite publications of metformin's lactic acidosis, no case had had been reported to Rouen pharmacovigilance center (PC) before 2007. In parallel, we noticed a lack of knowledge of those risks among emergency department (ED) professionnals and a positive impact on rate of case identification after informal information of one ED practitioner in 2007.
Objective: To improve identification and medical care of patients hospitalized for metformin's adverse effects.
Methods: In 2009, in collaboration with the PC, ED led a morbi-mortality review of reported cases and set up a protocol of care, dosing and notification.
Results: During the overall period, 45 cases were reported: seven before and 38 after MMR with a respective mortality rate of 43% and 23.5%. Hospitalisation in intense care was required in 19 cases (42%, 57% before MMR, 35% in 2011), dialysis in 8.
Metformin was took alone in 17 cases (38%), associated with other antidiabetics drugs in 28 (62%), one or two diuretics in 17 (38%), ACE in 15 (33.5%, associated with diuretics in 8). Among other risk factors, we identified digestives disorders in 13 cases (29%), cardiovascular disorders in 24 (53%), a background of respiratory disorders in 6 (13%), of renal failure in 5 (11%).
Hospitalization motives were digestive symptoms for 24 patients (53%), cardiologic troubles for 15 (33%), neurological for 14 (31%).
Biological investigations showed hyperkalaemia in 36 cases (80%), hyperlactatemia in 42 (93%), hyperglycaemia in 35 (78%), renal failure in 40 (89%). Measured in 41 patients, the Ph showed acidosis in 31 cases (69%). Dosed in 38 cases, metforminemia was upper therapeutic limits (1.3 mg/L) in 35 (78%), upper 5 mg/L in 29 (76%).
Discussion – Conclusion: A better knowledge of adverse effects can improve notifications and, more important, medical care: this study shows that a PC, close to physicians, can be a source of informations useful to patients. And, as most of these cases presented risk factors, it seems important to inform, not only ED, but all health professionals to allow the best use of metformin.
Reference:
1. Bailey CJ, Turner RC. Metformin. N Engl J Med 1996;334(9):574–9.

38-P153
PNPLA 3 rs738409 GG homozygote status is associated with increased risk of hepatocellular carcinoma in cirrhotic patients
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Background and aims*:* PNPLA3 rs738409 GG variant has been linked with the risk of cirrhosis in metabolic, alcoholic and hepatitis C liver diseases. Our goal was to study its connection with increased risk of hepatocellular carcinoma (HCC) in cirrhosis from different etiologies, taking other known HCC risk factors into account.
Methods: patients were included in the ongoing hospital multicentric case-control CiRCE study on environmental and metabolic risk factors of HCC. This study was based upon the first 304 cirrhotic French patients (270 men and 34 women): 152 with HCC (cases) and 152 without HCC (controls) matched for gender, age (±3 years), and date (±3 years) of cirrhosis diagnosis. Relationship between PNPLA3 rs738409 polymorphism and HCC was studied using unconditional logistic regression.
Results: One hundred and seventy seven patients (58.3%) were PNPLA3 rs738409 G allele carriers: 120 (39.5%) CG heterozygotes and 57 (18.8%) homozygotes GG. Age, time lag between diagnosis of cirrhosis and inclusion, hepatitis B or C infection, diabetes and PLPNA3 minor homozygote status were more frequent in cases than in controls. In multivariate analysis, PLPNA3 GG homozygote status was associated with HCC: OR 2.59 [1.29–5.20] ($P = 0.007$), after adjustment for matching factors, hepatitis B and C infection, diabetes, alcohol consumption and severity of the underlying cirrhosis. No interaction between PNPLA3 status and other covariates was significantly associated with HCC.
Conclusion: this study demonstrated a strong relationship between PNPLA3 GG homozygote status and HCC in cirrhotic patients, independently from cirrhosis etiology and severity. The usefulness of the PNPLA3 rs738409 allele in detecting cirrhotic patients at high risk of HCC, justifying close follow-up and aggressive preventive treatments, must be evaluated.

38-P154
A population pharmacokinetic study of the epinephrine/cisplatin combination during intraperitoneal perioperative chemotherapy: intraperitoneal clearance as a potential biomarker
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Objective: Intraperitoneal (IP) perioperative chemotherapy with cisplatin is an interesting option in ovarian cancer treatment. In a phase I trial, combination of cisplatin with IP epinephrine was shown to improve IP exposure to platinum and decrease systemic concentration. Such a combination was evaluated using a population pharmacokinetic analysis to provide a better understanding of this phenomenon and to determine to what extent pharmacokinetic parameters could be used as biomarkers.
Patients and methods: Data from 55 patients treated with cisplatin-based IP perioperative chemotherapy with ($n = 26$) or without ($n = 29$) epinephrine were analyzed using NONMEM. ROC curves were calculated for each PK parameter, allowing us to assess diagnostic values for renal toxicity.
Results: Binding of platinum (Pt) to protein was modeled leading to a unique model for both ultrafiltered and bound Pt. A three-compartment model with first-order elimination from the serum (central) compartment together with an additional compartment for the protein binding modeling best fitted the data of all patients. Epinephrine halves clearance between peritoneum and serum (IPCL) and increases the central volume of distribution of platinum. When epinephrine is administered, IP platinum is higher than 10 mg/L for longer and Pt penetration in tissue seems deeper. The IPCL is the pharmacokinetic parameter displaying the best predictive values with respect to renal toxicity. Interestingly, IPCL estimation using only one sample after either Bayesian estimation or direct calculation also gives good diagnostic values.
Discussion: Diagnostic values confirm that this parameter could be useful in assessing renal toxicity. Moreover, as IPCL is also linked to pharmacodynamic parameters (cisplatin penetration distance assessment and length of IP exposure), we assume that this parameter could be used as biomarker. As well as a Bayesian estimation, we propose a way to calculate this biomarker with a single sample taken just before the end of the administration. Prospective studies are nevertheless needed to confirm these data and validate IPCL as a biomarker of cisplatin during IP perioperative chemotherapy.

38-P155
Acquired resistance to oxaliplatin involved polyploidy and double strand break repair in the two human colon cancer cell lines HCT 116 and SW480
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Introduction: Oxaliplatin is a DNA-damaging agent currently used in clinic, but its efficacy may be restricted by occurrence of acquired resistance to an initial efficient treatment. Mechanisms of resistance may occured by a polyploidisation/depolyploidisation process. As cytotoxicity of platinum compounds is related to the formation of double strand breaks (DSB), we were interested in determining in two human colon cancer cell lines whether polyploidisation and DSB repair could be involved in oxaliplatin acquired resistance.
Methods: Human colon cancer cell lines HCT116 and SW480 were treated with oxaliplatin for 2 h at its IC_{90} concentration previously determinated by a clonogenic assay. Cells were analyzed at intervals during 14 days post-treatment. Cell proliferation was measured and apoptotic cell death was evaluated using annexin V coupled with FITC and caspase 3 western blotting. DSB formation and kinetic were measured by quantification of g-H2AX by FACs. DSB repair proteins and polyploidy were assessed respectively by western blotting and cell cycle analysis. CGH-array and clonogenic assay were performed on cells resistant to oxaliplatin initial treatment.
Results: Oxaliplatin induced cell proliferation arrest associated with early and temporary apoptotic cell death in HCT116 and SW480. DSB occurred earlier and in a transient manner in HCT116 whereas DSB remained low in SW480. However, DSB repair were activated in both cell lines. Polyploïdy was much higher in HCT116 than in SW480 and characterization of cells which survived to oxaliplatin exposure displayed recurrent chromosomal abnormalities only in HCT116 resistant cells. However, these 'escape' cells were more resistant to a second treatment by oxaliplatin than parental cells.

Conclusion: In our study, oxaliplatin leads to transient cell proliferation arrest, DSB formation and repair, polyploidy and generates escape cells more resistant to oxaliplatin than parental cells in both HCT116 and SW480, suggesting similar sequences of events involved in acquired platinum resistance. Future investigations will focus on understanding the underlying signaling pathways.

38-P156

Anti-cancer properties of cranberry juice in human colon cancer: characterization of the cellular and molecular mechanisms

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Objective: Polyphenols are natural compounds widely present in fruits and vegetables, and well known for their beneficial effects on health and diseases, including cancer. In the present study, several polyphenol-rich berry juices were evaluated for their antiproliferative effect in different human colon cancer cell lines. Our major objective was to characterize the cytotoxic properties of the most effective berry juice, and to elucidate the underlying cellular and molecular mechanisms.
Methods: Cytotoxicity was determined by the MTS assay. The cell cycle progression and the apoptosis rate were assessed by flow cytometry using propidium iodide and annexinV/TUNEL methods, respectively. Regulation at the expression level of major proteins of interest was examined by Western blotting.
Results: Chokeberry, blackcurrant, cranberry, blueberry and lingonberry juices significantly reduced the proliferation of four human colon cancer cell lines, HCT116, HT29, SW480 and Caco-2 cells. We observed that cranberry juice (CJ) was the most effective juice on cancer cells. The characterization of its cytotoxic effect revealed a cell cycle arrest in G2/M phase in HCT116 and HT29 cells in a concentration-dependent manner. Triggered apoptosis by CJ was demonstrated by using AnexinV and TUNEL assays, showing increased percentages in early apoptotic death and in the fragmentation of DNA. Moreover, the induced apoptosis was associated with an increased expression level of the apoptotic marker proteins, cleaved-caspase-3 and cleaved-PARP, and a downregulation of the antiapoptotic proteins, UHRF1 and Survivin. The G2/M cell cycle phase block was associated with Cyclin B1 downregulation. Enhanced gamma-H2AX phosphorylation was observed, suggesting activation of DNA damage response. Additionally, the stress-controlling and deacetylator of p73/KU70/FOXO proteins, SIRT1 showed a downregulation pattern concomitant with the upregulation of FOXO3a and p73. Finally, our findings showed that the non permeant SOD mimetic (MnTMPyP) prevented the apoptotic effect of CJ suggesting a key role of intracellular oxidative stress and in particular superoxide anions.
Discussion: Altogether, the present findings indicate that CJ inhibits proliferation and induce apoptosis of human colon cancer cell lines, likely by targeting the SIRT1 pathway. Hence, cranberry juice, a nutritional source of polyphenols, might open new therapeutic perspectives for the treatment of colorectal cancer.

38-P235

Risk of breast cancer recurrence is not increased by the use of local estrogen therapy among endocrine-treated patients

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Women with estrogen-sensitive breast cancers are to receive endocrine treatment for 5–10 years. An important side effect of the currently used drugs, tamoxifen and aromatase inhibitors (AI) is vaginal dryness. Local hormonal therapy represents the most effective treatment of vaginal dryness and is being prescribed despite its theoretical contraindication. This study aimed to assess the breast cancer recurrence risk among women receiving endocrine treatment and LHT compared to endocrine treatment alone.
We performed a nested case-controls study using the General Practice Research Database (GPRD). Included patients were female above the age of 18 years, with a first breast cancer, at least one AI or tamoxifen prescription between January 1, 1998 and Decembre 31, 2009 and at least 1 year follow-up. Cases were patients experiencing breast cancer recurrence and were matched to up to 10 controls based on birth year, date of cohort entry and follow-up duration. Logistic regression conditioned on age, cohort entry date and endocrine treatment type was used to estimate rate ratios (RR), and 95% confidence intervals. Adjustment on smoking, alcohol abuse, obesity, oophorectomy and previous use of certain drugs (hormone replacement therapy, anti-diabetic agents, NSAIDs, benzodiazepines, antipsychotic drugs, statins, with special focus on CYP2D6 inhibitors) was performed.
A total of 13 479 women were included, 10 806 receiving tamoxifen and 2673 receiving AI. Mean age at cohort entry was 63.7 (14.1) years, and mean (SD) follow-up was 3.5 (2.6) years. There were 917 cases, 811 with tamoxifen and 106 with AI. Cases seemed more likely than controls to have a BMI > 30 kg/m^2, to be current smokers, to have used anti-psychotic drugs and benzodiazepines, and less likely to have used CYP2D6 inhibitors. Local hormonal therapy (LHT) was found in 2.1% of cases and 2.8% of controls. The adjusted RR for recurrence was 0.79, 95% CI 0.49–1.27 for patients receiving LHT. For tamoxifen-treated patients, the RR was 0.83, 95% CI 0.51–1.35. None of the AI-treated patients who experienced breast cancer recurrence had received LHT, making subgroup analysis inconsistent.
The use of LHT is not associated with an increase in breast cancer recurrence among women receiving an endocrine therapy.

38-P247

Patterns and effectiveness of bortezomib use in elderly patients: the VESUVE cohort

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Bortezomib (BTZ) represents an important progress in the treatment of multiple myeloma (MM). BTZ combined with other agents is becoming a standard of care particularly in elderly patients not eligible for autologous stem cell transplantation. To date, no evaluation of BTZ in a real-life setting has been conducted in France. The objectives of this study were to describe and compare patterns and effectiveness of BTZ use in two age groups: ≤75 vs. >75 years.
VESUVE is a national cohort conducted in 60 French centres that included patients initiating BTZ from May 2004 to April 2006 using nominative hospital pharmacy dispensations. Patients treated for MM were followed for 2 years. Data were collected through medical files using standardized questionnaires. BTZ cycles were categorized as standard or not (dosage, number and rhythm of injections) according to market authorisation. Response was assessed by an independent committee according to adapted International Myeloma Working Group criteria.
Among the 793 patients included, 82.3% were 75 years old or less and 17.7% were older. Regarding usage patterns, median BTZ cumulative dosage per cycle was 8.0 mg [p25%-p75%] [6.9–9.1] in younger patients vs. 7.3 mg [6.0–8.2] in older patients ($P < 0.01$). The proportion of patients with at least one non-standard cycle was 68.0% in younger patients vs. 77.9% in older patients ($P = 0.02$). Adjunction of anti-myeloma agents after BTZ initiation was more frequent in younger patients (35.0% vs. 23.1%; $P = 0.03$) especially conventional chemotherapy (24.4% vs. 0.0%; $P < 0.01$).
Among the 588 evaluable patients for response, the overall best response rate was 57.1% in younger patients vs. 64.1% in older patients ($P = 0.19$).
The 2-year overall survival (OS) rate was 44.2% (95%CI 40.2–48.0; median 20.4 months) in younger patients vs. 36.3% (28.2–44.4; 14.1 months) in older patients ($P = 0.02$).
The 2-year progression-free (PFS) rate was 11.6% (9.3–14.3; 7.3 months) in younger patients vs. 13.8% (8.6–20.3; 6.5 months) in older patients ($P = 0.69$).
BTZ usage patterns were different according to age due to treatment adaptations in elderly patients. The OS rate was lower in patients over 75 years but similar PFS and treatment response rates were found between younger and older patients.

38-P400

Adverse drug reactions in patients treated for colorectal cancer: from the oncologists to the patients

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Background: Cancer is becoming a public health priority. Despite very different safety profiles, conventional chemotherapy drugs as well as new drugs, called targeted therapies, cause many adverse effects. Thus, treatment adaptations are often performed according to patient tolerance. This study was designed to estimate the frequency and to describe the characteristics of adverse drug reactions (ADRs) in patients treated for colorectal cancer in an oncology unit.
Method: Patients initiating a treatment by chemotherapy with or without targeted therapy for colorectal cancer were identified prospectively from 15 February to 15 April 2011 in the oncology unit of a teaching hospital and retrospectively from 1 January 2009 to 31 December 2010 by means of the hospital chemotherapy management software. Data regarding socio-demographic characteristics, the cancer, the treatments and the ADRs that occured during the treatment were collected through computerized medical and nursing records using a standardized form. For the prospectively included patients, data on ADRs were also collected through a patient self-administered questionnaire.
Results: In the retrospective part, 109 patients were included who had a total of 717 ADRs. Gastrointestinal effects (38.2%), general symptoms (13.1%), especially asthenia, and cutaneous effects (13.0%) were the most frequent. According to the criteria used in pharmacovigilance, 10.3% were severe, while 9.3% were classified as grade 3 or 4 according to National Cancer Institute Common Terminology Criteria for Adverse Events. In 15.3% of the cases, the ADR led to treatment adaptations (drug stopped, dosage reduced, etc). In the prospective part (19 patients included, 63 ADRs), data collected through medical and nursing records were similar to those obtained in the retrospective part. The 41 patients self-administered questionnaires reported 145 ADRs with a higher proportion of moderate and severe effects according to the patients.
Conclusion: The frequency of ADRs, especially severe, was high. Reporting of ADRs differed a lot between healthcare professionals and patients: if reporting from healthcare professionals was not exhaustive, it was more accurate in contrast to that of patients who reported almost everything they experienced. Nevertheless, the patients experience is an important factor to know and consider to improve global care and quality of life.

Impact of dialysis on pharmacokinetic and monitoring of tyrosine kinase inhibitors (example of imatinib an sunitinib)

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Introduction: In clinical practice, physicians are confronted with the question of whether patients with terminal renal failure and under dialysis can be safely offered targeted drugs such as tyrosine kinase inhibitors (TKI), while they are excluded from pivotal clinical trials. We present cases of patients requiring chronic hemodialysis and treated with imatinib or sunitinib.

Methods: Pharmacokinetic studies were performed on theses patients using UPLC/MS-MS to determine TKI concentrations. The first one, treated with sunitinib for recurring renal carcinoma, has been sampled to obtain an area under the curve (AUC) 18 days after the beginning of the first cycle of treatment, and then was monitored with trough concentration at different times of each seven cycles. AUCs of a second patient, diabetic, treated with 400 mg-imatinib for chronic myeloid leukemia, were performed the day before and the day with dialysis (D1 and D2 respectively) and imatinib was sought in dialysate. A third patient (with low-weight, 39 kg) was admitted for meningoencephalitis: imatinib dose was increased to 400 mg instead of 200 mg/day consecutively to starting dialysis.
Results: Patient treated with sunitinib obtained an AUC during the first cycle of 0.866 h/µg/mL, which was consistent with previously published data for the 37.5 mg dose. It was decided to increase dose to 50 mg/day but the patient presented G1-thrombocytopenia, G2-asthenia and G2-mucositis with a standard schedule (4 weeks with treatment and 2 weeks off). Then, the sunitinib administration was modulated into a different schedule (2 weeks on, followed by 1 week off) and the sunitinib concentrations ranged 50–100 ng/mL without G2-side-effects 2 weeks/cycle.
AUCs of the first dialyzed imatinib-patient were about 28 h/µg/mL with an half-life of 18.2 and 16.9 h on D1 and D2 respectively. Imatinib concentrations in dialysate were always below 2.5 ng/mL.
Patient with meningoencephalitis showed an imatinib concentration of 5570 ng/mL at admission under imatinib 400 mg/day whereas concentrations were ranged between 677 and 1114 ng/mL under 200 mg/day during the previous 6 months and after this event.
Conclusion: The pharmacokinetic parameters didn't seem to be under influence of the dialysis. Anyhow, these findings support that therapeutic drug monitoring is necessary for this kind of patients with abnormal conditions and treatment likely modifying TKI concentration.

39-O067
Statin-induced lupus: a case/non-case study in a nationwide pharmacovigilance database
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Background: Statin use has been advocated to prevent atheromatous complications in lupus patients and may be widely prescribed for these patients in future. Statin-induced lupus has also been described, though the risk is not confirmed. The goal of this study was to detect a safety signal regarding statin-induced lupus.
Methods: We conducted a case/non-case study in the French PharmacoVigilance Database from January 2000 until December 2010. Cases were drug-induced lupus reports. Non-cases were all reports of other adverse drug reactions (ADRs). Exposure to statins at the time of ADR was screened in each report.
Results: Among 235147 ADR reports, 232 were drug-induced lupus. Exposure to statins was present in 17 (7.3%) cases and in 10601 (4.7%) non-cases. Reporting odds-ratio (ROR) for statin exposure associated with lupus erythematosus was 1.67 (95% confidence interval [1.02–2.74]). The ROR was >1 for each statin but fluvastatin.
Discussion: This pharmacoepidemiological study suggests a link between statin exposure and lupus induction. The benefit-to-risk ratio of statin therapy in lupus patients should be evaluated through randomised controlled trials.

39-O068
ARITMO Project. Torsade de pointes and QT prolongation associated to antimicrobials, antipsychotics and H1-antihistamines: an analysis of the French spontaneous reporting database
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Background: The ARITMO project (http://www.aritmo-project.org/) aims to analyse the Torsade de pointes (TdP) and QT prolongation (QTP) potential of antipsychotics, antimicrobials and H1-antihistamines. As part of this project, French pharmacovigilance data were analysed.
Aim: To identify signals associating TdP or QTP events to the ARITMO project drugs of interest.
Methods: Reports collected in the French Pharmacovigilance database between January 2000 and August 2010 were analysed. Adverse reactions are coded according the MeDDRa dictionary. The cases were searched according to MeDDra preferred term codes, and through a review of the French pharmacovigilance database free text. Statistical signals of TdP and QTP were searched pooling these events for drugs belonging to the following classes, coded according the ATC classification: antipsychotics (ATC Code: N05A), antibacterials (J01 and J04), antimycotics (J02), antiprotozoals (P01), antivirals (J05) and H1-antihistamines (R06). A signal was defined as an association between the event and the studied drugs with at least tree cases and an reporting odds ratio (ROR) lower limit of the 95% confidence interval exceeding one. Signals were considered according to the mention or not in the Arizona CERT lists of the drugs causing the related events in patients without congenital QTP.
Results: In the subset of the French Pharmacovigilance database used, 213 TdP cases and 314 QTP cases were identified, 63 (30.6%) and 145 (46.2%) of which were related to ARITMO drugs, respectively. Among the 208 total cases related to ARITMO drugs, antipsychotics (48.1%) antibacterials (24.0%), and antiprotozoals (13.0%) were the most represented drug classes. The case non-case analysis generated 26 statistical signals, 15 of which concerned ARITMO drugs not included in the Arizona CERT lists. Of these, nine signals were found among antipsychotics (acepromazine, amisulpride, aripiprazole, cyamemazine, levomepromazine, loxapine, olanzapine, tiapride and zuclopentixol), three among H1-antihistamines (alimemazine, cetirizine and loratadine), two among antimycotics (amphotericin B and itraconazole), and one among antiprotozoals (quinine).
Conclusion: This study found signals of TdP or QTP for 15 drugs not mentioned in the Arizona CERT lists. These signals will be further evaluated taking into account concomitant medications that could explain the occurrence of related events.

39-O069
Is spontaneous reporting always the most important information supporting drug withdrawals for pharmacovigilance reasons in France?
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Because of design, objectives and number of included subjects, clinical studies are insufficient to assess the safety of new drugs. Sometimes, serious adverse drug reactions (ADRs) led to withdrawal of the drug from the market after approval. The objective of our study was to determine the nature of scientific evidence leading to drug withdrawals for safety reasons in France (2005–2011) and to compare results with a previous study (1997–2004).
Drugs withdrawn from the French market due to safety reasons (2005–June 2011) were identified from the website of the French Health Products Agency. Additional information from the literature and reports of Pharmacovigilance commissions allowed to classify these withdrawals according to the nature of the evidence (clinical trials [CT], case reports [CR], case-control studies [CC], cohort studies, animal studies, observational studies). We also identified the organ classes affected and type of ADRs (A or B).
A total of 22 products were withdrawn from the French market within the study period. The most frequently classes of these drugs were 'Nervous system' (7/22; 31.8%) with analgesics and anti-psychotics and 'Alimentary tract and metabolism' (5/22; 22.7%) with diabetes and obesity medications. The withdrawal rate has increased over the study period. The nature and type of the most frequently ADRs leading to drug's withdrawal were cardiovascular (11/22, 50%), neurological (5/22; 22.7%) and hepatic (3/22, 13.6%), A (14/22, 36.4%), B (8/22, 36.4%). CR (19/22; 86.4%) and CT (13/22; 5%) were the most frequently evidence involved. In 5/22 (57%) cases, CR were the sole evidence. 72.7% (16/22) of regulatory decisions were based on multiple sources of evidence, a combination of CR and CT in half the cases. In only seven cases, a CC or cohort studies was involved.
This study reveals an increase rate of withdrawals certainly explained by the 'benfluorex affair'. As previously observed, the part of spontaneous reporting in the regulatory decisions supporting withdrawal for safety reasons remains important (22.7%) but less than during the 1997–2004 period (57%). The evidence other than spontaneous reports is cited more frequently, particularly data from CT but unfortunately not pharmacoepidemiological studies that always seem to be difficult to implement.

39-O070
What are the factors influencing consent obtainment for clinical trial in stroke patients?
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Background: Despite the need to include stroke patients in clinical trial to improve knowledges of pathophysiology and to assess new therapeutic strategies, the obtainment of consent remains a problem because of motor and cognitive consequences of cerebral injury. This situation explains that consent is often obtained indirectly from a proxy decision-maker. Identification of factors associated to a direct or an indirect consent could improve information and recruitment of stroke patient in an emergency context.
Objective: The aim of the study was to identify factors associated with the need to obtain consent by a proxy decision-maker to propose red flags before inclusion in clinical trial avoiding to include patients unable to give their own consent
Methods: Among patients included in Biostroke cohort (PHRC 2004), two groups have been defined: a group of patients who gave directly their consent to participate; a group with consent obtained from a proxy decision-maker. Different parameters have been compared between the two groups using univariate analysis: demographic data, type of stroke (transient ischemic stroke, ischemic stroke, intracerebral hemorrhage), severity (NIHSS, Rankin scale), pre-stroke cognitive status (IQ-code), post-stroke cognitive status (MMSE), etiology of stroke.
Results: A total of 201 patients (89 women and 112 men) have been analyzed. Among them, 119 patients have directly given their consent while for 82 patients the consent was obtained from a proxy decision-maker. Patients with indirect consent were more aged (71 vs. 64, $P < 0.001$), have a more severe stroke (77% vs. 17%, $P < 0.001$), have more often an aphasia (61% vs. 13%, $P < 0.001$) and a pre-stroke cognitive impairment (58% vs. 35%, $P < 0.003$). The post-stroke disability and cognitive impairment were more severe in patient given their consent through a proxy decision-maker.
Conclusion: Age, aphasia, severity of stroke and pre-stroke cognitive impairment are factors that should alert investigators to rapidly contact family to obtain consent if they want to include a patient in a clinical trial.

39-P223
Risk of cancer on the five marketed TNF-alpha antagonists in rheumatoid arthritis at recommended doses: a meta-analysis of 33 randomized controlled trials comparing intention to treat to per protocol analyses
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Background: The risk of malignancies on TNF-α antagonists is controversial. The relative interest of modified intention to treat (mITT) or per protocol (PP) analyses to assess such sparse events remains unknown. The aim of this survey was to assess cancer risk on TNF-α antagonists in adult rheumatoid patients (RA), including the five marketed drugs (infliximab, etanercept, adalimumab, golimumab and certo-

lizumab) used in line with the New Drug Application (NDA).
Methods: Data sources were MEDLINE, CENTRAL, ISI Web of Science, ACR and EULAR meeting abstracts, scientific evaluation of the drugs leading to their marketing approval, and clinicaltrials.gov. We selected double-blind randomized controlled trials in adult RA patients, including at least one treatment arm in line with NDA. We performed random effect meta-analysis, with mITT and PP analyses.
Results: Thirty-three trials were included. There was no excess risk of malignancies on anti-TNF-α administered in line with NDA in the PP model (OR, 0.93 95%CI[0.59–1.44]), as well as in the mITT model (OR, 1.27 95%CI[0.82–1.98]). There was a trend for an excess non-melanoma skin cancer risk. mITT analysis overestimated the treatment effect. In contrast, PP analysis underestimated the treatment effect when assessing very sparse events and when many patients dropped out in placebo arms. In univariate metaregression, there was no differential risk among the five drugs.
Discussion: This study did not find any evidence for an excess cancer risk on TNF-α antagonists in adult RA patients the first years of treatment. Both mITT and PP analysis should be presented in such safety analyses.

39-P224
Use of cetuximab in a real-life setting in France with respect to KRAS status – preliminary results of EREBUS cohort study
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Background: Cetuximab demonstrated survival outcome improvement in metastatic colorectal cancer (mCRC). Cetuximab was first launched as a 2nd-line therapy in mCRC. In July 2008, this indication was extended to 1st-line therapy and restricted to mCRC patients with wild-type (wt) KRAS gene. We present here the preliminary results of the French EREBUS cohort study and describe cetuximab prescription patterns according to KRAS status in a real-life setting.
Methods: EREBUS is a cohort study conducted in 92 French centres. Patients initiating cetuximab between Jan and Dec 2009 were identified from nominative hospital pharmacy dispensations. The cohort included mCRC patients treated in 1st-line. They were followed for 12 months to evaluate the rate of metastases resection, usage patterns, safety and effectiveness of cetuximab.
Results: To date, 1038 patients treated by cetuximab for colorectal cancer have been identified. Cetuximab was mainly prescribed in mCRC (98.0%): 34.4% as 1st-line treatment, 34.5% as 2nd-line, 21.4% as 3rd-line and 9.7% as 4th or more. The investigation of KRAS status was performed in 94.4% of the patients and, of these, 94.9% had wt KRAS gene. Investigation of KRAS mutation status and wt status were similar whatever treatment line (investigation: between 93.3% and 100.0%; wt status: between 93.9% and 100.0%). The investigation was performed on primary tumour (82.6%), on metastases (16.3%) or both (1.1%). The main reasons of absence of KRAS status investigation were: previous treatment by cetuximab (42.9%) and absence of available tumour material or technical issue with analysis (33.3%). Investigation of EGFR expression was performed for only 2.4% of the patients.
Conclusions: EREBUS is the first post-marketing cohort study conducted in France to describe the usage patterns of cetuximab. Extensive investigation of KRAS status and the high proportion of patients with wt status indicates adherence to market authorisation, although EGFR expression remains rarely investigated.

39-P225
Risk assessment of drug-induced DRESS syndrom: a disproportionality analysis using French pharmacovigilance database
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Drug reaction with eosinophilia and systemic symptoms (DRESS) is an uncommon severe adverse drug reaction (ADR). This ADR includes: skin rash, fever, eosinophilia and/or atypical lymphocytosis and participation of at least one internal organ. Few drugs are involved but for each drug, the risk of DRESS is currently unclear.
Aim: The aim of this study was first to identify the drugs which are more frequently associated with DRESS and spontaneously reported in the French PharmacoVigilance system (FPVD) and then to compare the risk between these drugs.
Method: All cases of DRESS reported from September 1st 2007 to August 31st 2010 were included. For the drugs most frequently involved in DRESS, a disproportionality analysis was performed considering that cases were all reports of DRESS and non-cases all the remaining ADR reports for the same drug. This method allows comparison of drug exposure among cases and non cases using the proportional reporting ratio (PRR with its 95% confidence interval).
Results: Three hundred and twelve cases of DRESS were included in the study. Patients have a median age of 57 years and 52.6% were women. Average onset of the 1st symptoms after drug introduction was 30.6 days (median 22 day) and 17 patients (8%) died. The drugs most frequently involved (>20 cases/drug) were: allopurinol, vancomycin, carbamazepine, sulfamethoxazole and sulfasalazine. However using the PRR, the risk of DRESS was higher for sulfasalazine (PRR = 53 [32;87]), allopurinol (PRR = 47 [36;63]), minocycline (PRR = 43 [20;92]) and carbamazepine (PRR = 20 [13;29]); moderate for vancomycin (PRR = 16 [11;23), strontium ralenate (PRR = 9 [4;19]), colchicine (PRR = 7 [3;13]), lamotrigine (PRR = 6 [3;12]) and cotrimoxazole (PRR = 5.3 [3.4;8.4]).
Discussion: The disproportionality analysis can be used to compare the risk of ADR between some drugs. However, this analysis is limited by the difficult exclusion of various biases, particularly those due to unequal ADR reporting among different drugs (ADR notoriety, ….) and to the over-representation of specific ADR for some drugs.
Conclusion: Despite the limits of this study, our results are an interesting approach to compare the risk of DRESS among the drugs which are usually involved in this pathology.

39-P226
Emergency admissions for major haemorrhage-related adverse effects of antithrombotic therapy
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Objective: The aim of the study was to describe the frequency and type of major haemorrhage related to antithrombotic therapy, to report the clinical characteristics, management and outcomes of patients admitted to a teaching hospital emergency department (ED).
Methods: Patients older than 16 years admitted in our ED with acute major haemorrhagic while treated by any antithrombotic agent were selected by computer requests from diagnostic codes and specific emergency therapies. Major haemorrhage was defined by at least one the following criteria: unstable hemodynamic, haemorrhagic shock, uncontrollable bleeding, need of transfusions, need of haemostatic procedure, or a life threatened location of bleeding (intracranial, gastrointestinal, pulmonary or peritoneal bleeding, compressive muscular hematoma).
Results: Between January 1 and October 31, 2011, 355 cases were selected which represented more than 1 patient per day. Median age was 82 years ± 10.3 (21–100). One hundred and seventy-four patients were taken vitamin K antagonists (24 in combinations with other antithrombotic agents), 164 patients antiplatelet medications (dual antiplatelet therapy in 14 cases), 17 others antithrombotic agents (heparin, LMWH). Major haemorrhagic accidents were: gastrointestinal tract bleeding in 40.5%, intracranial bleeding in 31.5%, muscular hematomas in 6.8%, epistaxis in 4.9%, haematuria in 3.5%, scalp bleeding injuries in 2.7%, others in 9.9%. Transfusion was needed in 55% of cases. The mean length of hospital stay was 7.7 days. The overall mortality was 12.3%, mostly in the intracranial bleeding group, and was independent to the type of antithrombotic. In the vitamin K antagonist group, only 41% of patients have received K vitamin treatment (dosage >10 mg in 75%), 33% have received prothrombin complex concentrate, 26.5% received both treatments. The mean delay between admission time and reversal time was 4 h 30 ± 3 h 40. Vitamin K antagonist treatment was definitely stopped in 3/4 of cases. In the antiplatelet medication group, no specific treatment was done. Antiplatelet treatment was definitely stopped in 2/3 of cases.
Conclusion: This register shows the magnitude and the severity of haemorrhage-related adverse events in patients treated with antithrombotic agents in a ED, suggesting a great vigilance in risk benefit imbalance in elderly.

39-P227
Adverse drug events (ADEs) caused by self-medication (SM) Preliminary results of a prospective multicentric survey in 11 emergency french departments (EDs) (APNET study group)
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Background: The regular use of self-medication to treat minor common illnesses is probably frequent in French population. EDs are an ideal place to observe ADEs and data are available on their frequency among EDs' admission, but little is known about that related to SM (ADEs-SM).
Objective: To measure the frequency of ADEs-SM among EDs' patients, and describe their characteristic.
Method: During 2 months, periods of study were randomized in 11 EDs centres. Every adult patients admitted at theses dates were interviewed, interviewers were medical and pharmaceutical students. The patients unable to answer, or refusing were excluded. The data on medical admission, on pharmaceutical history, on self-medication habits and taken drugs and on clinical outcome were collected. The drug causality assessment was helped by the Naranjo algorithm. All doubtful files, and systematically the ADEs-SM files, were reviewed by an expert committee. The comparison between groups was made by Chi-square for quantitative data, and by t test for qualitative data.
Results: Four thousand six hundred and sixty-one patients were admitted in the EDs during studys' periods. Three thousand and twenty-seven were included.
The reasons of the 1634 (35.06%) exclusions were: self-poisoning (2.17%), patients' refusal (10.62%), patients' inability and refusal of their relatives (1.80%), patients' inability and no relatives (20.47%).
The median age of included patients were 43 years old (18–99), with 46.45% of females, and 16 pregnancies. One thousand eight hundred and fourteen patients have taken at least one prescribed drug, and 1927 declared to have taken at least one self-medication drug during the last 2 weeks. Among the included patients, there was 296 ADEs (9.78%), with 2/3 involvement of drugs in multifactorial pathologic conditions, and 1/3 adverse drug reactions. Fifty-two patients (1.72%) have experienced an ADE related to self-medication, with self-modification of prescribed drug for 19 patients, therapeutic break for 14 and a non prescribed drug for 17 patients.
Conclusion: SM is frequent among EDs' patients and clearly underestimate. The frequency of ADEs-SM is enough to be taken into account. A way to detect self-medication related pathologies should be based on intervention at the bedside of clinical pharmacist and pharmacologist. Therapeutic education in primary care could be a way to prevent ADEs-SM.

39-P236

Relationship between severity of motor symptoms, impairments in activities of daily living, disease duration, age of onset, dopaminergic treatment and levodopa-related motor complications in Parkinson's Disease

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Objective: To evaluate the relationships between severity of motor symptoms, impairments in activities of daily living (ADL), levodopa-related motor complications, disease duration, age at disease onset and dopaminergic treatment.

Methods: Idiopathic non-demented PD out-patients underwent standardized clinical examination in this cross-sectional study. PD characteristics were assessed by the Unified PD Rating Scale, parts II (impairments in ADL), III (severity of motor symptoms) and IV (severity of levodopa-related motor complications). Levodopa Daily Equivalent Dose (LDED) was calculated by the usual formula. Structural Equation Modeling (SEM) was used to assess the relationship between all possible pair of variables in one single statistical model, which is not possible with other regression techniques. Associations were measured by means of standardized regression weights (SRWs), which represent the average amount the dependent variable increases when the independent increases one standard deviation and other independent variables are held constant.

Results: Four hundred and fifty patients were recruited (age: 69 ± 1, 57% males, disease duration: 6 ± 1, UPDRS II+III: 29 ± 1). Age of disease onset correlated significantly with disease duration (swr = −0.42, $P < 0.001$), severity of motor symptoms (swr = 0.26, $P < 0.001$), LDED (swr = −0.18, $P < 0.001$), severity of levodopa-related motor complications (swr = −0.14, $P = 0.005$) but not with impairments in ADL (swr = 0.02, $P = 0.6$). Disease Duration correlated with impairments in ADL (swr = 0.20, $P < 0.001$), motor symptoms (swr = 0.44, $P < 0.001$) and motor complications (swr = 0.33, $P < 0.001$). LDED correlated significantly with the severity of levodopa-related motor complications (swr = 0.17, $P < 0.01$) but not with the severity of motor symptoms (swr = 0.05, $P = 0.6$) or impairments in ADL (swr = 0.02, $P = 0.8$). Similarly, neither severity of motor symptoms nor impairments in ADL correlated with levodopa-related motor complications (swr = 0.03, $P = 0.8$ and swr = 0.04, $P = 0.4$).

Discussion: A meaningful statistical model accounting for the relationships between parameters of PD evolution, dopaminergic treatments and levodopa-related motor complications could be developed by using SEM.

39-P237

Outcome of pregnancies exposed to oseltamivir

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Introduction: Limited data on oseltamivir safety during pregnancy is available. In France, since the pandemic influenza A (H1N1v) 2009, oseltamivir has been recommended as a preventive or curative treatment in pregnant women whatever the trimester of pregnancy. The purpose of this study was to investigate whether oseltamivir is associated with adverse birth outcomes.

Patients and methods: All prospectively assessed pregnant women exposed to oseltamivir after the 3rd week following the last menstrual period (LMP) who were collected by 17 pharmacovigilance centers were included for follow-up. Cases were excluded if the treatment period was not clearly mentioned, and the outcome of pregnancy unknown.

Results: Three hundred and twenty-five pregnancies (nine pairs of twins) met these criteria. The mean age of the patients ($n = 289$) was 28.8 ± 5.5 years and 57/217 were primigravida. Testing for influenza A (H1N1v) was positive in 96 (30%) women (89 after the 1st trimester). Thirty-nine patients also received a pandemic influenza A vaccine during pregnancy. Oseltamivir was given as a prophylactic treatment in 40 patients or a curative treatment in 154 (unknown in 131). Forty-seven patients (group 1) were treated between 4 and 12 weeks after LMP with the following outcome: 5 (10.6%) spontaneous abortion, one fetal death, and 43 live births (two pairs of twins). None of the child had major malformation and only two minor anomalies were noted (dolichochepalia and ear anomaly). The outcome of the 278 pregnancies exposed after the 12th week post-LMP (group 2) was one fetal death at 38 weeks (triple circular of umbilical cord), one medical abortion for polymalformation and 282 live births, including six with major malformations. The gestational age at delivery (twins excluded) in live births was similar across groups 1 and 2 (39.3 weeks ± 2.5 vs. 39.7 ± 1.9) as was the birth weight in full-term neonates (3262 g ± 507 vs. 3291 ± 517).

Conclusion: Despite the limited sample size of this study, particularly early exposed pregnancies, this data suggests that oseltamivir exposure during pregnancy is unlikely to be associated with an increased risk of adverse birth outcomes.

39-P259

French medical residents' attitudes towards drug companies

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Context: While exposure to and attitudes about Drug Company (DC) interactions among physicians have been studied extensively, relatively little is known about relationships between DC and medical residents in France.

Objective: To measure the attitudes of French medical residents towards DC.

Methods: In September 2011, a 59-item anonymous survey was sent to 3492 residents (all specialties) from five French Medical Universities (Bordeaux, Toulouse, Montpellier, Marseille, Grenoble), exploring their exposure and position towards DC. The questionnaire was based on three different publications (JAMA 1990;264:1693; JAMA 2005;294:1034; EJCP 2010; 66:727) after expert consensus (all authors). Five groups of questions were asked: formation about pharmaceutical sale representative visit (PSRV) and conflicts of interest, practice of PSRV and advice about it, quality of information, opinions about pharmaceutical sponsoring and behavior since Mediator affair.

Results: Overall response rate was 603/3492 (17.3%) with range among universities between 13.3% and 18.8%. Most of residents (72%) thank that their formation was inadequate about conflicts of interest. During the last 6 months, 53% residents met at least six times a PSRV, 61.4% received at least one gift from DC and 85% attended at least one sponsored lunch. Only 8.8% reported having a generally positive attitude towards DC. This positive attitude differ according to specialties: 24% for surgeons, 13% for medical specialists, 9% for psychiatrists, 8% for pediatricians, 5% for GP, 0% for anesthesiologists. Two out of three residents thank that their prescriptions are not influenced by PSRV. Among respondents, 64.5% estimated that they were not enough prepared to discuss DC messages, whereas 53.3% agreed with the organization of residents' medical congresses. About DC information, 13% residents found it valuable. Since Mediator affair, 18% changed their means for drug information. Among all residents, 54% used Vidal dictionary, 17% independent drug journals, 13% drug consensus of national agencies (HAS, AFSSaPS).

Conclusions: This study shows that most (90%) of medical residents have negative opinions towards DC. Resident experiences and attitudes suggest that they not enough critical and curious with respect to DC. Educational initiatives in order to modify residents' attitudes should modify the residents' behavior.

40-O071

A sequential Gs then Gi pathway activation is required to induce the B3-AR proliferative effects on myometrial smooth muscle cells

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Background: B3-AR stimulation is currently being tested for the prevention of bladder instability, depression, and in our team for the handling of preterm labor. We have previously shown that B3-AR stimulation by the selective agonist SAR150640 induces myometrial cell (MC) proliferation via Gs protein activation. The B3-AR is not internalized upon prolonged stimulation, but nothing is known about its potential negative regulation by a switch from Gs to Gi protein coupling, as it has been documented in the B2-AR.

Objective: We assessed the effect of a sustained B3-AR stimulation on a potential Gs/Gi coupling switch and its effect on proliferation.

Methods: Primary MC lines were established from myometrial biopsies obtained from women undergoing elective caesarean delivery for uncomplicated pregnancies before the onset of labor. After 24 h adhesion and 72 h starvation, cells were treated with SAR 100 nM (for 3, 8 and 48 h). Proliferation was assessed by cell counting using flow cytometry. Erk1/2 and Akt activation were assessed by western blotting. G-protein pathways involved were assessed using pharmacological agents: Melittin (100 nM) and pertussis toxin (PTX, 200 ng/mL) to inhibit Gs and Gi respectively, and U0126 (5 μM) and Triciribin (10 μM) to inhibit Gs and Gi respective targets: Erk1/2 and Akt.

Results: SAR induced a 2.9* and 1.6*folds increase in Erk1/2 phosphorylation after 3 and 8 h stimulation respectively, and a 2.03-fold* increase in Akt phosphorylation after 48 h of stimulation (*$P < 0.05$ vs. control). All activations were fully antagonized by melittin co-stimulation, and PTX only failed to antagonize Erk1/2 activation at 3' (2.49 fold increase vs. PTX alone, $P < 0.05$). Finally, 48 h SAR stimulation induced a 21%* increase in cell number vs. control (*$P < 0.05$), an effect fully antagonized by U0126, melittin or PTX but only partially by triciribin (+15.41% cells only vs. Triciribin alone, $P < 0.05$).

Conclusion: This study shows that a Gs/Gi coupling switch occurs upon B3-AR sustained stimulation in MC. Instead of inhibiting its effect, this switch is required to induce the B3-AR-dependant proliferation via: First Gs/Erk then Gi/Erk and finally Gi/Akt activation pathways. These data bring new insights on the potential effect of a sustained B3-AR stimulation in the myometrium, and also potentially in other indications.

40-O072

Poly (ADP-ribose) polymerase inhibition induces Sirtuin1 translocation into cytoplasm after in vivo cerebral oxidative stress

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Introduction: *In vitro* studies have shown that oxidative stress (OS) activates poly(ADP-ribose) polymerase1 (PARP1), decreases sirtuin1 (SIRT1) activity, NAD levels and finally leads to cell death. PARP1 and SIRT1 are both NAD-dependent enzymes[1,2]. PARP1 hyperactivation induced by cerebral OS contributes to cell death[1]. *In vitro*, its inhibition restores NAD levels and increases SIRT1 activity[3]. SIRT1 induction protects neurons from cell loss induced by OS *in vitro*[4]. The aim of this study was to assess whether PARP inhibition could restore NAD loss and increase SIRT1 expression/activity after *in vivo* cerebral OS.

Materials and Methods: *In vivo* cerebral OS is induced in anaesthetized male Sprague-Dawley rats by intrastriatal infusion of malonate, a mitochondrial toxin that promotes free radical production. The PARP inhibitor, 3-aminobenzamide (3AB; 54 μg) and its vehicle, were injected 30 min before malonate infusion[1]. NAD levels were measured at 4 and 24 h after malonate, SIRT1 cytoplasmic and nuclear expression at 6 h. SIRT1 activity was evaluated by western-blot of acetylated-histone H3, a nuclear SIRT1 substrate.

Results: Malonate induced NAD loss at 4 and 24 h (respectively 68 ± 8 and 39 ± 4 pmol/mg tissue vs. 109 ± 6 pmol/mg tissue for sham-operated, $P < 0.001$) which was not reduced by 3AB (4 h: 61 ± 4 pmol/mg tissue; 24 h: 52 ± 6 pmol/mg tissue). Whereas SIRT1 cytoplasmic/nuclear expression ratio was not modified by malonate (14 ± 1AU vs. 14 ± 1AU for sham-operated), 3AB promoted SIRT1 cytoplasmic translocation (21 ± 3AU) without modifying histone H3 acetylation (25 ± 2AU vs. 30 ± 3AU for malonate + vehicle of 3AB group).

Discussion: PARP inhibition does not reduce NAD loss following *in vivo* cerebral OS. For the first time, we show that 3AB induces SIRT1 translocation from the nucleus into the cytoplasm after *in vivo* cerebral OS. This may induce an increased SIRT1 activation in cytoplasm leading to NAD consumption and may explain the

absence of NAD restoration after PARP inhibition. Moreover, this translocation may induce cytoplasmic mechanisms that may participate in the neuroprotective effects of PARP inhibition after OS.

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40-O073

The role of Toll-Like Receptors in chemokine production by human lung macrophages

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Introduction: Toll-Like Receptors (TLRs) are the majors players involved in the innate immune response of macrophages to pathogens. TLRs have the ability to identify a range of conserved microbial motifs, and then induce the expression of a wide range of immune and inflammatory genes such as chemokines genes to fight against various infectious and non-infectious agents. This study examined the effects of TLRs stimulation on the production of chemokines by human lung macrophages (LM).

Methods: LM were isolated from resected lungs and stimulated with maximal concentrations of agonists specific for TLR1/2 (Pam3CSK4), TLR2 (HKLM), TLR3 (Poly(I:C)-HMW and Poly(I:C)-LMW), TLR4 (*E. coli K12* LPS), TLR5 (*S. typhimurium* flagellin), TLR6/2 (FSL-1), TLR7 (Imiquimod), TLR8 (ssRNA40) and TLR9 (ODN2006). Chemokines transcript expression was assessed at 6 h with RT-qPCR, while protein expression was measured in supernatants with ELISA after 6 and 24 h.

Results: All agonists except ODN2006 (TRL9) induced an increase in the expression of chemokines transcripts. Imiquimod (TLR7) induced a weak increase in the transcript expression of three chemokines (max. fourfold), Poly(I:C)-LMW of 14 (4- to 504-fold), whereas the others TLRs agonists affected between 23 and 33 chemokines. CXCL1, CXCL10 or CXCL11 were the most increased chemokines for the majority of the TLRs agonists, between 64- and 3286-fold. Only two chemokines (CCL17 and CXCL1) were increased with all TLRs agonists (except ODN2006), while 26 were never affected. There was a perfect relationship between the observations at the transcriptional levels and the cytokines measured at the protein level (TNF-α, IL-1β, IL-6, CCL1, CCL4, CCL18, CCL22 and CXCL1).

Conclusion: These results suggest the expression of the TLR1-8 on human lung macrophages, the activation of which induces the production of chemokines involved in the innate immune response. The most affected chemokines are responsible for the recruitment of inflammatory cells, notably neutrophils (CXCL1), monocytes/macrophages (CXCL10), or T-cells (CCL17, CXCL10 and CXCL11). Since the most studied TLRs-related effects on macrophages focus on the TLR2 and TLR4 agonists, our work describes a potential role for the others TLRs in the regulation of the reaction to pathogens (i.e. virus…) by human lung macrophages.

40-P281

The polymorphism associated with drug abusers changing two amino acids in the n-terminus of the serotonin 5-ht2b receptor leads to a gain of function

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Introduction: The possibility that 5-HT2B receptors participate in drug addiction in humans was postulated when a double single nucleotide polymorphism (SNP) variant affecting two amino acids in the N-terminus of the 5-HT2B receptor was identified at a higher rate among drug abusers than in a control group (Lin, 2004). Based on (i) our preliminary molecular pharmacologic studies of the 5-HT2B receptor variant linked with drug abuse, and (ii) our recent report describing the pivotal role played by 5-HT2B receptors in behavioral and physiological responses to 3, 4-methylenedioxymethamphetamine (ecstasy, MDMA), our working hypothesis is that 5-HT2B receptors contribute to the behavioral and physiological effects of drugs of abuse, and that variant 5-HT2B receptors do so to a greater extent than do wild type 5-HT2B receptors. This work intends to elucidate the role of a 5-HT2B receptor variant in mediating behavioral responses to drug of abuse and addictive behaviors.

Methods: We have corroborated the study from Lin (2004), identifying this single nucleotide polymorphism (SNP) in an independent cohort of drug abusers. Based on a molecular modelisation of the 5-HT2B receptor and molecular dynamics simulations, we performed site-directed mutagenesis studies to elucidate how, at a molecular level, the 5-HT2B receptor SNP affects intracellular responses to drugs of abuse.

Results: Studying this new cohort of drug abuser, we found that the 5-HT2B receptor SNP is significantly more frequent among drug abusers than in a control population. Our pharmacological analysis demonstrates that the 5-HT2B receptor SNP associated with drug abuse is a gain of function mutation for agonist activation. We also found that the N-terminal extremity has an inverse agonist-like effect on basal and stimulated 5-HT2B activity, which is lost in the 5-HT2B receptor SNP.

Conclusion: Our line of investigation will likely illuminate how the 5-HT2B receptor polymorphism affects molecular, biochemical, and behavioral processes germane to drug abuse, and may suggest novel strategies for pharmaceutical interventions in drug abusers.

40-P282

Involvement of purinergic receptors and NLRP3 inflammasome pathway in the ATP-induced cytokine release and MMPs/TIMP imbalance in macrophages

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Adenosine triphosphate (ATP) has been described as a danger signal activating Nod-like receptor family, pyrin domain containing 3 (NLRP3) Inflammasome and the proinflammatory cytokine IL-1β release in lung. This pathway has been previously described to be involved in various inflammatory diseases including fibrosis.

We investigated the role of ATP as a danger signal and purinergic receptors in the activation of NLRP3-Inflammasome pathway in human macrophages. Moreover, we evaluated the influence of the activation by ATP in the release of pro-inflammatory cytokines and the balance MMPs/TIMP.

Monocytes from healthy donors were obtained from buffy coat (EFS, Rennes) using human CD14 Microbeads (Miltenyi) separation kit. Macrophages were obtained after differentiation from monocytes by incubation with rhGM-CSF for 7 days. Cytokines and MMPs production was measured in the supernatant using ELISA or zymography. NLRP3 expression was evaluated by immunohistochemistry.

LPS and ATP elicited an increased production of IL-6, IL-18 or IL-1β at 4 h 30 and 24 h. TIMP-1 secretion, a pro-fibrogenic cytokine was also increased at 4 h 30 and 24 h. Gelatinase expression, mainly MMP-9 was increased at 24 h as observed in zymography and confirmed by ELISA. In addition, NLRP3 protein expression was increase at 24 h in LPS+ATP primed cells. To investigate the involvement of purinergic receptors, macrophages were pretreated with suramine, an antagonist of P2 receptors.

Suramine significantly reduced the production of TIMP-1 at 4 h 30 and 24 h as well as IL-1β and IL-6. These results showed that ATP could be a major endogenous danger signal that engages NLRP3 and P2 receptor leading to inflammatory process and cytokine release. This probably alters the MMPs/TIMP equilibrium which influences the extracellular matrix deposition and fibrosis.

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P009

Beneficial effect of insulin elevation on pressure-induced vasodilation through a Ca^{2+}-independent PI3K-NO pathway during early development of insulin-resistance

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Objective: Pressure-induced vasodilation (PIV) is a physiological mechanism relying on the interaction between mechanosensitive C-fibers and calcium (Ca^{2+})-dependent vasodilation. This study is designed to determine whether neurovascular response is modified by progressive installation of impaired glucose tolerance in diet-induced obesity and the related underlying mechanisms.

Methods: Male C57BL/6J mice were fed an obesogenic diet (OD) for 2 and 4 weeks (2OD, 4OD) or a control standard diet (C). We assessed cutaneous microvascular response using laser Doppler flowmetry in response to local increasing pressure application and to iontophoretic delivery of acetylcholine and sodium nitroprusside. These microvascular responses were also assessed by using IRS-1/PI3k/eNOS pathway inhibitors.

Results: Body weight was significantly increased in 2OD and 4OD groups. Glucose tolerance and insulin sensitivity were not changed in 2OD group whereas they were both impaired with a two-fold increase in plasma insulin level in 4OD group. Endothelium-independent vasodilation was unchanged by OD, whereas Ca^{2+}-endothelium-dependent vasodilation was significantly attenuated in 2OD and 4OD groups. The nervous function of C-fibre and Aδ fibres are unaltered. Neurovascular interaction measured by PIV was decreased in 2OD group. In contrast, in 4OD group PIV was restored at control level, and significantly decreased by IRS-1/PI3k/eNOS pathway inhibitors.

Conclusions: Impairment in both Ca^{2+}-dependent endothelial function and neurovascular function is occurring earlier than insulin-resistance installation triggered by obesogenic diet. Increased plasma insulin level appears beneficial to PIV response via IRS-1/PI3k/eNOS pathway that allow Ca^{2+}-independent NO production and restore neurovascular interaction could be a surrogate mechanism to protect the microcirculation under progressive installation of glucose/insulin tolerance impairment.

P010

FOS expression from blood leukocyte transcripts in patients with coronary disease is a biomarker of smoking exposure

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Objective: Analysis of leukocyte transriptome, in particular Finkel-Biskis-Jinkins Osteosarcoma (*c-Fos*) gene with a prominent role in inflammation, provides new insights into atherosclerosis mechanisms. Although smoking is a major risk factor, how tobacco use links coronary artery disease (CAD) remains unclear. We aimed to analyze the relationship between smoking status and *c-Fos* expression in circulating leukocytes of patients with CAD.

Methods: *c-Fos* expression was measured by RT-Q-PCR, from blood leukocyte of 239 consecutive patients after acute myocardial Infarction (MI). Patients were asked about their self-reported smoking status and stratified in three groups: Current Smokers (CS) (N = 85), Past Smokers (PS) (N = 78) and Never Smokers (NS) (N = 76).

Results: NS had a higher risk profile including hypertension and CS were younger than PS and NS (−13 and 17 years respectively). CRP levels showed only a trend toward a decrease in NS and PS when compared with CS. Mean *c-Fos* transcript level was gradually increased in CS when compared with PS and NS (0.924 vs. 0.908 and 0.861 AU, respectively; $P = 0.005$). By univariate analysis, neither age, sex, CRP nor white blood cell were associated with cFOS transcript levels. By multivariate analysis, CS (vs. PS + NS) was the strongest predictor *c-Fos* transcript level, (B = 0.042 ± 0.014, $P = 0.003$), even after adjustment for confounding (i.e. hypertension, chronic medication, familial history of CAD, and prior MI).
Conclusion: Our work suggests that c-Fos transcript level in blood leukocyte could be considered as a cumulative biomarker of smoking. As *c-Fos* gene has been evoked as new factor of progression and severity of atherosclerosis, they could be considered as a novel potential pathway of tobacco toxicity in coronary artery diseases.

P011

Modulation of endothelial progenitor cells in high-fat diet mice: role of PPAR-?

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Bone marrow-derived endothelial progenitor cells (EPCs) promote neovascularization. Metabolic pathologies such as obesity and diabetes are associated with decreased level circulating EPCs. Experimental studies suggest that stimulation of peroxisome proliferator-activated receptor (PPAR-α) may stimulate cell differentiation. Here, we evaluated the effects of the PPAR-α pathway activation on the phenotype and the function of EPCs from mice fed with a high-fat diet. Male C57BL/6N (8 week-old) wild-type (WT) and PPAR-α-null (KO) are used. Mice received either a standard diet or a high-fat diet (HFD, 42% Kcal from fat) for 12 weeks. Body weight was recorded throughout the feeding period. After 12 weeks, intraperitoneal glucose tolerance test was performed. Bone marrow-derived cells were obtained by isolating mononuclear cells from bone marrow of mice. Cells were cultured in the absence or in the presence of specific agonist PPAR-α, WY-14643 (5 μM) for 7 days. To analyze cell phenotype, extensive characterization of isolated cells at day 0 and after 7 days of culture was performed. Labelled EPCs were analyzed by flow cytometry using the anti-Sca-1-PE/Cy7 and anti-Flk-1-PE antibodies. We observed that the weight of mice and blood glucose levels are not affected by the HFD, however, increased epididymal adipose tissue deposition is observed only in WT mice (0.34 ± 0.11 vs. 0.62 ± 0.12 g in WT mice receiving standard diet and HFD, respectively, $P < 0.05$). Regarding progenitor cells, the deletion of PPAR-α increased percentage of EPCs both at day 0 and after 7 days of culture. In addition, at day 0, HFD had no effect on the EPCs of WT mice but induced a reduction of EPCs in KO mice. After 7 days, HFD reduced the subpopulation of EPCs in WT mice but had no effect in KO mice. Finally, the agonist WY-14643 increased the differentiation of EPCs from WT mice fed with HFD as well as their ability to promote in vivo bone marrow-derived cell-mediated angiogenesis. Our study demonstrates that PPAR-α modulates the differentiation of bone marrow-derived progenitor cells. Moreover, they highlight the default differentiation of EPCs induced by HFD that is restored, at least in part, by PPAR-α pathway activation.

P012

Mechanisms of aortic endothelial dysfunction in adjuvant-induced arthritis in rats

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Introduction: Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by articular and extra-articular events including cardiovascular diseases. Macrovascular endothelial dysfunction (ED) has been reported in RA patients and is suspected to contribute to atherogenesis. However, the precise mechanisms of ED in RA are not understood. The aim of this study was to elucidate these mechanisms in conductance vessels from adjuvant-induced arthritis (AIA) in rat.
Method: Experiments were performed on the AIA model performed on male Lewis rats. Three weeks after the onset of arthritis, vascular function was evaluated on isolated aortic rings. Experiments determined the vasodilating response to acetylcholine (Ach) in norepinephrine-constricted rings in the presence or not of different drugs.
Results: As a reflection of ED, Ach-induced relaxation was decreased in AIA rats compared to controls. Incubation of rings with L-NAME, Apamin-Charybdotoxin and indometacin revealed that AIA is associated with a reduced contribution of NO synthase and EDHF pathways and with an overactivation of the cyclo-oxygenase pathway. More precisely, incubation of rings with the selective COX-2 inhibitor NS398, the PGI2 synthase inhibitor tranylcypromine and the TX synthase inhibitor furegrelate identified the deleterious contribution of COX-2, PGI2 synthase and TX synthase activation in AIA-associated ED. The SOD-mimetic Tempol and the NADPH-oxidase inhibitor apocynin significantly improved vasorelaxation in AIA but not in controls rats. Conversely, the iNOS inhibitor 1400 W did not modify ACh-induced vasodilation in AIA or control rats.
Conclusion: Our study identified decreased NO and EDHF production, increased superoxide anion and activation of COX-2 pathways and its downstream effectors as new mechanisms involved in endothelial dysfunction in AIA. Our data provide new elements for a better treatment of this cardiovascular risk factor in RA.

P013

Postnatal overfeeding in mice leads to overweight and to cardiometabolic and oxidative alterations in adulthood

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Introduction: Several studies in mice have shown that postnatal overfeeding (OF) induces permanent moderate increase of body weight in the adult life; however, cardiovascular repercussions of postnatal OF are less known.
Methods: Immediately after birth, and during 3 weeks, litters of C57BL/6 mice were either maintained at 10 (normal-fed group, NF), or reduced to 3 in order to induce an OF situation. At weaning, mice of both groups received a standard diet. Measurements of phenotypic characteristics and metabolic parameters (cholesterol, insulin and leptin) were performed in the plasma at 7 months. Tissue oxidative stress was assessed by Electron Paramagnetic Resonance in the heart using CP? spin probe and both SOD and catalase activities were measured by spectrophotometry. Cardiac function was measured by echocardiography and the susceptibility to myocardial global ischemia and reperfusion was assessed *ex vivo* in isolated perfused heart.
Results: OF led to an increase in body weight (+30%) as compared to NF group. Significant increases of plasma cholesterol, insulin and leptin levels were observed in OF mice as compared to NF mice. Myocardial CP? radical, SOD and catalase activities were increased in OF mice compared to NF mice. *In vivo*, diastolic (97 vs. 114 mmHg, $P < 0.05$) and systolic (126 vs. 140 mmHg) blood pressure were significantly higher in OF than NF mice. Moreover, LV shortening and ejection fraction were decreased in OF mice. *Ex vivo*, after 30 min of ischemia, hearts isolated from mice that underwent postnatal OF showed lower recovery of coronary flow (35% vs. 55%, $P < 0.05$) and developed ventricular pressure. Moreover, infarct size evaluated after 2 h of reperfusion was increased in OF group (31% vs. 54%, $P < 0.05$) as compared to NF.
Conclusion: These results show that OF induces metabolic, oxidative and functional disturbances but also a higher susceptibility to cardiac functional damage after ischemia *ex vivo*. Complementary data are required to understand the cellular pathways implicated in these metabolic and cardiovascular modifications.

P014

Beneficial effects of polyphenols (PP) on cyclosporine-induced endothelial dysfunction

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Aim: To evaluate PP effects on cyclosporine (CyA)-induced endothelial dysfunction (ED) in rat and the role of oxydative stress.
Materiels et Methods: Male Wistar rats (14 weeks old, 8 per group) were investigated in four groups. CyA was given to CyA and CyA-PP groups (10 mg/kg per day), oil vehicle to control group *via* gastric gavage for a period of 14 days before euthanization. PP were given at a concentration of 100 mg/kg per day, during 3 weeks.
Systolic (SBP) and diastolic (DBP) blood pressures were measured by tail-cuff plethysmography, endothelial function was assessed on mesenteric arteries by myography. Reactive Oxygen species (ROS) and peroxynitrite were assessed by fluorescent tissues staining.
Results: CyA induced ED. NO-mediated component of relaxation was decreased in CyA group compared to control (maximal global relaxation: 66.0 ± 9.2% vs. 97.8 ± 0.2%, $P < 0.05$). Moreover, the EDHF-mediated component of relaxation was clearly altered in CyA group compared to control (19.6 ± 11.3% vs. 92.9 ± 1.5%, $P < 0.01$). Co administration of PP to CyA improved significantly EDHF, but not NO-mediated component of relaxation compared to CyA group (76.7 ± 5.7% vs. 19.6 ± 11.3; $P < 0.001$; and 66.0 ± 9.2% vs. 83.9 ± 3.3).
SBP and DBP were significantly increased by CyA treatment from 153.5 ± 2.9 and 85.9 ± 3.8 mmHg in control group to 178.5 ± 4.1 and 109.5 ± 2.4 mmHg ($P < 0.05$). PP prevented partially the development of CyA-induced hypertension ($P < 0.05$).
Inhibition of oxidative stress improved EDHF-mediated ED induced by CyA (82.9 ± 1.7% vs. 19.6 ± 11.3%; $P < 0.01$). ROS and nitrotyrosine staining levels, were statistically increased in CyA group compared to control. PP prevented CyA-related ROS and nitrotyrosine fluorescence raising levels (51.4 ± 7.1 vs. 71.27 ± 12.5 UI, $P < 0.05$; $n = 6$ and 36.8 ± 9.1 vs. 57.8 ± 12.9, $P < 0.05$, $n = 3$ respectively).
Conclusion: PP prevented CyA-induced hypertension and EDHF-component mediated ED, by decreasing oxydative stress.

P015

Obesity and metabolic syndrome in a population of eastern Algeria

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Considered a major risk factor for cardiovascular disease, metabolic syndrome (MS) is little, or not taken into account, under the supervision of obesity. In the adult population of this study, the SM generates high frequencies and different according to the definitions (NCEP ATP III: 43.63%, IDF: 49.51%). The objective of this study was to describe obesity and estimate its position in the onset of MS.
A cross-sectional survey was conducted in 2011 on the adult population. Biochemical assays, measurements of blood pressure and anthropometric measurements were made possible to classify the subjects SM+ and SM-as defined by the IDF and NCEP ATP III.
A total of 320 subjects participated in the survey; they are divided into 23.87% men and 76.08% women. The mean BMI 28.42 kg/m^2 women vs. 25.65 kg/m^2 men. Weight status is characterized by a high incidence of obesity and overweight women (40.83% and 66.53%). The overall prevalence of obesity in the sample is 35, 84%. The prevalence of obesity was significantly higher in women (40.83% vs. 22.08%) (P 0.0045). The average number of metabolic complications is higher in

obese than in normal weight. The impaired fasting glucose, hypertriglyceridemia, low HDL cholesterol are respectively more frequent in obese (32.4%, 53.2% and 77.1%) than in normal weight (10.6%, 12.9% and 75.3%). The metabolic syndrome was significantly more frequent ($P < 10^{-3}$) in the obese (according to IDF: SM 63.92% and according to NCEP ATP III: 60.7%) than in normal weight (according to IDF SM: 30, 57% and SM according to NCEP ATP III: 23.27%). SM is present in the IDF at 54.45% women vs. 44.57% men according to NCEP ATP III in 48.47% of women vs. 38.8% men.
The incidence of SM is very high in the population Constantine. As part of monitoring of obesity, particular emphasis should be placed on the control of central obesity as well as body. On the other hand, given the differences observed on the frequency of central obesity according to both definitions, studies must be considered in determining the threshold values appropriate to the Algerian population.

P016
Sequential physiological analysis of daily-life recordings
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Introduction: Non invasive monitoring of chronic patients or sports(wo)men needs the development of real-time evaluation of individual physiological state.
Our hypothesis is that the evolution of a subject physiological state can be described as a sequence of elementary physiological situations, each corresponding to a specific combination of physiological functions.
Our objective is to demonstrate the possibility to divide a daily life moment into a sequence of distinct situations from physiological (cardio-respiratory and actimetric) signals.
Methods: Twelve healthy volunteers participated in the 'daily life' study, which consisted in the simultaneous recording of ambulatory sensors data from a pair of subjects. Each pair came once in the lab for 2 h at lunch-time and was accompanied by the experimenter. Each subject was equipped with a EQ-01 sensor unit (Equivital™, Hidalgo), which consists of a monitoring belt and a sensor electronics module. Blood oxygen saturation, heart and respiration rates and skin temperature were recorded with a 5 s sampling period. Signals from 3D-accelerometer were simultaneously recorded (Fs = 25.6 Hz). A Bluetooth wireless data network allowed to stream real time physiological data from both sensor units.
During the recording period, subjects were asked to accomplish the various activities encountered in daily work conditions: reading-writing, walking, speaking, drinking-eating, rest, flexing, etc. Each signal of each recording was then annotated in accordance with a formal scenario structure. We assessed the agreement between changes in dynamic characteristic of the signals, visually estimated, and annotations.
Results: On almost all signals and all subjects, we observe a good concordance between changes in dynamic characteristic of the signals and annotated occurrences of situation changes. When a situation change is not detected on one signal, it may be systematically located on at least another one.
Discussion: This qualitative visual approach confirms our hypothesis of the sequential evolution of the individual physiological state. Transitions between successive elementary physiological situations are detectable as soon as a series of different signals is collected. This strongly suggests that an a posteriori signal processing may be developed to automatically segment the recordings.
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P017
Study of ApoB-LDL/ApoA-HDL ratio in hypertensive population (Tlemcen Algeria)
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the apoB/apoA1 ratio represent the balance of the proatherogenic and antiatherogenic lipoprotein, the purpose of this study was to determinante the apoB of LDL and apoA1 of HDL in hypertensive population, in the aim to have a better power of information for detection of atherogene risk.
Methods: plasma lipids, lipoproteins, apoB, apoA1 were measured in 30 hypertensive men and women, above 68.59 age and 28.37 BMI, compared with 20 controls in apparently good health.83.33% of this population has a limit HTA,75% has a diabetes millitus and 87.17 in obese.
Results: The results show significant differences between hypertensives and controls. The parameters sera were measured by immunoturbidimetric method.
Conclusion: we conclude that the measure of apolipoproteins give more information about a risk of hypertension that apoA1, lipoproteins and plasma lipids. In other part, the index of atherogenecities (CT/CHDL, CLDL/CHDL, ApoB/ApoA1) give the same information, when we class the hypertensive population according to the genre, gender, BMI and oldness of HTA. However the ApoB/ApoA1 ration is a better index to predict a coronary risk associated with HTA
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P018
Cardio-metabolic and oxidative consequences of postnatally-induced overweight in adult mice
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Several studies in rodents have shown that postnatal overfeeding (OF) induces permanent moderate increase of body weight in the adult life; however, cardiovascular and oxidative repercussions of postnatal OF are less known.
Immediately after birth, litters of C57BL/6 mice were either maintained at 10 (normal-fed group, NF), or reduced to 3 in order to induce an OF situation. At weaning, mice of both groups received a standard diet. Measurements of phenotypic characteristics and of plasma metabolic parameters were performed at 7 months. Tissue oxidative stress was assessed by Electron Spin Resonance in the heart and both SOD and catalase activities were measured. Cardiac function was assessed by echocardiography and the susceptibility to myocardial global ischemia and reperfusion was measured *ex vivo* in isolated perfused heart. Determination of cardiac gene expression profile was performed with oligonucleotide chips at early and late stages of development.
OF led to an increase in adult mice body weight (+30%) as compared to NF group. Significant increases of plasma cholesterol, insulin and leptin levels were observed in OF mice. Myocardial tissue oxidative stress, SOD and catalase activities were increased in OF mice. *In vivo*, diastolic (97 vs. 114 mmHg, $P < 0.05$) and systolic (126 vs. 140 mmHg) blood pressures were significantly higher in OF. Moreover, LV shortening and ejection fraction were decreased in OF mice. *Ex vivo*, after 30 min of ischemia, hearts isolated from mice that underwent postnatal OF showed lower recovery of coronary flow (35% vs. 55%, $P < 0.05$) and developed ventricular pressure. Moreover, infarct size was increased in OF group (31% vs. 54%, $P < 0.05$) as compared to NF. Several genes expression were modified in OF mice, such as members of the apelin pathway.
These results show that OF induces metabolic, oxidative and functional disturbances but also a higher susceptibility to cardiac functional damage after ischemia *ex vivo*. Complementary data are required to understand the cellular pathways involved in these cardio-metabolic and oxidative modifications.

P019
Vascular effect of an extract of Balanites aegyptiaca
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Background: Balanites aegyptiaca is a plant used in Senegal notably for its alimentary interest. Fructs of this plant is presented to have antihypertensive properties.
Objective: The aim of this study was to determine whether an hydro-alcoolic extract of fructs of Balanites aegyptiaca is able to induce a relaxant effect in the rat aorta and so the mechanism underlying this effect.
Methods: Rat aortic rings were suspended in organ chambers for recording of changes in isometric forces. Rings with endothelium were incubated or not with L-Nitro Arginine Methyl Ester (L-NAME) to block NO synthase, before contraction with adrenalin and a concentration relaxation curve to an hydro-alcoolic extract of fruct of Balanites aegytiaca. In some experiments, endothelium was removed before contraction with adrenalin and concentration relaxation to the extract. Prevent effect of the extract was determine with contraction curve to adrenaline in rings with and without endothelium incubated with the extract of Balanites aegyptiaca.
Results: The hydro-alcoolic extract of Balanites aegytiaca induces a vasodilatary effect in the rat aorta contracted with adrenalin. This effect is not endothelium-dependent and is not mediated by NO. Furthermore the extract incubated with aorta rings, is able to prevent the contraction induced by adrenalin.
Conclusion: Balanites aegyptiaca induces vascular relaxation which can explain the benefic effect of this plant in treatment of high blood pressure in Senegal.

P020
Evaluation of food habits in healthy subjects and patients with cardio-vascular disease (Constantine – Algeria)
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Like many countries in the developing world, contemporary Algeria saw a health situation marked by the dramatic decline of communicable diseases coupled with the emergence and rapid development of chronic noncommunicable diseases (cardiovascular diseases (CVDs), diabetes, cancer ...etc.).
Currently, CVDs appear at the top of most killer diseases in Algeria. However, according to WHO reports, our country would still be ill-prepared to handle such burden. The major causes of this problem is the lack (or even inexistence) of education programs, lack of nutrition and lack of dissemination of information relating to food and health.
Faced with this unfavorable situation, Primary prevention based on global programs oriented towards population is the best approach to achieve slow this emerging epidemic. The basis for this prevention is the identification, prevention and control of major risk factors, the most common.
In this study, we examined food habits and lifestyles of two samples comprised 50 patients with CVDs and 50 healthy subjects. Several aspects are reviewed by our survey such as information related to diet and lifestyle, knowledge of relation health-food and quality of some aspects of support for patients with these diseases compared to the normal population.
Our study revealed an unhealthy diet, lack of awareness and nutrition education and lack of knowledge of major risk factors of CVDs in both samples. This imbalance is mainly due to the drastic change in lifestyle of Algerian public saw the changes in food habits in response to social and economic upheaval and rapid urbanization experienced by the country. Our survey also revealed the inefficiency of programs structured around the management of this public health problem.

The lack of public awareness programs and other communication initiatives/ nutrition education are among the causes of the lack of public awareness in relation to this important issue for the well being of individuals and human development. There was a distinct lack of qualified personnel as well as manifestations of awareness organizations represented by national associations that take part of this responsibility.

P021

Role of smooth muscle cell mineralocorticoid receptors in vascular tone regulation

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Background: Arterial stiffness is a risk factor for the development of hypertension and vascular injury. This leads to arterial remodeling and increased damage after heart attack.

Objective: Aldosterone is a main regulator of renal sodium reabsorption, modulating blood pressure (BP) by binding to mineralocorticoid receptors (MR). In clinical trials, MR antagonists have beneficial effects on cardiovascular without changes in BP suggesting effects of MR in the cardiovascular system.

Methods: MR specific contribution in vSMC to regulate muscular tone remains to be established. To address this question, we generated a mouse model with conditional inactivation of the MR in SMC (MR^{SMKO}).

Results: We first confirmed SMC-specific deletion of MR in vessels. The mutant mice had a similar carotid MCSA as control mice, but with reduced BP (107.4 ± 2.1 vs. 117.3 ± 1.8 mmHg in control, $P < 0.01$) and decreased systolic diameter of carotid (0.55 ± 0.02 vs. 0.61 ± 0.02 mm in control, $P < 0.05$). Inhibition of nitric oxide (NO) signalling by L-NAME had no effect on the arterial distensibility while that of control group was decreased (10.1 ± 0.9 vs. 8.0 ± 1.2 mmHg in control, $P < 0.01$). Interesting, L-NAME effect on BP (deltaBP) was greater in MR^{SMKO} than of control mice. *Ex vivo* aortic contraction induced by phenylephrine was increased in the mutant mice (Emax: 4.79 ± 0.62 vs. 3.21 ± 0.38 mN in control, $P < 0.05$) while contraction induced by potassium chloride was reduced (Emax: 8.71 ± 0.51 vs. 10.38 ± 0.48 mM in control, $P < 0.05$). Relaxation induced by sodium nitroprussiate but not acetylcholine (ACh) was reduced (92 ± 3 vs. 101 ± 1% in control, $P < 0.05$). However, the relaxing effect of acetylcholine was significantly decreased in mutant compared to control mice in presence of indomethacin (Emax: 87 ± 3 vs. 104 ± 2% in control, $P < 0.01$). The results show an alteration of vascular tone via a defect in the NO response.

Conclusion: These findings support that MR expression in vSMC plays a major role in regulating vascular tone and may to be a determinant of arterial stiffness. The involvement of vSMC-MR on aldo/salt-induced remodeling and vascular dysfunction is currently evaluated.

P022

Reduced leukocyte telomere length is associated with lower serum ADMA levels in patients with coronary artery disease

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Introduction: Asymmetric dimethylarginine (ADMA), is an endogenous competitive inhibitor of NO synthase (NOS), whereas in the closely related compound symmetric diméthylarginine (SDMA) does not inhibit NOS. ADMA leads to a decreased NO bioavailability, increasing oxidative stress and endothelial dysfunction. Recent data suggest that leukocyte telomere length (LTL) may be a possible reliable marker for cardiovascular pathologies such as acute myocardial infarction (MI). The aim of our study was to identify the possible association of plasma ADMA and LTL as biomarkers of oxidative stress in early postmyocardial infarction period.

Methods: Blood samples from 33 consecutive patients hospitalized <24 h after symptom onset for acute MI and admitted to the coronary care unit of from Dijon University Hospital were taken. Serum levels of L-arginine and SDMA were determined using high-performance liquid chromatography. For ADMA, ELISA kits were used. LTL was evaluated by extracting leukocyte DNA from venous blood samples and performing real time PCR. The L-arginine/ADMA ratio was used as a biomarker of endothelial dysfunction.

Results: LTL was significantly reduced in patients with the lowest L-arginine/ ADMA ratio (1.15 vs. 1.27 ratio T/S^{-1}, $P = 0.005$). A trend for a positive correlation between L-arginine/ADMA ratio, but not with SDMA, and LTL was noted ($r = +0.339$, $P = 0.053$). Moreover, positive associations were found between serum levels of SDMA and age ($r = +0.468$, $P = 0.006$), homocystéine ($r = +0.462$, $P = 0.012$) and a negative association with creatinine clearance ($r = -0.603$, $P < 0.001$).

Conclusion: Our study showed that in patients with acute MI, ADMA may be a useful biomarker of cardiovascular diseases and that reduced LTL was associated with higher serum ADMA levels. Further studies are now needed in order to explore the exact relationship between L-arginine metabolism pathways and mechanisms of telomere shortening.

P024

Chronic intake of red wine polyphenols by young rats prevents aging-induced impairment in soleus muscle mitochondrial function and reduces oxidative stress

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Purpose: Aging is associated with oxidative stress-mediated muscle dysfunction. We determined whether chronic intake of red wine polyphenols (RWPs), a rich source of natural antioxidants, prevents aging-related impairment of muscle mitochondrial function trough a reduced oxidative stress. Indeed, RWPs has been shown to protects against aging-induced endothelial dysfunction and decline in physical performance (1).

Methods: Young rats were compared to rats receiving from week 16 to 40 either solvent or RWPs. Soleus muscle maximal mitochondrial respiration (Vmax, complex I, III, and IV activities) and oxidative stress through DHE staining were determined, as previously reported (2).

Results: Soleus mitochondrial maximal oxidative capacities was decreased in old vs. young rats (6.17 ± 0.52, 8.88 ± 1.44 µmol O_2/min/g dw, respectively, $P < 0.05$). DHE staining, marker of oxidative stress, was increased in old rats as compared to young one (100 ± 9.03%, 28.47 ± 2.81, respectively, $P < 0.001$). RWPs normalized oxidative stress and mitochondrial function in old rats (for the oxidative stress, 40.39 ± 4.76%, P = NS compared to the young group, and for the mitochondrial function, 8.62 ± 0.63 µmol O_2/min/g dw, P = NS compared to the young group).

Discussion: Thus, intake of RWPs protects against aging-induced soleus muscle mitochondrial dysfunction. These effects likely involve the ability of RWPs to normalize oxidative stress.

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P025

Impact of obesity on the balance oxidant/antioxidant in children western Algeria

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In many developing countries, overweight and obesity coexist with undernutrition. This is a double burden for those countries whose efforts to overcome these problems must be carefully balanced. Algeria is not immune to this scourge of modern times, since the prevalence of overweight and obesity among children 6–12 years reaches 25%. The long-term obesity is a risk factor for the development of several chronic diseases such as cardiovascular disease, diabetes or certain cancers.

Objective: Determination of anomalies in lipid metabolism and oxidant/antioxidant status in obese children compared to children who witness the same age group in the region of Tlemcen (Algeria).

This study was a cohort of 100 children aged between 10 and 14 among them 50 obese with a BMI that exceeds 30 kg/m^2 compared to an average of 50 witnesses. Lipid parameters (cholesterol, triglycerides) were been measured in serum and the different lipoprotein fractions. Lipid peroxidation was been estimated by measuring the hydroperoxides and malondialdehydes (MDA) by spectrophotometry. Plasma carbonyl proteins (marker of protein oxidation) were assayed by 2,4-dinitrophenylhydrazine reaction. For antioxidant systems, it was determined the enzymatic activity of catalase and superoxide dismutase (SOD). The oxygen radical absorbance capacity of plasma (ORAC) was estimated by the ability of erythrocytes to resist the hemolysis induced by free radicals in vitro in the presence of plasma.

The results found show a state of dyslipidemia in obese, characterized by hypertriglyceridemia associated with hyper-LDL-cholesterolemia and hypo-HDL-holesterolemia. In addition, an alteration of the oxidant/antioxidant status was been observed in these same subjects showed significantly lower ORAC, SOD and catalase activities, and higher plasma hydroperoxides, MDA, and carbonyl proteins levels, compared to control children. These alterations are because obese children have a high fat and protein, subject's radical attacks.

Childhood obesity presents complex metabolic complications and oxidative stress. Data were important because they allow the patient to insist on preventive measures, recommending a diet rich in antioxidants.

P026

Computer-based modeling – new approach in evaluation of the reactivity of perfused vessel segments

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Introduction: Even after the replacement of analog recorders of vascular segment responses to vasoactive stimuli by digital ones evaluation of vessel reactivity relies mainly on several classical descriptive parameters such as amplitude of contractile responses and area under the curve. Involvement of current digital measurement software allows incomparably more accurate assessment of a wide set of contractile response parameters, whose determination was not possible by routine descriptive methods.

Aim: To quantify and assess computer-based model parameters of digitally recorded contractile responses of perfused rat arterial segments in comparison with parameters available by conventional descriptive evaluation.

Materials and methods: Segments of renal arteries of control and diabetic rats were subjected to series of contractions induced by successively increasing bolus doses of noradrenaline (0.1; 0.5; 1; 3; 6; 10 μg). The relaxation was induced by single bolus dose of acetylcholine (20 μg) after precontraction. The digitally recorded contractile responses were processed by computational modeling using methods of Levy and Monte Carlo.
Results: Apparent reduction of relaxatory responses in the diabetic group compared with controls was found. Evaluation of contractile responses by descriptive methods did not reveal significant differences between groups at any used dose of noradrenaline. In contrast, modeling of responses by the appropriate software procedures allows calculations of the characteristic parameters, which in present study indicated a significant increase in sensitivity of arterial segments and the value of Akaike information criteria in the diabetic group of animals compared with the control group.
Conclusion: Software design proposed for measuring, recording and computational modeling of perfused artery contractile responses meets the expectations of accuracy and high reproducibility of data extraction. This model presents a new approach and improvement in evaluation of vascular reactivity when compared to routine methods.
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P027

Impact of Iron oxide nanoparticles on brain, heart, liver, kidney and lung mitochondria

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Purpose: To determine if iron oxide nanoparticles impairs mitochondrial respiratory chain complexes activities in organs characterized by different oxidative capacities.
Methods: Twelve young male rats (15 weeks) were used. Tissue biopsies were taken. Mitochondria were extracted by different centrifugations and the isolated mitochondria technique was used to study the maximal mitochondrial respiration (Vmax, complex I, III, and IV activities) and respiratory chain complexes activity using substrates such as succinate (Vsucc, complex II, III, and IV activities) on brain, heart, liver, kidney and lung (1,2). We observed the effect of the exposure of Iron oxide nanoparticles (NP) at different concentrations (100, 200, 300, 500 μg/mL) on the different types of mitochondria.
Results: Baseline maximal oxidative capacities were 26.32 ± 4.68, 48.89 ± 4.59, 26.91 ± 2.48, 13.37 ± 1.67, 11.25 ± 1.27 μmol O_2/min/g protein for brain, heart, liver, kidney and lung mitochondria, respectively. Data were significantly different between brain, heart, liver, kidney and lung mitochondria.
As compared to baseline values, exposure of all these organs to NP, whatever the concentration used did not significantly alter their mitochondrial respiratory chain complexes activities.
Discussion: There were no mitochondrial alterations after an exposure of NP, whatever the concentrations used. Thus, in young rat mitochondria, this type of NP might not show mitochondrial toxicity.
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P028

A new efficient method of ultrasonic non viral gene delivery to the rat myocardium

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Objective: Cardiac gene transfer is a powerful molecular tool to improve our understanding of the role of new proteins and mutants in cardiac pathophysiology. There is a need for a simple efficient myocardial gene delivery technique in order to study the physiological role of proteins in their native environment. Here we tested a new method of myocardial non viral gene delivery, by using the combination of ultrasound energy (USE), liposomes and high pressure injections to the rat heart.
Methods and results: Wistar rats were subjected to intra-myocardial injections of liposomes mixed to LacZ DNA or tagged scrambled siRNA. The heart was exposed after an inter-costal incision, and then injections were conducted between two sets of USE heart exposure. Ultrasound application resulted in much higher transfection efficiency than the liposomes-DNA or siRNA alone. The ultrasonic based liposomes-DNA/siRNA delivery resulted in low inflammatory response, as well as in low cardiac fibrosis as shown by total collagen staining. Quantitative real time polymerase chain reaction (PCR) showed that the ultrasonic delivery resulted in cardiac specific transduction. Following the delivery of a GFP plasmid or the tagged scrambled siRNA, sufficient calcium tolerant isolated transfected cardiac myocytes were obtained to perform single cell physiological measurements and biochemical experiments on homogenates.
Conclusion: We developed an interesting safe method for local gene transfer in the heart using ultrasound and liposomes gene delivery. This method is particularly useful to study the effect of gene transfer on cardiac myocytes maintained in their normal environment in animal models.

P029

The transcription factor isoform CREM-Ibˀ-X is an important regulator of the normal structural and function organization of the atrial myocardium in mice

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The substrate of atrial fibrillation (AF) is often composed of complex interplay between structural and functional changes of the atrial myocardium. Transgenic mice (TG) with cardiomyocyte-directed expression of CREM-IbΔC-X, an isoform of transcription factor CREM, develop atrial dilatation and spontaneous-onset AF without ventricle remodeling. Here we investigated the pathophysiology of the formation of the AF substrate in this TG mice. The goal was to identify the role played by CRE transcription pathway at the atrial level.
Methods and results: Telemetric Holter electrocardiogram recordings showed atrial ectopies which increased exponentially after 7 weeks of age and preceded the development of sustained AF in TG mice (n = 7). In parallel, there was progressive atrial dilation as seen at the echocardiography together with an increased atrial weight (WT: 4.9 ± 0.9 mg, n = 6; TG: 8.5 ± 0.9 mg, n = 7, $P < 0.05$). High-resolution optical mapping to record optical potentials during pacing from the left atrium (LA) of Langendorff perfused heart showed a delay of the activation of LA indicating of inter and intra atrial conduction blocks (n = 6). Whereas in WT mice (n = 6) well polarized action potentials (AP) were recorded in all atrial trabeculae using the glass microlectrode technique, in TG mice (n = 6) most AP were depolarized or non excitable or generated a slow-response AP. Next, we studied the histological nature of this progressive arrhythmogenic substrate. Picro-sirius staining of atrial slice revealed extremely thin atrial wall containing just a few trabeculae. Well striated myocytes (a-actinin staining) were markedly elongated with some areas of interstitial fibrosis (WT: 9.5 ± 2.2% vs. TG: 15.1 ± 3.2%; n = 6). Enzymatically isolated atrial myocytes were not hypertrophied but rather elongated (WT: 98 ± 0.2 μm (n = 17 myocytes) vs. TG: 208 ± 0.6 μm (n = 17 myocytes); $P < 0.05$). The mRNAs encoding for classical myocardial hypertrophic markers including the atrial natriuretic peptide, the brain-type natriuretic peptide or the alpha-myosin heavy chain were down-regulated in TG vs. WT atria.
Conclusion: Expression of CREM-IbΔC-X in TG hearts is responsible for an abnormal growth of the atria resulting in an elongated and thin myocardial tissue which constitutes a substrate of persistent AF. CRE transcription pathway appears as a key regulator of the normal structural and function organization of the atria.

P038

Implication of Cx37 in obstructive nephropathy

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Chronic kidney disease (CKD) is promoted by a variety of factors that induce chronic inflammation and fibrosis. Inflammation and excessive scaring have been recently associated with disruptions of the gap junction-mediated intercellular communication. Thus, alterations of the expression of the gap junction protein connexin 37 (Cx37) in the vascular level, have been associated to the development of inflammation in chronic and acute vascular pathologies.
We have recently demonstrated that decline of Cx37 expression is an early signal of CKD in mice. In this study we first characterize different cell types that express Cx37 in the renal cortex of healthy mice. Immunofluorescence experiments for Cx37 and appropriate co-markers showed that this Cx was abundantly expressed in the glomerular and peritubular endothelium (co-localized with MECA-32). In addition, a positive co-localization of Cx37 was observed with both NCCT and AQP2 which are specific markers for distal convoluted tubules and collecting ducts respectively. Interestingly, by using both qPCR and immunofluorescence studies, we noticed a dramatic reduction of Cx37 7 days after unilateral ureteral obstruction (UUO).
To further investigate the role of Cx37 in CKD, 3 month-old Cx37 KO and wild type mice underwent UUO for 7 days before tissue collection. Our data showed that Cx37 KO mice were protected in terms of tubular dilation and the degree of inflammation. Moreover, these mice showed less tubulo-interstitial proliferation and apoptosis.
Our study highlights the importance of the gap junctional intercellular communication in obstructive nephropathy, and suggests that Cx37 may play a major role in the development of CKD.

P039

Mice lacking periostin expression are protected against the development of renal disease

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Introduction: Chronic Kidney Disease (CKD) is characterized by progressive decrease in renal function related to a progressive accumulation of fibrosis. The lack of efficient treatment makes urgent the finding of novel targets for therapy. Our purpose is to investigate the implication of periostin (POSTN) in CKD progression. Classically, this protein is involved in the regulation of osteoblasts' adhesion and differentiation. Recent studies have shown that cardiac fibrosis, as different types of cancer as well, are associated to increased expression of POSTN, and proposed that POSTN activation is a major signal involved in the development of vascular remodeling and fibrosis.
Materials and methods: We studied POSTN in a model of tubulointerstitial disease after 15 days of unilateral ureteral obstruction (UUO) in WT and POSTN KO/beta-galactosidase knock-In mice. The expression of POSTN was evaluated by quantitative RT-PCR, Western Blot and its localization was determined by the reporter gene LacZ in KO mice. The expression of other genes, well-known for their pro-fibrotic and inflammatory action was also studied by RT-PCR, WB and IHC.

Results: The UUO WT mice showed a high expression of POSTN mRNA and protein. POSTN was localized at the collector tubes at the beginning of the disease and then at epithelial cells and around altered/dilated tubules. Histological studies showed that KO mice had less dilation and fibrosis. At the same time, the activation of other genes involved in the development of renal lesions like Collagen III, vimentin and MCP-1 was blunted KO mice. Interestingly, proliferation in tubular cells was increased in the KO mice and they also displayed higher expression of E-cadherin. The detrimental actions of POSTN were, at least partially, mediated by the activation of ERK1/2, but not the p38, pathway.
Conclusion: Increase of POSTN expression is an initial and important event of CKD. Genetic inhibition of POSTN expression is accompanied by a better preservation of the renal tissue in the UUO model. Our results provide initial evidence that POSTN can be a promising target against the development of CKD progression

P040

Renin inhibition reverses the decline of renal function in hypertensive transgenic mice

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Blockade of the RAS is the main target of treatments for hypertension and chronic kidney disease. Aliskiren (ASK), a direct renin inhibitor, is a novel anti-hypertensive drug that has been administered in combination with angiotensin II receptor blockers and angiotensin-converting enzymes inhibitors.
To gain a better understanding of the mechanisms involved in the progression and regression of renal disease we administered ASK to renin transgenic mice (RenTg) that ectopically express high levels of active renin. The RenTg strain is characterized by elevated blood pressure leading to a slow decline of renal function, mimicking well the progression of hypertensive nephropathy.
Ten months old transgenic mice that exhibited high blood pressure (≥140 mmHg) and proteinuria (≥80 mg/mmol albumin/creatinine) were treated with ASK (25 mg/kg/day) for 28 days while RenTg PBS mice receiving placebo were controls. Age-matched wild type mice treated or not with ASK were considered as normotensive controls.
ASK reduced blood pressure from as early as day 14 to wild type levels (141 ± 6 at day 0 vs. 100 ± 7 mmHg at day 14). Proteinuria (95 ± 30 mg/mmol at day 0 vs. 5 ± 2 mg/mmol at day 28) and cardiac hypertrophy were also normalized. Renal inflammation and interstitial fibrosis were significantly decreased in RenTg ASK. Profibrotic p38 and erk map kinases were highly activated in RenTg PBS animals. ASK treatment cancelled this activation. Antifibrotic smads 1/3/5 were reactivated in RenTg ASK mice, leading to elevated BMPs 4 and 7. Tubular cell cycle arrest observed in RenTg PBS animals was also reversed in response to Aliskiren.
Our results indicate that ASK normalizes blood pressure and proteinuria and induces a shift from pro- to anti-fibrotic and inflammatory mediators leading to regression of renal fibrosis and reestablishment of renal function and structure. Moreover, ASK normalized cardiac hypertrophy indicating that ASK exerts both, renal and cardio-protective actions.

P041

The Na+-driven Cl-/HCO3- exchanger Ndcbe plays an important role in the regulation of sodium balance

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We recently identified a novel mechanism of NaCl absorption in the cortical collecting duct, which is electroneutral, inhibited by hydrochlorothiazide and amiloride-insensitive. Ndcbe (Slc4a8), a Na^+-driven Cl^-/HCO_3^- exchanger, coupled to pendrin (slc26a4), a Cl^-/HCO_3^- exchanger allow NaCl to cross the apical membrane. Our purpose was to demonstrate the role of Ndcbe in the regulation of Na^+ balance. We first showed that Ndbce protein was upregulated in Na^+-depleted and DOCP-treated mice. To test whether the *Ndcbe*$^{-/-}$ mice have an impaired renal ability to conserve Na^+ and Cl^-, urinary Na^+ and Cl^- excretions were measured in *Ndcbe*$^{-/-}$ and *Ndcbe*$^{+/+}$ mice that were paired fed a normal salt and a NaCl-free diet. Under normal salt diet, urinary excretions of Na^+ and Cl^- were similar in both groups. When switched to a NaCl-free diet, *Ndcbe*$^{-/-}$ and *Ndcbe*$^{+/+}$ mice decreased similarly urinary Na^+ and Cl^- excretions to almost zero within 3 days, indicating that *Ndcbe*$^{-/-}$ mice had normal renal ability to conserve Na^+ and Cl^- in response to NaCl restriction. Under a normal salt diet, *Ndcbe*$^{-/-}$ mice did not exhibit hallmarks of vascular depletion. However, blood pressure was slighly lower in *Ndcbe*$^{-/-}$ mice than in *Ndcbe*$^{+/+}$ mice (105.2 ± 2.0 vs. 111.6 ± 1.2 mmHg, $N = 9$ in each group; $P < 0.01$). After 6 days of NaCl-depleted diet, urinary aldosterone excretion increased in both *groups of mice* but was higher in *Ndcbe*$^{-/-}$ mice than in *Ndcbe*$^{+/+}$ mice (38.4 ± 8.7 pmol/24 h ($n = 9$) and 17.4 ± 1.9 pmol/24 h ($n = 9$), respectively; $P < 0.03$). These results suggest that Ndcbe disruption is likely to be compensated for. Injection of amiloride increased natriuresis to the same extent in both groups. In contrast, following the injection of hydrochlorothiazide, an inhibitor of NCC, urinary Na^+ excretion in *Ndcbe*$^{-/-}$ mice was 160% that of *Ndcbe*$^{+/+}$ mice, indicating that NCC activity was higher in *Ndcbe*$^{-/-}$ mice. These results are consistent with immunblot analyses showing overexpression of both NCC and phospho-NCC in *Ndcbe*$^{-/-}$ mice. In conclusion, Ndcbe disruption is compensated by an increase in NCC activity, indicating its importance in the regulation of Na^+ balance and hence, in vascular volume and blood pressure regulation.

P042

Williams beuren syndrome hypercalcemia: is TRPC3 a novel mediator in calcium homeostasis?

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Williams-Beuren syndrome (WBS) is a neurodevelopmental disorder associated with hypercalcemia of unknown origin. This syndrome results from the deletion of contiguous genes on chromosome 7, including the general transcription factor IIi gene (GTF2I). GFT2I encodes TFII-I, which suppresses cell-surface accumulation of TRPC3 channels, involved in calcium transport in lymphocytes.
We report the case of a WBS patient with hypercalcemia associated with abnormal TRPC3 expression. Analysis of peripheral lymphocytes revealed a sharp increase in TRPC3 expression, compared to control patients. To investigate the potential role of TRPC3 in calcium homeostasis, we performed specific immunostaining on the intestine and the kidney, major calcium-regulating tissues. We provide the first demonstration that TRPC3 is expressed in normal digestive epithelium and renal tubules in control patients, and overexpressed in the intestine in the WBS patient. Taken together, these data suggest that calcium metabolism abnormalities observed in WBS may be due to TFII-I haploinsufficiency and subsequent TRPC3 overexpression, thereby increasing both digestive and renal calcium absorption. This original observation prompts further investigation of TRPC3 as a novel actor of calcium homeostasis.

P043

Type 2 diabetes mellitus in mice worsens renal impact following hemorrhagic shock

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Introduction: To determine if type 2 diabetic mice would present a severer renal impact following a hemorrhagic shock (HS) based on a recently described model of acute kidney injury (Mayeur et al., Crit Care Med 2011) and to describe the impact of HS on renal response to hypoxia.
Methods: We performed HS or sham procedure in type 2 diabetic and obese db/db mice. Creatininemia, histologic injury score and KIM-1 mRNA were used to study renal impact of HS. Tissular hypoxia and its impact were quantified by pimonidazole immunostaining and mRNA quantification of HIF, VEGF-R1, VEGFR-2, Tie-2, eNOs and iNOS.
Results: The early increase (H6) in creatininemia and histological findings underline the intensity of AKI in diabetic mice. Moreover, diabetic shocked mice exhibited overexpression of KIM-1 when compared to control shocked mice. Diabetic mice exhibiting a mild diabetic nephropathy already express hypoxic signals at baseline. Moreover, in dbshock, hypoxia is severer than in Cshock, as illustrated by the increase in blood lactates, pimonidazole staining and HIF-1a quantification. Moreover, endothelial NOS were highly overepressed in diabetic shocked mice when compared to non diabetic shocked mice.
Discussion: Taken together, these observations demonstrate that the impact of AKI following hemorrhagic shock in type 2 diabetic mice is severer. The role of hypoxia in the development of diabetic CKD appears critical and is increasingly studied (Rosenberger C, Kidney Int 2008). Our work confirms hypoxic impact of diabetes melitus in the diabetic mice kidney, even in the absence of any acute injury. HS renal impact was severer in type 2 diabetic mice with an increase in tissular hypoxia and an altered response to hypoxia.
Conclusions: renal impact of a HS in type 2 diabetic mice is more intense than in non diabetic. Prerequisite hypoxia during diabetes could result in a renal preconditioning that modifies endothelial and tissular response to AKI.

P044

Potassium retention during pregnancy requires stimulation of H,K-ATPase type 2

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Renal reabsorption of K^+ during dietary K^+ restriction requires the stimulation of H,K-ATPase type 2 by adrenal progesterone (Elabida et al. 2011, Kidney Int. 80, 256–262). Since renal K^+ retention has been observed during pregnancy we investigated whether this pathway could be functional in this physiological state.
During the late part of gestation in mice, the decrease in renal K^+ excretion is accompanied by stimulation of HKA2 mRNA and activity. In this condition, kidney function is disconnected from the dietary conditions since gravid mice increase their food consumption (and their K^+ intake) by 15%. HKA2-null mice are unable to efficiently retain K^+ during gestation compared to wild-type littermate (WT) but maintain normal plasma K^+ values (around 3.9 mM). This occurs in parallel with a decrease of the fertility rate (86% vs. 25% of successful gestation in WT and HKA2-null mice, respectively) and a higher mortality rate during gestation in HKA2-null mice compared to WT. Both phenotypes are reversed when gravid HKA2-null are given a K^+ supplementation in their drinking water. All together, our results provide evidence that pregnancy is a physiological state where kidney is switched from a K^+ secretive to a K^+ reabsorptive state because of stimulation of H,K-ATPase type 2. Moreover, inability to do so, have an impact on the development of the gestation.

P045

Protective effect of vitamin E on renal dysfunction in diabetic albino wistar rats fed low zinc diet

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Vitamin E is a critical component of antioxidant system and may be used as a potential therapeutic agent to reduce clinical disease associated with increased free

radical activity. Our purpose was to investigate the benefit effect of vitamin E on functional and histological alterations of kidney in diabetic rats fed low zinc diet. Forty male alloxan-diabetic albino (Wistar) rats weighing 250–300 g were fed a complete purified diet containing either 54 μg zinc/gram diet (control) or 1 μg zinc/g diet (deficient). Half of the animals in each diet group received supplemental vitamin E (500 mg/kg diet). On day 21, after an over-night fasting, animals were killed; plasma levels of glucose, urea, creatinine and uric acid as zinc levels in kidney and serum histology were examined. Zinc concentrations of the low-zinc diabetic animals were significantly lower than those of the control diabetic animals, at the end of the protocol. Dietary zinc intake significantly increased blood glucose, serum urea, creatinine, uric acid of low zinc diabetic rats. Vitamin E ameliorated all the previous parameters. To conclude, the present study demonstrates that vitamin E presumably acting as an antioxidant, significantly reduced the severity of diabetes disease and improved renal function.

P046

Lead toxicity and the hypothalamic-pituitary-testicular axis

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Environmental exposure to toxic lead occurs in a number of industries with potential adverse effects on the reproductive capacity of exposed men. Clinical and animal studies indicate that abnormalities of spermatogenesis result from toxic lead exposure. The experiment were performed on 30 adult male rats divided in three equal groups and were orally given by gavage 250 and 500 mg/L of lead acetate diluted in mineral water for 90 days and the other group was used as control. This study was designed to associated blood lead concentration and reproductive discorders. The results revealed a significant decrease in the weight of both the testes, epididymides and pituitary. This reduction in weight of sex glands and pituitary was accompanied by an alteration of the normal histological structure. The results showed a significant ($P < 0.001$) reduce of epididymal and testicular sperm counts including daily sperm production. Moreover, lead obviously affected sperm density and sperm viability and a significant increase of sperm abnormalities in rats treated compared with the controls. We have observed a significant difference for circulating testosterone, LH and FSH following lead poisoning. Therefore, lead exposure resulted in oxidative stress and this was well extrapolated from the increase in lipid peroxidation products (LPP). The results indicate that there was a significant ($P < 0.001$) increase of (LPP) in exposed rats than their corresponding control both in pituitary testis, and epididymides. Results clearly show that lead has a deleterious impact on the reproductive system witch impairs hypothalamic-pituitary axis functions

P055

Influence substrate oxidation ratio on food liking, food wanting and food consumption in humans

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Several carbohydrate-based models of feeding have been described. However, the influence of substrate balance on liking, wanting and macronutrient selection has never been studied in humans in line with these models. Therefore, a randomized 4-condition crossover study was conducted in 12 normal-weight men (age: 24 ± 3 years). The sessions differed by the composition of the breakfast, which was rich in carbohydrates (HCB), low in carbohydrates (LCB), rich in fat (HFB) and low in fat (LFB). Two hours and 20 min after the breakfast, energy expenditure (EE) and the respiratory exchange ratio (RER) were measured before evaluation of olfactory liking for four food items. The subjects were then allowed *ad libitum* energy intake during a snack (sweet and fatty toast). Whatever the composition, the rich energy diets induced higher EE ($P < 0.01$) and lower energy intake during the snack ($P < 0.05$) than did the low ones. A negative correlation was found between EE and the amount of sweet toast eaten ($P < 0.01$). The HCB and LCB induced a higher RER ($P < 0.001$) and lower olfactory liking for sweet food items ($P < 0.05$) than did the HFB and LFB. A negative correlation was found between the RER and olfactory liking for the sweet food items ($P < 0.05$) whereas this correlation was positive for the fatty food items ($P < 0.05$). These results indicate that a high fat oxidation rate induces a strong liking for carbohydrates and a low liking for fats, which brings new support for carbohydrate-based models of feeding in humans.

P056

Effect of olive oil consumption on lipid and anthropometric profile of moroccan postmenopausal women

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Introduction/Background: Olive oil is an integral part of the Mediterranean diet and therefore of the Moroccan one. Several studies associated olive oil to low mortality from cardiovascular disease probably due to its high content of oleic acid (71%) and linoleic acid (13.2%) known for their cardioprotective virtues.

Aim: To study the effect of a regular consumption of olive oil on the anthropometric and lipid profiles of healthy postmenopausal women.

Methods: Seventy-five postmenopausal women (55.41 ± 6.15 years) were assigned to consume 25 mL of olive oil during 8 weeks of nutritional intervention. Anthropometric (weight, height and BMI) and lipid profile (total cholesterol, HDL-c and LDL-c) have been determined at 0, 4 and 8 weeks.

Results: Results show an improvement on anthropometric profile: BMI decrease significantly ($P = 0.019$) from 28.4 ± 4.1 to 28.2 ± 4.1 after 8 weeks of olive oil consumption and in the lipid profile, we observed a significant decrease on total cholesterol (2.10 ± 0.34–2.05 ± 0.31 g/L; $P = 0.006$) and LDL-c (1.29 ± 0.32–1.25 ± 0.31 g/L; $P = 0.036$).

Conclusion and perspective: These results suggested that regular consumption of olive oil can have an impact to prevent cardiovascular disease in to postmenopausal women and help to decrease cardiovascular risk. A study over a longer period would be recommended to confirm these results.

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P057

Effect of fatty acids of Citrillus colocynthis seed on body weight development & hyperlipidemia in rats male wistar fed a high fat diet

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Objective: The objective of this study was to investigate the body-lowering and hypolipidemic effects of *Citrullus colocynthis* oil on the rats Wistar fed a high fat diet.

Materials and methods: *C. colocynthis* oil was extracted using chloroform solvent in a Soxchlet apparatus. The solvent was then evaporated and the lipid fraction was recovered.

Male rats Wistar of 1-month-old were divided into three groups, each of which was fed with one of the three diets for 2 months:

Diet 1: Control 3.6% oil,

Diet 2: High Fat Diet 28% sunflower oil,

Diet 3: 20% sunflower oil + 8% colocynth oil,

Results: The current results indicate that supplementation of colocynth oil decreased body weight and increased lipid plasma levels. We also conclude that the age of rat wistar is another important factor to consider with the composition of the diet, in the development of dietary obesity.

Discussion: Many studies have demonstrated that normal rats become obese when offered a high fat diet ad libitum ([1, 2, 3]). In contrast, other studies demonstrated that body weight of rats being fed high fat diet ad libitum is a little different ([4]) or even smaller ([5]), which is our case, we noticed that in our study and of Borst & Conover ([4]), the rats have been young, unlike the rest of studies who used adult rats wistar.

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P058

Influence of the ratio urinary/plasma polyphenols provided by consumed tea prepared under realistic Tunisian habits on cholesterol profile in type-2 Tunisian diabetics

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Introduction: Accumulating evidence from in vitro, animals and epidemiologic studies suggests that high tea- polyphenols consumption may be associated with the improvement of type-2 diabetes control. However, the influence of tea consumption under realistic Tunisia habits on total cholesterol profile in type 2-diabetes is still somewhat controversial. Our objective was to study the influence of absorbed and metabolized polyphenol compounds provided by tea decoction with sucrose on total cholesterol, LDL- cholesterol and HDL-cholesterol in Tunisians type 2-diabetics.

Methods: Over than two hundred obese type- 2 diabetic patients aged from 35 to 65 years attending in the National Institute of Nutrition in Tunis area were participated in the present study. The diabetics were divided into two groups based on higher consumers of tea and marginally or non-consumers of tea. Fasting blood was collected, centrifuged then the plasma was removed for analysis of total cholesterol, HDL and LDL-cholesterol by appropriate method. The total polyphenols were determined in blood and urine by the Folin-Ciocalteu methods. The links between plasma or urinary retention of polyphenols and cholesterol profile was applied after adjusting for factors that interfering with diabetes such as sex, age, BMI, physical activity, previous medical history, medication, smoking, drinking coffee, family history by a partial Spearman correlation coefficient.

Results: Our results showed the presence of a positive link between tea decoction with sucrose and plasma or urinary polyphenol concentration (respectively, $P = 0.006$, $P = 0.005$). Similarly, among all variables studied, only the total cholesterol and LDL- cholesterol were inversely correlated (respectively, $P = 0.042$, $P = 0.016$), whereas HDL-cholesterol was positively correlated ($P = 0.07$) with the intake of tea after all adjustments.

Conclusion: The spontaneous tea decoction consumed with sucrose can promote long-term absorption and concentration of polyphenols in plasma and urine. This one seems to improve the cholesterol profile involved in the balance of type 2-diabetes in Tunisia.

P059

Hydration status of diabetic subjects

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Objective: In diabetes mellitus, the impact of hydration status is a consequence of metabolic disorder.

Typically, osmotic polyuria generates a polydipsia for the regulation of the water balance. This regulation may be less long-term operational in diabetics and the elderly.

In type 2 diabetes, patients can lose a lot of weight during the evolution of their disease and their treatment.

Thus, body composition should be monitored to ensure that body fat decreases, without the lean body mass and total body water decreases excessively.

Patients and methods: Our work has focused on water balance of the diabetic patients (47) compared with normal controls (32) in adult population not very old with a BMI > 25. % Body fat, fat mass (kg), % lean body mass, lean body mass (kg), weight (kg), % water, volume of water (L), BMI are measured in each subject by Bodystat 1500 MDD (bioelectrical impedance).

Results: Therefore, for all subjects in our study, there is an increase in fat mass slightly hydrophilic at the expense of lean body mass more hydrophilic.

The deficit in the water content of the subjects uncontrolled diabetes could be linked to losses, in particular, urinary greater in this group of subjects, due to the osmotic polyuria.

It is not possible, to blame the water loss by decreased fluid intake, given that patients have an age in which the phenomenon of thirst is preserved.

The study of body composition in body fat and fat-free mass did not reflect significant differences between the groups studied, so the characteristics of body composition and tissue distribution have no responsibility for the observed difference in terms of water content.

Discussion: The abundance of body fat, taking certain drugs involved in the decrease in water retention in the body.

An appreciation of the importance of different factors in the water deficit in diabetic patients requires the assessment of body composition and knowledge of the effects induced by the therapy instituted.

Assessment of total body composition by bioelectrical impedance analysis could be incorporated into prevention programs for type 2 diabetes and monitoring weight loss during disease manifests.

P060

Obesity and oxidative stress

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Today everything seems to indicate that the prevalence of overweight and obesity in Algeria are increasing at an alarming rate. The aim is to determine the frequency of overweight and obesity in a cohort of adults representative of Constantine, to characterize the risk factors and to assess markers of oxidative stress in the obese. It is a cross-sectional study with cluster sampling and stratification on sex (540 individuals aged 18–64 with 296 women and 244 men). The plasma used for determination of lipid parameters and markers of oxidative stress. The pellet is used for the preparation of erythrocyte lysat to assay the parameters of oxidative status/ intracellular antioxidant by HPLC. 40.37% have a BMI between 18.5% and 24.9%, 3.14% had a BMI <18.5. 32.03% suffer from overweight, 24.44% of obesity BMI> 30. 21.14% are obese class I, 6.50% obesity class II and 2.44% obesity class III.

The statistical analysis revealed that BMI is highly correlated ($P < 0.001$) with sex, age, educational level and occupational category, income is not correlated with BMI ($P > 0.05$). The frequency of a waist size of 100–109 cm is 24.39% for males and 35.36% in women. People rate with TRH ≥ 1.0 in men and ≥0.85 HRT in women, respectively 37.71% and 74.4%. The percentage of body fat significantly greater in30% women than in men 17%, it is the same for the sum of four skin folds. Obese people have levels of plasma lipids and lipoprotéines High It Is the same for reporting indicators of atherogenic risk. There is an elevation of plasma hydroperoxides, MDA and protein carbonyl erythrocyte plasma and red cell with a decrease in antioxidants such as vitamins C and E and carotenoids, and erythrocyte catalase. Obesity is associated with metabolic disorders and oxidative stress that lead to many complications.

These results suggest that obesity and overweight are increasing trends in our country. causes are food and physical inactivity. In our country Obesity will be a real public health problem. And should be a priority of our country to guide public health actions.

P061

Nutritional approaches of pedopsychiatric disorders in the autistic child: interests of estimating the impact of the nutrition on pervasive development disorders

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The purpose of the research is to estimate the eating behavior of the autistic child as well as the influence of nutritious diets on the improvement of the behavior of the autistic child, i.e. the correlation which may exist between nutrition and autism.

Being exactly understood that child autism tends to be connected with a neurological disorder from the first stages of brain development, our works focused on the biological origin of this disease as well as the eating disorders of the autistic child.

Our study was carried out on a sample of 10 autistic children that we followed during 6 months, and whose parents answered a questionnaire on food habits and behavior of their children and the changes observed after the adoption of a new nutritional approach.

Obviously, the autistic child suffers from nutritional deficit because of their refusal of certain food, therefore it necessary to define a specific and individual diet, without forgetting the beneficial contribution of food supplements such as Vitamin B6 in certain cases of nutritional deficiencies.

The results which we obtained turn out decisive; they allow us to glimpse a new approach of the therapy of the autistic child and deserve to be explored further.

P062

Evidence for interactions between aroma compounds and the CB1 receptor: a way to regulate food intake?

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Obesity is a major cause of morbidity and mortality worldwide, characterised by a chronic imbalance of energy homeostasis. The regulation of dietary intake appears to be an effective way to regulate this imbalance. Furthermore, it is now well established that the endocannabinoid system influences appetite *via* the cannabinoid receptor type 1 (CB1): CB1 agonists can promote food intake while CB1 antagonists tend to decrease appetite (1). Interestingly, recent studies showed that CB1-like receptors are expressed in the olfactory epithelium of *Xenopus laevis* tadpoles (2). Elsewhere, it has been demonstrated that aroma perception is implicated in the process of satiety (3). Collectively, these studies suggest that aroma compounds could have an impact on food intake through interactions with the endocannabinoid system. We therefore aimed to explore this hypothesis by performing *in silico* and *in vitro* studies to identify aroma compounds able to interact with the CB1 receptor.

In silico screening of around 3000 aroma compounds described in the Flavor-Base (Leffingwell and Assoc.) was performed using the agonist and antagonist pharmacophore models previously derived from literature data (4). *In vitro* studies were carried out in HEK293 cells expressing the mouse CB1 receptor. Interactions between candidate molecules and the CB1 receptor were measured using a functional assay (Glosensor assay, Promega). In addition, expression of CB1 mRNA in mouse tissues was assessed by quantitative real-time RT-PCR.

From a set of aroma compounds predicted as candidates by the *in silico* study, three molecules were found to be moderate inverse agonists of the CB1 receptor. Furthermore, the CB1 receptor was significantly expressed in the olfactory bulb and olfactory mucosa of mice.

On the basis of these findings, interactions between the endocannabinoid system and aroma compounds could be proposed as a mechanism involved in the establishment of satiety.

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P064

Bisphenol A effects on the intestinal lipid metabolism: in vivo and in vitro study

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Purpose: Determine the impact of the bisphenol A exposure on intestinal lipid metabolism in *in vivo* and *in vitro* models.

Methods: *In vivo*, C57Bl/6 mice was exposed to 0.2 µg/kg bw/d BPA in drinking water. Treatment began at gestational day 6 and continued in offspring up to 36-weeks old. Four weeks after weaning, mice from treated-dams were fed with a high fat diet. During the BPA exposure, body weight, fat body mass and energy expenditure were checked. At 30-weeks old, we performed a lipid load test in male offspring mice. Mice were force-fed with corn oil (0.5 mL) and the time-course changes in triglyceridemia was analysed. *In vitro*, human colorectal adenocarcinoma caco-2 cells were exposed to BPA doses (10^{-5}–10^{-15} M). Expression of genes involved in lipid metabolism, L-FABP, CD36, ERα and β, ERRα and γ were investigated. The mRNA levels were quantified by quantitative RT-PCR.

Results/discussion: Results showed a sex-dependent effect of BPA exposure. Body weight of male pups born to BPA-exposed dams was significantly higher (15–30%) compared with controls. It appears in positive correlation with adipose tissue and in negative correlation with O2 consumption and energy expenditure. The lipid load test indicated that plasma triglyceride levels were decreased in BPA-treated mice suggesting that BPA affects either lipid uptake or the intestinal lipoprotein processing. *In vitro*, BPA altered the mRNA expression of studied genes suggesting that the intestinal lipoprotein processing was activated.

These data suggest that BPA could facilitate the lipid storage and consequently the obesity.

P065

Pro-inflammatory status in gestational diabetes placenta

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Introduction: Gestational diabetes mellitus (GDM) is a status of glucose intolerance during pregnancy and it represents a risk factor for neonatal obesity (macrosomia) which is influenced by maternal hypergycemia and insulinemia through placental circulation. The study was undertaken to investigate the implication of pro-inflammatory factors in the placenta of GDM women.

Methods: Thirty women with GDM, and they all take off of macrosomic babies, and 30 healthy age-matched pregnant women were recruited in the present study.

Serum concentrations of fasting glucose, insulin and biochemical parameters were analyzed. mRNA encoding for IL-6, TLR4, TGF-β, leptin and adiponectin were quantified in placental samples by using RT-qPCR.
Results: GDM women exhibited higher fasting glycemia, insulinemia and glycated hemoglobin compared with control pregnant women, reflecting a decrease in insulin sensitivity in these individuals. The placental mRNA expression of the pro-inflammatory factors was up regulated (IL-6, TLR4 and TGF-β); these observations probably reflect the increase in placental inflammation in diabetic women compared with controls [1]. Leptin and adiponectin mRNA was also significantly increased in GDM group compared with control group. Placental leptin participates in the regulation of energy metabolism and body fat in the placenta and the overproduction of leptin could alter the lipidic metabolism and may indirectly stimulate fetal growth [2].
Conclusion: Our study has allowed us to link gestational diabetes to many metabolic disturbances that can profoundly alter the intrauterine environment; this change has affected the placental gene expression, with an increase of markers and mediators of inflammation. These up regulation of inflammation in the placenta of the GDM women and might be involved in the incidence of macrosomia.
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P066
Evaluation of nutritional status and frequency of monoclonal antibodies in patients with multiple myeloma subject to chemotherapy
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Objective: To evaluate the diet and determine the frequency of monoclonal antibodies and circulating levels of CRP, complement C3 and immunoglobulins in patients with multiple myeloma (MM) in chemotherapy and in healthy controls.
Materials and methods: Thirty (30) new patients with MM (17 men, 13 women, age 66.1 ± 1.51 years) and 30 controls (18 men, 12 women, age: 47.06 ± 1.5 years) were recruited in the service of Hematology Center university hospital of Tlemcen.
Results and discussion: The average daily intake of nutrients and micronutrients in patients with MM was significantly decreased compared to controls. Studies of myeloma in relation to dietary factors observed an inverse association between the risk of myeloma and certain foods (Fernandez et al., 2000; Brown et al., 2001). The levels of vitamin C and catalase activity were significantly reduced in patients compared to controls, while the serum level of MDA was significantly increased in patients (Sharma et al., 2009). Also, serum levels of the molecule C3 were significantly reduced in patients compared to controls ($P = 0.007$), this may be due to chemotherapy, which paralyzes immune cells increasing the risk of infections (Facon, 2004). However, those of gamma globulin were significantly higher in patients compared to controls ($P = 0.045$). These results are consistent with the literature (Karlin et Coman, 2009; Facon et *al.*, 2003; Atu et *al.*, 2003; Bidet et *al.*, 2007; Baur Chaubert et *al.*, 2006; Raab et *al.*, 2009; Harousseau, 1998). Similarly, serum concentrations of CRP were higher in patients compared to controls, but the difference between the two groups does not reach statistical significance ($P > 0.05$) (Alexanian et *al.*, 1969; Palumbo et *al.*, 2006; Mateos et *al.*, 2006; Facon, 2004). The frequency of monoclonal spikes was 45% of all patients. Also, results of immunofixation revealed a frequency of 77.78% to 22.22% IgG and IgA (Harousseau 1998; Coutet et al., 2004; Baur Chaubert et al., 2006; Boudjerra 2009; Atu et al., 2003; Karlin et Coman, 2009).

P067
Intrauterine growth curves based on Tunisian data
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Introduction: The aim of this study is the construction of fetal growth curves. The fetal growth curves are variations in birth weight according to gestational age from a large sample, representative of the Tunisian population. It is an essential component of a good antenatal care, and seeks to detect and prevent problems during pregnancy by definition of small-for gestational age, appropriate-for gestational age and large-for gestational age.
Methods: All births are registered in the maternity of University Hospital of Sousse Farhat Hached for a period of 8 years. No births had been excluded except born dead, fetal deaths in utero and multiple pregnancies. Polynomial regression was performed for modelisation of means and standard deviations to construct 3rd, 10th, 50th, 90th and 97th percentiles of distribution of birth weight according to gestational age.
Results: Sixty two thousand two hundred twenty five weight measurements were included in the period from 2000 to 2008. Retained gestational ages were between 26 and 43 weeks of gestation. We performed the smoothing curves by polynomial regression, which retained were the third degree and sixth degree, respectively for mean and standard deviation. Mean and standard deviation are modeled by following function: 1- Mean, $BW = 45619.798 – 4324.844 * GA + 134.103 * GA^2 – 1.309 * GA^3$; ($R^2 = 0.98$), 2- Standard deviation, $BW = -261268.084 + 46353.591 * GA – 3374.677 * GA^2 + 128.865 * GA^3 – 2.718 * GA^4 + 0.030 * GA^5 + 0.001 * GA^6$; ($R^2 = 0.97$).
Conclusion: These curves will be used for clinicians to be able to classify small for gestational age and large for gestational age, and guide them to take initiatives to improve fetal growth.

P068
Food behavior of 100 type 2 diabetes patients presenting a ponderal overload, residing in constantine, Algeria
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The prevalence of type 2 diabetes and obesity, regarded today as two worldwide epidemics, does not show any sign of attenuation. The 'industrialized' lifestyle known as, encouraging sedentariness, as well as food imbalance, mainly caused by unhealthy food are the first marked ones.
In this study we tried to study the relation between type 2 diabetes and obesity in a sample of patients residing the Wilaya of Constantine, Algeria. Thus, this investigation aims to find out the impact of ponderal state, visceral fatty mass and food habitudes on the evolution of type 2 diabetes within the studied population.
The study included a population of 100 type 2 diabetic subjects, with a BMI > 25 kg/m^2, the studied parameters were: anthropometric profile, risk factors of obesity and diabetes, food behavior and consumption frequencies of the various food groups. These data were collected by the means of a questionnaire and a 24 h recall.
The results obtained confirm the existing bond between ponderal overload and type 2 diabetes, and obesity with strong android character. This type of obesity is influenced by sex, age, and genetics. Ponderal excess and the visceral distribution of grease seem to be independent risk factors for type 2 diabetes.
Interviewed patients had a very reduced physical-activity, and suffered from food disorders, namely the change of appetite and nibbling. These factors, related with a poor dietetic support, and food unbalancing with an excessive ingestion of certain hypercaloric products, enormously support the uptake of weight and consequently disturbs glycemia, worsening a little more health condition of the patients.

P069
Effects of alpha-linolenic acid enriched diet on metabolic syndrome: improvement of liver insulin resistance by modulating insulin signaling pathway
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The metabolic syndrome (MS) is a complex disease defined as the coexistence of three or more of the following components: abdominal obesity, hypertriglyceridemia, and high fasting glucose. Insulin resistance is the pathogenic denominator that links all components of the MS. The mechanisms responsible for the onset of the MS involve a combination of genetic and environmental factors. Among them, dietary lipids play an important role. However, not all types of fatty acids promote MS, and omega-3 poly-unsaturated fatty acids (PUFA) even appear protective. However, the mechanisms sustaining these protective effects remain unclear.
Because hepatic insulin resistance, characterized by a defect of insulin signaling in hepatocytes, is a key component of the pathogenesis of fasting hyperglycemia, we investigated the effects of n-3 PUFA enriched diet on hepatic insulin signaling pathway (more specifically phosphoinositol-3-kinase pathway [PI_3K]) during the establishment of MS.
For that, fatty Zucker rats were used as model of MS in comparison with their lean littermates, and were fed a control or a linseed oil diet from gestation until sacrifice at 3-months-old.
Our results showed that an α-linolenic acid rich diet significantly decreased glucose intolerance and tended to reduce hyperinsulinemia observed in fatty Zucker rats fed the control diet. In the liver, the early steps of PI_3K insulin-receptor signaling (insulin receptors substrates 1 and 2) were disturbed in control fatty rats whereas no difference was observed in the late steps (total and phosphorylated Akt/protein kinase B). The n-3 PUFA rich diet tended to restore liver PI_3K insulin signaling pathway in fatty Zucker rats.
This study evidences that an α-linolenic rich diet is able to modulate liver insulin sensitivity during the establishment of MS. These results suggest new nutritional approaches for early insulin resistance improvement.

P070
Role of Src kinases in docosahexaenoic acid induced calcium influx via TRPC 3, 6 channels in human T-cells
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Introduction: During the recent past, there has been an upsurge of information on the role of polyunsatured fatty acids (PUFA) in the regulation of immune cell functions. PUFA have been shown to exert immunosuppressive effects in humans and animal models. In fact, DHA which is metabolized by the precursor of n-3 PUFA (linoleic acid, 18:3 n-3) by an altering series of position specific desaturase and elongase steps is found in large amounts in different parts of human body. It has been demonstrated that DHA induces increases in [Ca^{2+}]i via the ER pool and the opening of CRAC (Ca^{2+} release-activated Ca^{2+}) channels in human T cells. As DHA influence the calcium influx, we undertook the present study to elucidate role of src kinases in the regulation of T cell signaling via TRPC channels.
Methods: We have used Fura-2/AM to monitor intracellular calcium signaling. Western blotting technique is used to evaluate the degree of phosphorylation of protein kinases, and shRNA technology to inhibit the expression of TRPC 3 and 6.
Results: Our results show that inhibition of tyrosine kinases upregulate the calcium influx in Jurkat human T-cells. The DHA-evoked response in the increase of [Ca^{2+}]i while inhibiting src kinases was significantly curtailed in cells transfected with shRNA for TRPC 3 and 6. DHA induced the phosphorylation of Fyn without any effect on the phosphorylation status of Lyn and Yes. Besides, the Gab2/PI3K pathway negatively regulates the DHA-induced increases in free intracellular

calcium concentrations, $[Ca^{2+}]i$. DHA induced Gab2/PI3K interaction is regulated by tyrosine kinases (Fyn). Fyn mediated activation of Gab2 is necessary for its interaction with PI3K during calcium influx.
Conclusion: Our results suggest that DHA mediated calcium influx via the opening of TRPC channels is negatively regulated by the activation of Fyn/Gab2/PI3K pathway.

P071

Study of the impact of millet (Pennisetum glaucum) on the glucidic and lipidic metabolism in diabetic rats

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Introduction: Millets are indigenous African cereals that, unlike wheat or rice, are well adapted to African semi-arid and sub-tropical agronomics conditions. Accordingly, our choice was made on this cereal in order to check its impact on the glucidic and lipidic metabolism in Streptozotocine-induced diabetic rats.
Observation: Diabetic rats fed with corn starch-based diet underwent a reduction in glycemia estimated at 36.56%, while diabetic rats fed with Millets-based diet presented a significant reduction arrived up to 68.95%. Significant decreases in triglycerides and total cholesterol (1.06 and 1.6 g/L, respectively) were finally observed in rats receiving the Millets -based diet compared with rats fed with corn starch-based diet (2.29 and 2.37 g/L respectively).
Discussion: Most evidence points to the effect of dietary fiber on hunger and satiety, based on different mechanisms, through its intrinsic effects and hormonal responses. Dietary fiber has consistently been shown to have a higher satiety value when compared with digestible complex carbohydrates and simple sugars [1–2]. The mechanisms involved in the cholesterol-lowering effects are not well-known. However satiation and satiety induced a slight decrease in cholesterol in a moderate or low insoluble fiber to bind with bile acid. The effect of certain dietary fibers on lowering triglycerides in blood is probably slowing the absorption of the triglycerides at the small intestine, as the role of fiber in lowering the glycemic index may help reduce the blood levels of triglycerides [3].
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P072

Mancozeb and Metribuzin modulate in vitro lymphocyte proliferation and redox status of human lymphocytes

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Introduction: Human populations are generally exposed to pesticides via food intake. These compounds are of major health concern since epidemiological and experimental studies revealed adverse effects. In this work we evaluated in vitro the effects of different concentrations (1–100 μM) of Mancozeb (fungicide) and Metribuzin (herbicide), on the proliferative responses of human lymphocytes stimulated by concanavalin A (ConA, mitogen), the Th1- (IL-2, INFc) and Th2- (IL-4) cytokine secretion and on the intracellular oxidative status.
Methods: Human lymphocytes were used as the test system. Proliferation was monitored by MTT assay. Interleukin-2, -4 and INFc quantification was performed by ELISA kits. Glutathione (GSH) and Superoxide dismutase were used as Lymphocyte oxidant/antioxidant markers.
Results: The results showed that Mancozeb significantly reduced ConA lymphocyte proliferation in a dose-dependent manner in humans. It also decreased IL-2, INFc and IL-4 secretion with a a shift away to Th1 phenotype. Metribuzin at low concentrations (1–10 μM) resulted in activation of ConA stimulated lymphocyte proliferation and cytokine production in human cells. However, at high concentrations (25–100 μM), Metribuzin induced a dose-dependent inhibition of lymphocyte proliferation and cytokines. Changes in intracellular levels of reduced Glutathione, hydroperoxides and carbonyl proteins and in the activities of catalase and SOD were observed after Mancozeb and Metribuzin exposure reflecting oxidative stress and DNA damage especially at high concentrations.
Conclusion: Mancozeb and Metribuzin had significant immunomodulatory properties with oxidative stress induction at high concentrations.

P073

Investigation of obesity and overweight; Impact of eating habits in school children: Constantine 2010–2011

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Now Childhood obesity is recognized as a public health problem due to prevalence, and rapid development in recent decades.
The aim of study was to estimate the prevalence of overweight and obesity among school children 8–9 years living in the region of Constantine and clearly identify their eating habits.
An epidemiological study was conducted in 2011 involving 325 school children (165 girls and 160 boys) aged 8–9 years in the town of Constantine. We distributed an anonymous questionnaire, it is reset to be completed by the parents at home, given were treated with EPI info version 6.4.
The measurements were performed according to standard procedures (OMS 2007), body mass index (BMI) shows that 3.10% of children are in a state of emaciation, 66.80% are normal, 18.20% are overweight, 12% of children are obese, sex was significantly associated with obesity ($P < 0.001$).
Afternoon snack for children recognized as a factor promoting weight gain and found that 50.80% of children who take the afternoon snack are overweight and 48.70% are obese.
93.20% of children are foods high in calories and outside the main meals. 97.40% of obese and overweight children practice saying snacking. Decision-sweetened beverages were significantly correlated with obesity.
The results of our survey show that 83.4% of children do not practice physical activity. The relationship between physical inactivity and obesity is significant ($P < 0.001$).
The correlation between the type of breastfeeding (breast, bottle, or mixed) and BMI in obese children and not significant ($P > 0.05$).
These results suggest that obesity and overweight are a growing epidemic in progress, the unbalanced diet and physical inactivity are key factors that must be controlled by the establishment of a program of early prevention of this silent disease.

P074

The taste for children aged 5–10 years

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Introduction: Taste, have an essential role in food choice and therefore the quality of diet that determines the nutritional status of the consumer.
In thecontext of better nutrition education and to help child to have nutritionally adequate consummation, we are interested in the study of taste among children through the following objectives; to determines the rate of recognition and knowledge of food children's food preferences by sex and age.
Method: The study population is targeted aged 5–10 years enrolled in primary schools in the wilaya of Constantine Algeria, 784 children were in this study, 395 were girls and 389 were boys a food preference questionnaire was used including a list of foods grouped into eight food categories according of the objectives of our study.
Results: The results of this study indicate that children aged 5–10 years show positive attitudes towards the majority of food categories, sweet and fatty foods have the highest degrees of the recognition (about 97%), we don 't found a signification difference between hedonic scores of girls and boys for all food groups. By age children aged 6–7 years prefer more dairy, sugar and fat than other ages, children aged 5 years had the lowest score hedonic towards vegetables.
Conclusion: The preference for specific foods is largely a reflection of the cultural and personal experience, knowing the factors involved in establishing food preferences my enable the development of education strategies.

P075

Antibacterial effect of lactic acid bacteria against multidrug-resistant staphylococcus aureus isolated of milk cows mastitis

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This study aims to highlight the antagonistic power of lactic acid bacteria towards *S. aureus* isolated from mastitis cow's milk from different farms in the area of Amizour (Bejaia).
An isolation and identification of staphylococcal strains from 17 samples of cow's milk mastitis after antibiotic treatment were conducted
Of antibiograms of strains *S. aureus* were performed to select the strain most resistant to antibiotics.
A screening of six strains of lactic acid bacteria was carried out on the basis of their antibacterial effect against the strain of *S*. aureus selected.
The strain of lactic acid bacteria that has demonstrated the best antibiosis was selected for testing in vitro against *S. aureus*.
Finally, an in vivo study of nine holoxenic rabbits infected with multidrug-resistant *S. aureus* was conducted. After the rabbit's dissection, histological sections of intestinal and colonic portions were performed.
The isolation allowed enrolling 10 strains to *Staphylococcus aureus*. The antibiograms of the strains identified as *S. aureus*, were used to select the strain L5, which is more resistant to antibiotics.
The test of spots showed that the *Lb. paracasei* is the strain which showed the best antibiosis against *S. aureus* L5.
The monitoring of the antibacterial activity of *Lb. paracasei* towards *S. aureus* (L5) in skim milk showed that the growth of *S. aureus* (L5) is reduced by 1.5 log in the presence of *Lb. paracasei* after 24 h of culture.
The histological sections performed on Intestinal and portions of colon, have showed a restoration of the intestinal villi and colonic crypts in rabbits treated with *Lb. paracasei* compared to control diarrhea and diarrheal untreated rabbits receiving sterile skim milk. In the latter, the villi and crypts appear atrophied and deteriorated by the action of *S. aureus* (L5).
These results support the use of *Lb. paracasei* as a probiotic against the antibiotic-associated diarrhea caused by *S. aureus*.

P076

Importance of traditional diet containing barley 'Telbina diet' on the hyperglycemia of wistar rates

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Objective: Testing the effect of a diet containing barley (Hordeum vulgare) inspired of receipt of Telbina on the hyperglycemia of the diabetic rats compared to a control diet.

Materials and methods: This study was conducted on male wistar rats at which one induced the diabetes by intra-péritonéal injection of (60 mg/kg) of Streptozotocin. The rats, weighing 220 ± 5 g, were subjected either to the control diet (compound mainly of casein, vegetable oil, starch and cellulose), or with the experimental mode containing 55% of Telbina, and this during 4 weeks.
Results: Our results show a major reduction in the glycemia (approximately 80%) in the diabetic rats under diet containing Telbina, and one sees this rate arriving at the normal (approximately 1 g/L) at the end of 4 weeks. Also, one recorded a profit of weight in these rats estimated at 18 g/month. On the other hand, in the diabetic rats under control diet, the rate of the glycemia reaches the 5 g/L. The body weight decreases considerably at this group of rats, estimated at −45 g/month. The statistical analysis shows that the difference between the effect control diet and the diet containing Telbina on the diabetic rats is highly significant ($P \leq 0.01$) dice the 1st week. Moreover, it is noted that the diet containing Telbina involved a regression of some symptoms of the diabetes of which polydipsy. Indeed, the urinary analyses revealed the absence of the ketonic bodies and proteins, whereas, these symptoms persist in the diabetic rats subjected to the control diet.
Conclusion: The diet containing Telbina could prevent or modulate the hyperglycemia of diabetic rats.

P077
Optimizing extraction of tomato lycopene by solvent mixtures
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Lycopene in tomatoes is a natural antioxidant which is part of the carotenoid family, as well as the β-carotene, capable of reacting with free oxygen to compensate for the oxidation reactions responsible for the bleaching of pigments and disease (Arab and Steck, 2000; Hadley, 2003). Each year, millions of tons of tomatoes are processed and large amounts of by-products are discarded. Rather than discard these wastes will benefit consumers through their wide range of new jobs and can make better use by using them as food additives.
The objective of this work is to optimize the extraction of lycopene from tomato by mixtures of solvents (dichloromethane, hexane and petroleum ether), the data is processed by the statistical software Minitab 15. This study has opted for optimal mixture composed of 62.64% of dichloromethane, 13.12% of hexane and 24.24% of petroleum ether to give a better extraction efficiency. This mixture has given the content of lycopene in tomato skins, which is 837.14 µg/g. The lycopene content of tomato peels was estimated at 837.14 µg/g. This result shows the interest of the valuation of the tomato peels, to expand their range of uses in the food industry as a food additive and explore all the therapeutic properties of lycopene from tomatoes.
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P078
Antioxidant effect of three parts (leaf, root and seed) of Zizyphus lotus L. in diabetic rats
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The use of medicinal plants is now the form of medicine most widely used around the world. *Zizyphus lotus L.*, like other plants of the same family, was used ancestrally to treat diabetic diseases in Algerian population. We found that the rate of vitamins A, E and C, considered being antioxidants, decreased significantly in diabetic animals. Root extract increases all these vitamins in diabetic rats. In addition, it appears that the root and leaf extracts regenerate the glutathione in the liver of diabetic animals. We also observed that the activity of glutathione peroxidase (GSH-Px) increases in red blood cells, pancreas and liver of diabetic animals. When considering the total antioxidant status by determining the activity of the ORAC and KRL in the blood, we observed again, that the diabetic animals exhibited a decline in these indicators and the three extracts of Zizyphus lotus L. appears to normalize these parameters.
All these observations indicate that hyperglycemia of diabetic animals lead to a decrease of antioxidant status; furthermore, the extract of Zizyphus lotus root of L. has beneficial effects in this pathology by improving the antioxidant status.

P079
Effects of Oleaster oil on plasma and liver lipids in rats fed a cholesterol-rich diet
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Several studies were conducted on the effect of olive oil, main source of fat in the Mediterranean Basin, on cardiovascular diseases whilst little attention was attributed to the study of the *Oleaster* (the ancestor tree).
Objective: With the aim to select new vegetable products with therapeutic properties, the present study focused on the chemical composition and the effect of Algerian Oleaster oil on plasma and liver lipids in rats.
Material and methods: The chemical composition (fatty acids) was determined by CPG/SM (AOCS, 1989). Male Wistar rats divided in four diet groups ($n = 5$): Sunflower group, Oleaster group, or supplemented with cholesterol, Chol/Sunflower and Chol/Oleaster groups. The experiment lasted 4 weeks. Total lipids livers were determined by Folch *et al.* method. Lipid parameters of liver and plasma were determined with kits. Differences between groups means were assess using Student test '*t*'. Differences were considered to be significant at $P < 0.05$.
Results and discussion: Administration of Oleaster oil lowred slightely the serum levels of total cholesterol, while increasing the serum level of high density lipoprotein cholesterol. Those results are in accordance with investigations of Belarbi *et al.* (2011), determining the effect of oleaster oil on humans. Furthermore, the content in total lipids in liver, decreased in rats fed Oleaster oil compared to those fed sunflower oil. Kris-Etherton *et al.* (1984) suggested that the intake of monoinaturated fatty acid increased levels of plasma and hepatic cholesterol in rats compared to dietary polyunsaturated fatty acid. Similar findings have been reported by Beynen (1987) and Chang and Huang (1999).
In conclusion, Oleaster oil positively affects plasma and liver lipids. This new oil can be used as alternative to olive oil in human diet after toxicological studies.
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P080
Study of children food neophobia and factors influencing
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Introduction: Food neophobia is an unwillingness to try a food unknown, which leads to a restriction of food directory and poorer adjustment to the dietary recommendations that may affect the nutritional status of children. To help the child to eat food which they reject, we are interested in the study of taste in children through the following objectives; to detect the level of food neophobia by sex, age and factors that may reduce neophobia in children.
Method: The study population is targeted to children aged 5–10 years enrolled in primary schools in the wilaya of Constantine Algeria, located in different urban areas, 784 children were included in the study, which 395 were girls and 389 were boys. A questionnaire for children was used to assess the food neophobia and the factors that influence it.
Results: In this study children aged 5–10 years are moderately neophobic which there is no significant difference between girls and boys. Children aged 5 years had a lowest score of the food neophobia while aged 10 have the higher score of food neophobia compared to other ages. Children aged 5 years are more influenced by imitation, visual appearance and environment of food intake than other ages.
Conclusion: It appears that children aged 5–10 years are moderately neophobic, whose gender was not a factor influencing the phenomenon of food neophobia and secondly it is possible to overcome neophobia child based on different factors and strategies that can influence this behavior.

P081
Sex differences in eating habits and lifestyle among medical students
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The objective of this study was to determine eating habits and lifestyle characteristics among a group of students.
Patients and methods: The students of the first and second year at the faculty of medicine of Monastir (Tunisia) completed a self-administered questionnaire including data about socio-demographic characteristics, lifestyle and dietary habits.
Results: A total of 235 women and 108 men with a mean age 20.26 ± 0.74 and 20.52 ± 0.96 years, respectively, were included in this study. Men had significantly higher ($P < 0.01$) weight, height and body mass index values. The majority of women and men were of normal weight (79.6% vs. 71%). A significantly higher ($P < 0.001$) percentage of men than women had a regular physical activity. Smoking and alcohol intake were not common among students. Equal frequency (68%) in women and men reported taking meals regularly. Forty five percent to 76% of women and 34% to 62% of men consume at least one meal at home. Breakfast was the most ($P > 0.05$) skip meal in men. A significantly larger ($P < 0.01$) percentage of women (65.5%) than men (51.4%) reported daily intake of vegetables. The daily fruit intake was equally reported by women and men. Seventy three percent of students consumed <1.5 L of water and particularly women than men ($P < 0.001$). No significant differences by sex were observed with regard to consumption of milk, coffee, tea, soda or fruit juice.
Conclusion: Several sex differences were observed in eating habits and lifestyle. Nutrition interventions are recommended to improve student's lifestyles and diets.

P082
Hypoglycemic and hypolipidemic effects of ethanolic Artemisia herba-alba extract in diabetic type 2 rats
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Diabetes mellitus is the major endocrine disorder in our society. Hence, we are interested in evaluating the pharmacological action of Tunisian traditional plants used as medicines.
The objective of this study was to evaluate the antidiabetic and antihpyerlipidemic effects of ethanolic Artemisia herba-alba extract on diabetic type 2 models that were established by combination of high fat diet fed and two low dose of streptozotocin (STZ).

Male Wistar rats (100–130 g) were fed with high fat prepared diet a (15% fat) for a period of 2 weeks. Than rats was injected intraperitoneally with two low doses of streptozotopcin (30 mg/kg body weight) separated by 1 week. Hyperglycemia was confirmed after 3 days of the injection date of STZ and only the rats with the blood glucose exceeds 250 mg/dL were considered diabetic. The ethanolic extract of Artemisia herba-alba was given orally (2 g/kg body weight) daily for a period of 45 days.
Our results showed that the ethanolic Artemisia herba alba extracts reduces the blood glucose level in diabetic group. The plasma lipoproteins HDL, LDL- cholesterol and the triglyceride levels were altered in high fat fed and STZ induced diabetic rats and these levels were also reverted back to near normalcy by Artemisia herba alba treatment.
It may be concluded that Artemisia herba alba possesses hypolipidemic and hypoglycemic effects; witch may be due to the presence of flavonoids in the extract.

P083

Study of the nutritive value of Opuntia ficus indica

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The *Opuntia ficus indica*, commonly known as 'prickly pear' is a Cactaceae native to Central America that is well suited to arid and semi-arid areas such as Algeria. This cactus, long ignored, is attracting more and more interest in recent decades.
Objective: Our objective was to determinate the nutritive value of seeds of Opuntia ficus indica.
Materiel and methods: Chemical composition of crude fiber was estimated by method of (AOAC, 1993), protein by (AOAC, 1995), lipid (ISO 659, 1988), sugar and minerals by (Dubois and al., 1956) and (Audigié et al., 1980).
Results: The study of seed composition shows high crude fiber content estimated at 71.91% and significant levels of fat and protein 10.19% and 9.19% respectively. The rate of minerals amounts to 1.74%, which is not negligible, while the sugars are found in trace amounts only.
Conclusion: Seeds of Opuntia ficus indica can be a good source of crudes fiber, protein and lipid.
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P085

Longitudinal study of the prevalence obesity Children in the district of CONSTANTINE (2009–2010)

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Childhood obesity is now the most common metabolic disease in the world. Its prevalence has been increased rapidly over the past two decades.
Obesity is a case which characterized by excess body fat, distributed across the board in the various areas of the body.
The importance of this subject in the prevention and the knowledge of risk's factors that promote the development of this silent pathology, we have released an epidemiological study during 2009–2010 attached to an anonymous questionnaire, of 305 children attending school in the town of Constantine aged between 7 and 8.
The work took place in different schools in the previous town that selected after the consultation with the Office for National Statistics (ONS).
Rates of overweight, obesity, normal and underweight are calculated according to new standards of WHO growth in 2007.
In 2010, the prevalence of obesity in school children aged between 7 and 8 old is 6.2% while the percentage of overweight was 16.7%, and we see that taking sugary drinks with meals is a factor that promotes weight gain, because we found 88.5% in children, obese children and overweight pose a significant percentage (78.90%, 88.20%).
As for snacking, 98.4% of children reported snacking between meals often, so the relationship between snacking and (BMI) is signified too important.
Our study shows that the trend of increasing prevalence of childhood obesity over time is confirmed and this requires to the establishment of a prevention program.

P086

Effect of a mode hypergras on the composition in lipids of the bodies and the activities of lipases at the rat wistar during gestation (Liver, fat Fabric, Muscle, Intestine, Brain and Heart)

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The objective of this work is to determine the effects of the mode cafeteria (hyperlipidic and hypercaloric) on the metabolism of the lipids and the activities of lipases in rats during pregnancy and lactation (1).
Materials and Methods: the study was undertaken on rats of the wistar type. The rats pregnant were divided into two great groups, a pilot batch and an experimental batch which consumes the mode cafeteria. Samples taken on tube EDTA are used to proportion glucose, triglycerides, cholesterol. Aliquot parts of bodies were preserved for lipidic, proteinic proportionings and determination of their composition in fatty-acids.
The lipase activity is determined starting from the hydrolysis of the TG of synthetic substrate, by measuring the quantity of CASE released by titrimetry according to technique pH-STAT.(3)
Results and discussion: In addition, the increase in the serum total cholesterol and triglyceride rates is related to an increase in synthesis and secretion of the lipoproteins (2). Our results show that the mode cafeteria induces an important activity LPL on the level of the liver, fat fabric, muscular fabric and intestines in the rats witness receiving the mode cafeteria compared with initiating rats receiving the mode to J0 like in J21.(4)
Our results show that during the period of gestation-breast feeding, the polyinsaturés fatty-acids of the series omega-6 behave in vitro like powerful agents 'adipogenic' and in vivo like factors supporting the development of fat fabric. For the periods of gestation and breast feeding, there exists an effect-amount between the quantities of omega-3 in the food and the accumulation of these compounds in the brain, until the optimum is reached.
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P087

In vitro n-3 and n-6 polyinsaturated fatty acids modulated mitogen stimulated T cell lymphocytes and oxidant antioxidant status in obese pregnant

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The aim of this work is to determine in vitro the effects of polyunsaturated fatty acids (PUFA n-3 PUFA and n-6) (DHA, EPA and LA) at different concentrations on lymphocytes T proliferation and the variation of some markers oxidant/antioxidant status intracellular (MDA, carbonyl proteins) in control women, pregnant and obese pregnant. Some biochemical parameters (urea, creatinine, uric acid, triglycerides and total cholesterol) and hematologic (blood count and differential count) were also assayed.
T lymphocytes are isolated from the blood of control women, pregnant and obese pregnant in the region of Tlemcen. These cells are cultured with mixed of polyunsaturated fatty acids at 30 µM (30 µM DHA/EPA 15 µM/LA 0.2 µM) and 15 µM (15 µM DHA/EPA 7.5 µM/0.1 µM LA), and then stimulated by the mitogens (concanavalin A 'Con A'; insulin). At the end of treatment, the cells are counted and used to determine the parameters of oxidative status. The plasma is used to determine the biochemical and hematological parameters.
A decrease in cell proliferation, basal or stimulated by Con A, and/or insulin was observed in pregnant women and pregnant women compared with obese controls. The mixture of fatty acids used reduced lymphoproliferation among both pregnant women and pregnant women than in obese controls. The rate of MDA and protein carbonyl levels are elevated in lymphocytes in pregnant women and pregnant obese. Our results indicate that fatty acids can modulate activity of T cells, but alter intracellular oxidant status.
The levels of triglycerides and total cholesterol were increased significantly in pregnant women compared with controls and very significant in obese pregnant women compared with controls.
The number of red, white and hemoglobin were significantly increased in obese pregnant women compared with controls.

P089

Nutrition and teeth erosion

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Teeth are not only threatened by caries, caused by bacteries, additionally the erosion of dental enamel of non bacterial origin is becoming a wide spread problem caused by exposing the teeth to acids from exogenous as well as endogenous origin:
- Exogenous acids as results of unhealthy dietary habits, mainly by frequent uptake of acidic beverages as soft drinks (orange juice, cola) vinegar, wine and acidic medication.
-Endogenous acids can be caused by reflux from the stomach (Lussi A. and all 2004).
Regardless of the origin of the acidic challenges there is a general consent in the scientific community that erosive damage resulting are irreversible; this is precisely why early diagnosis and a data on prevalence and progression of dental erosion are required to make comparison between different countries.
Over the past 20 years several studies evaluating the prevalence of tooth wear have been conducted in developed countries (Barlett D 2009).
We have undertaken a study in Algeria on a sample of 662 children aged between 6 and 11, attending two schools in the same area, these children were at the stage of mixed dentition. We used the BEWE index and measured especially the front teeth (upper, lower).
The results of this study are as follows: 42% showed evidence of tooth wear with 17% having dentine exposure.
To record the progress of the erosion, photographs and models were taken periodically every 2 months.
We again recorded a very high level of dental caries (70%), every child having at least four teeth with an untreated decay.
In the case of minor erosion, prevention by a normalisation of eating behavior and regular brushing with a fluoridated gel, which increases the enamel resistance to erosion, seemed sufficient (Ganss C.2009).
For larger defects in dental hard substance, prosthetics measures are indicated to protect the teeth and restore the masticatory function.

P090

Partial substitution of milk proteins by crosslinked starch (E 1422) and its effect on the rheological behavior and texture of an immitation cheese manufactured and marketed in algeria

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The immitation cheese is preparing a much more recent, which has a much more extensive stabilization of milk proteins, while retaining more or less the finished product looks like a cheese. The rising price of cheese is the result of the high costs of raw materials. Switching of caseins by other non-dairy could probably benefit from a product at a lower cost

This study was conducted in order to study the effect of the addition of a crosslinked starch (E 1422) in formula (at 3%) of immitation cheese. But first, we followed the evolution of physicochemical parameters and phenomena that occur during the manufacturing process and hydrothermal behavior of crosslinked starch were followed by microscopic analysis. The impact of this substitution on the texture was revealed in response to sensory analysis, namely the triangular test, the test of rank order and the hedonic test, these tests were respectively analyzed statistically by the binomial distribution, Friedman test, ANOVA and PCA.

During melting, swelling of starch intervened and volume increases due to the absorption of water, which is confirmed by the rheological study has shown that changes in apparent viscosity during the melting process. We have noticed a gradual increase in viscosity due to swelling of crosslinked starch grains contained in the formula. Moreover, the results indicate that the presence of this modified starch reduces the ability to redesign the immitation cheeses. The results of sensory analysis showed through the triangular test the specialty cheese was significantly different from another specialty cheese, without cross-linked starch and a high index of acceptability. The product obtained is characterized by a texture sliceable, more brittle and less liquid texture. Moreover, it appears that the final product showed no significant changes ($P > 0.05$) on the bitter, pungent, sweet, salty and sour and smell.

P091

Influence of the mutation on the growth rate of corynebacterium glutamicum

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The influence of the mutation on the growth rate of *Corynebacterium glutamicum* was investigated in this study, while following fermentation during 72 h in a medium of fermentation having glucose as source of ammonium carbon and chloride as source of nitrogen. Throughout this fermentation, we followed the production of biomass (by turbidimetry) as well as the production of metabolites (lysin) by the wild and mutated *Corynebacterium glutamicum, while measure consumed glucose, in order to estimate the* growth rate of each one. We found that the maximum growth rate μ max of the mutated *Corynebacterium glutamicum is 0.26/h, whereas that of the wild type is 0.52/h, the* consumption of glucose is more significant for the wild type than for the mutated type and the production of lysin of this one is significant compared to the wild stock which is almost null.

P099

Dynamic lung hyperinflation and exertional dyspnoea in patients with pulmonary arterial hypertension

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Objective: Despite the absence of overt spirometric obstruction, patients with pulmonary arterial hypertension (PAH) may exhibit reduced expiratory flows in tidal operating range, which could promote exercise-induced dynamic lung hyperinflation. This, in turn, could contribute to increased exertional dyspnoea. The purpose of this study was, therefore, to examine the impact of putative changes in dynamic operating lung volumes during symptom-limited incremental cardiopulmonary cycle exercise testing (CPET) on the intensity of dyspnoea in patients with PAH.

Materials and methods: Twenty-five non-smokers PAH patients with no evidence of spirometric obstruction and 10 age-matched non-smokers healthy control subjects performed a CPET to the limit of tolerance. Ventilatory pattern, operating lung volumes [derived from inspiratory capacity (IC) measurements], and dyspnoea intensity (by Borg scale) were assessed throughout CPET.

Results: IC decreased progressively throughout CPET (dynamic lung hyperinflation) in PAH patients (average 0.15 L), whereas it increased in all the healthy subjects (0.45 L). Among PAH, 15 patients (60%) exhibited reduced expiratory flows at low lung volumes. In this subgroup IC decreased throughout exercise by 0.50 L, whereas it increased by 0.36 L in the remaining 10 patients (40%). Dyspnoea intensity and ventilation (V'_E) were greater in PAH patients than in controls at any stage of CPET: at standardized work rate of 60 watts, dyspnoea ratings and V'_E were 3.4 ± 1.7 Borg units and 36.7 ± 7.5 L/min respectively (Mean±SD) in PAH patients compared with 0.6 ± 0.6 Borg units and 23.1 ± 5.1 L/min respectively in controls.

Discussion: Dynamic lung hyperinflation and excessive ventilatory demand did occur in the majority (60%) of non-obstructive PAH patients and contributed to exertional dyspnoea during symptom-limited incremental CPET.

P100

Low sigh frequency is associated with airway hyperresponsiveness in severely obese women

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Background: While asthma prevalence is increased in obesity, hyperresponsiveness prevalence remains debated. Consequently, obese patients may not fulfil physiologic criteria for asthma, which may be influenced by comorbidities as sleep disordered breathing and gastroesophageal reflux disease. The bronchoprotective role of sighs, promoting airway opening, may be critically relevant in obesity since overweight leads to distal airway closure, a phenomenon that promotes hyperresponsiveness.

Objectives: To assess the pathophysiology of hyperresponsiveness and the diagnostic criteria for asthma in severe obesity.

Methods: Severe to morbidly obese women referred for bariatric surgery (n = 118, mean ± SD BMI = 46.1 ± 6.8 kg/m^2) underwent pulmonary function testing (tidal ventilation monitoring [with measure of sigh percentage of total breaths], spirometry, lung volumes, fractional exhaled NO [determination of bronchial and alveolar NO], methacholine challenge allowing calculation of the dose-response slope), overnight polygraphy and oesogastro-duodenal fibroscopy.

Results: Among obese women, 57 (48%, 95% CI: 39% to 57%) exhibited hyperresponsiveness (dose-response slope ≥2.39% decrease/μmol) that independently correlated with FRC % predicted, $Raw_{0.5}$ (positive relationships) and sigh frequency (negative relationship) (model: $r^2 = 0.41$). After clinical and functional assessment, 22 women (19%, 95% CI: 12–26%) were classified as asthmatics (hyperresponsiveness plus suggestive symptoms), 36 (30%) as unlikely asthma and 60 (51%) as nonasthmatic. No confounding effect of sleep disordered breathing and gastroesophageal reflux disease on asthma diagnosis was evidenced.

Conclusions: A high prevalence of hyperresponsiveness is evidenced in severely obese women, which is partly related to sigh frequency. Obese women fulfil physiologic criteria for asthma, without confounding effect of sleep disordered breathing and gastroesophageal reflux disease.

P101

Does hypoxia at altitude alters the increases in maximal flows due to low air density?

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An increase in total lung volume by dilution (VA) is constantly reported in articles comparing VA at sea level and various altitudes. This change in VA is attributable to that of the residual volume. This increase can be due: (i) to a methodological error (ii) to an increase in dynamic compliance due to hypoxia (iii) to an obstructive altitude-induced disease.

The aim of the study was to test the last hypothesis by measuring flow-volume curves at altitude and comparing maximal flows to those obtained with a mixture simulating the density of air at altitude.

Methods: Eighteen healthy sujects performed flow- volume curves at sea level and at Cerro de Pasco in Peru at 4300 m altitude. Increases in % compared to sea level were calculated for FEV1, FVC, PEF, MEF25, MEF50, MEF 25–75. Flows were measured with a Hyper'compact (Medisoft).

Six other subjects performed flow-volume curves at sea level while inhaling air, or three helium oxygen mixtures simulating 2000, 4000 and 5500 m altitudes. The increase in the same variables were calculated by comparison of the intrapolated values at 4300 m to air breathing. Flows were measured with a Fleisch no 4 PTG fixed outside the wall of a body box. Calibrations were made by making the assumption that vital capacity at the mouth and in the box were equal.

Results: The mean increases at altitude were 7.0% vs. 6.0 in simulated condition for FEV, 3.8 vs. 3.9 for FVC, 21.9 vs. 22.8 for PEF, 28.7 vs. 18.1 for MEF25, 23.4 vs. 24.1 for MEF50 and 23.4 vs. 18.1 for MEF25–75. No significant difference in any variable was observed.

Discussion: The occurence of a hypoxic-induced bronchoconstriction at altitude is unlikely. Therefore the two remaining hypothesises should be evaluated. A methodological error seems unlikely as the increase in VA tended to decrease with time of exposure at altitude using the same apparatus. The hypothesis of an increase in lung compliance was made by Mansell A et al (J Appl Physiol, 1980) and was supported by measurements of pulmonary compliance. An hypoxic-induced secretion of surfactant reducing the lung elastic recoil could be a cause of the phenomenon.

P102

Decreased ventilatory response to carbon dioxide by steady state in patients with myotonic dystrophy type 1 compared to healthy subjects

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Background and objective: Ventilation is exquisitely sensitive to increased PCO_2. Carbon dioxide produces its effects by stimulating both central and peripheral chemoreceptors. The testing of such ventilatory response to CO_2 can be achieved either by steady state or rebreathing (Read) methods. In order to test the hypothesis based upon abnormality of the central ventilatory control mechanisms in myotonic dystrophy, contributing to chronic alveolar hypoventilation, we compared the ventilatory response to CO_2 between control subjects and patients with myotonic dystrophy type 1 (MD1).

Methods: Ventilatory response to CO_2 was achieved in a steady state while breathing gas mixtures containing 3% and 6% of CO_2. Each concentration was successively inhaled during 5 min following spontaneous breathing room air for at

least 10 min. While seated in a comfortable chair, ventilation and $PETCO_2$ were continuously recorded.
Results: Twenty height controls and 52 MD1 patients were studied. Ventilatory responses to CO_2 were 1.66 ± 1.03, and 0.86 ± 0.74 L/min/mmHg respectively in controls and MD1 patients. Ventilatory response to CO_2 was significantly lower ($P < 0.0001$) in MD1 patients than in controls.
Conclusion: This control study confirms the decreased ventilatory response to CO_2 in MD1 patients using the alternative steady state method. Further studies are needed to define more precisely the role of the impairment of the central ventilatory control in the course of the disease.

P103
A simple but fundamental reason why positive and negative pressure artificial respiration works differently
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The bronchial tree is close to a theoretical optimal distribution system in several senses. An optimal fluid transport system should carry air with minimal energy dissipation, meaning small muscular effort for breathing. A different requirement is that exterior air reaches the acini before the start of expiration. Consequently, the transit time from the mouth-nose to the gas exchangers should be minimized to allow for cyclic respiration.
However, any arborescent distribution system suffers from an inherent and dangerous property: its extreme sensitivity to morphological defects. If, at each successive bifurcation of a tree, the flow is divided unevenly, this effect multiplies along the tree and the final distribution of air will be strongly heterogeneous.
And anatomical studies reveal that the bronchial tree presents a systematic branching asymmetry (1). There is then a natural question about how, in normal breathing, an extreme heterogeneity of the distribution does not appear. The reason is that inspiration is driven by the dilatation of the acini that can be considered, in first approximation, as creating a *uniform* distribution. In other words; if all acini are identical and move in the same manner, the flow imposed by that same motion at the terminal bronchioles level is also uniform. And the same at expiration. So in normal breathing it is the pressure that is distributed unevenly. And this will be also the case of negative pressure artificial respiration.
On the opposite, in the case of positive pressure artificial respiration, the situation is very different. It is the external pressure at mouth-nose which drives the system and in that case, the tendency to heterogeneity will, in principle, prevail.
In the case of a rigid tree, such phenomena can be described in the multi-fractal formalism.
Reference:
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P104
Hypercapnic chemoreflex and muscle metaboreflex interactions enhance cardiac work
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Purpose: The goal of this study was to clarify through a new experimental model if, in humans, metaboreflex and chemoreflex really interact.
Methods: Subjects were 10 healthy males. A 5-min control resting period was followed by three 6-min experimental conditions in random order: (i) Hypercapnic Chemoreflex Activation (HCA) characterized by 6-min rest with +10 mmHg end-tidal CO_2 partial pressure ($PetCO_2$) from normocapnia, (ii) Muscle Metaboreflex Activation (MMA) characterized by a 1-min isometric handgrip exercise of dominant upper-arm at 50% maximal voluntary contraction (MVC) followed by 5-min rest with normocapnia, and (iii) both reflexes activation (HCA+MMA) characterized by 1-min isometric handgrip exercise of dominant upper-arm at 50% MVC followed by 5-min rest, and by +10 mmHg $PetCO_2$ from normocapnia simultaneously. Throughout all 6-min experimental conditions, dominant upper-arm ischemia and voluntary hyperventilation (minute ventilation = 30 L/min) continued. We measured MAP, heart rate (HR), and cardiac output (CO) and calculated stroke volume (SV) and total vascular resistance (TVR). Data were averaged on 4-min stationary periods: control and experimental condition.
Results: All the variables were similar across the three experimental conditions during the control resting period. Then, compared with control, MAP increased during the three experimental conditions: MAP, HR, CO, and SV were higher in the HCA+MMA condition than in HCA or MMA, while TVR was increased only in the MMA condition. In HCA+MMA condition, rise in MAP was the result mainly of the increases in HR and SV (MAP: +31.3%; CO: +40.7%, HR: +27%; SV: +11.25%; TVR: +0.3%). On the contrary, on one hand MMA increased MAP mainly via the rise in TVR, and on the other hand, HCA increased MAP via the rise in HR with sustained SV and TVR.
Conclusion: Our study shows that hypercapnic chemoreflex and muscle metaboreflex interact leading to a greater response when both are activated rather than each one separately. Interactions seem to modify the nature of the response, increasing the cardiac work through heart rate and stroke volume enhancement simultaneously to systolic as diastolic arterial pressure increase.

P105
An integrated, nonlinear model for oxygen transfer into the blood
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We propose an integrated model for oxygen transfer into the blood, coupled with a lumped mechanical model for the ventilation process. Chemical reactions are accounted for explicitly by mean of Hill's curve, which quantifies the saturation of hemoglobin. The nonlinearity of those effects has strong consequences on the oxygen transfer rate, and allows to reproduce features which cannot be recovered by the linear framework which defines the commonly used Lung Diffusion capacity (or Transfer Factor). We shall pay a particular attention to the sensitivity of oxygen uptake with respect to the different parameters of the model, and highlight the fact that this sensitivity may be highly dependent of the considered regime. We show in particular that membrane permeability does not affect this oxygen transfer in the normal regime but, as it decreases (e.g. in the case of emphysema) below a critical value, it becomes a significant parameter. In the same spirit, the model recovers the fact that patients suffering from a deterioration of the membrane might not feel any trouble at rest, but they may experience difficulties as soon as they perform exercise. Another striking difference of our approach with the straight use of the Lung Diffusion Capacity is that oxygen transfer is almost independent of oxygen concentration in the alveolar air, as far as it does not enters a critical zone. This model can be extended straightforwardly to account for oxygen heterogeneities in oxygen distribution overall the respiratory tract, and thereby to investigate the influence of the non-uniform distribution in the different regimes.

P106
Usefulness of spirometry, lung volumes and psychological status assessments of the dyspneic patient
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Rationale: Spirometry and lung volume measurements are recommended for the assessment of dyspnea, together with an evaluation of psychological status.
Objectives: To assess these statements in non-overlapping conditions characterized by potentially abnormal ventilatory capacity.
Methods: Patients with severe obesity (BMI ≥ 35), chronic obstructive pulmonary disease (COPD) or interstitial lung disease (ILD) underwent spirometry, lung volume and psychological status (Hospital Anxiety-Depression, Fatigue Impact Scale) assessments. The relationships between dyspnea, psychological status and heath-related quality of life (SF-36 score) were evaluated using principal component analysis. Patients with mild dyspnea (MRC score ≤2) were compared with those with moderate/severe dyspnea (MRC score ≥3).
Main results: Three hundred and twenty-eight patients were prospectively enrolled, of whom 107 (33%) exhibited moderate to severe dyspnea (45/128 COPD, 28/78 ILD, 34/122 obese). Principal component analysis demonstrated that anxiety, depression and fatigue had no effect on dyspnea, and were related to the mental component of SF-36. Dyspnea only affected the physical component of SF-36. Severe dyspnea was related to airflow limitation and lung hyperinflation in COPD, to restrictive defects in ILD, and to increased resistance in obese patients. In non-obese patients, a $FEV_1 < 31\%$ predicted exhibited a 92% positive predictive value for severe dyspnea (99% specificity).
Conclusions: Dyspnea quantified by MRC is not affected by psychological status in patients with COPD, ILD or obesity. Spirometry and lung volume measurements have limited usefulness for predicting dyspnea in these patients since only a very severe impairment of FEV_1 can be used to confidently predict frank dyspnea.

P107
Pulse transit time allows a reliable non-invasive measurement of respiratory effort under non-invasive ventilation
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Rationale: Among respiratory events which may occur during nocturnal non-invasive ventilation (NIV), differentiating between central and obstructive events requires appropriate indicators of respiratory effort.
Objective: To assess pulse transit time (PTT) as an indicator of respiratory effort under NIV in comparison with esophageal pressure (Pes).
Methods: **1** During wake period, PTT was compared to Pes during spontaneous breathing and under NIV with or without induced leaks in 11 healthy individuals. **2** To evaluate the contribution of PTT vs. Pes for differentiating central from obstructive respiratory events occurring under NIV during sleep, 10 patients with obesity hypoventilation syndrome (OHS).
Results: **1** From spontaneous breathing to NIV without leaks, respiratory effort decreased significantly and with increasing level of leaks, there was a significant increase in respiratory effort. In both situations changes in PTT accurately reflected changes in Pes.
2 In OHS patients during nocturnal NIV, intraclass correlation coefficients between Pes and PTT were 0.970 for total number of events and 0.970 for percentage of central events.
Conclusion: PTT accurately reflects the unloading of respiratory muscles induced by NIV and the increase in respiratory effort during NIV. PTT during sleep is also useful to differentiate central from obstructive respiratory events occurring under NIV.
Clinical trial registration number: NCT00983411.

P108

Role of cellular effectors in the emergence of ventilation defects during allergic bronchoconstriction

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Rationale: It is not known how local factors within the airway wall or parenchyma may influence the emergence and spatial distribution of ventilation defects, thereby modulating the dynamic system behaviour of the respiratory system during bronchoconstriction. The goal of this study was to assess the relation between the distributions of cellular effectors and the emergence of defects in regional ventilation distribution following allergen challenge.

Methods: We performed high-resolution K-edge subtraction synchrotron imaging (KES; 47 μm pixel) during Xenon inhalation and measured the forced oscillatory input impedance (Zrs) in OVA-sensitized Brown-Norway rats ($n = 6$) at baseline and repeatedly following OVA challenge (5% solution in NaCl 0.9%, 10 min). Histological slices best corresponding to the CT image were prepared and stained with a modified May-Grunwald Giemsa (MGG) and immunohistochemical (IHC) staining with monoclonal anti rat CD68. Slides were digitized and bronchi and blood vessels were randomly selected within and outside of ventilation defects, based on Xe-KES images. Total cells and eosinophils were counted in MGG images within 100 μm of the bronchial and vascular basement membrane (BM) and normalized to BM length using computer assisted image analysis, in the imaged animals as well as three controls.

Results: Eosinophil and CD68+ counts were significantly higher in the airway and vascular walls within the ventilation defects (VD's), compared to their counterparts outside of the defects (Figure); #: $P < 0.05$ vs. control; *: $P < 0.05$ vs. Outside, by ANOVA. The minimal central airway diameters following OVA challenge were tightly correlated to eosinophil and total cell counts in the airway walls within the poorly ventilated zones: $R = -0.85$, $P = 0.031$).

Conclusions: Our data suggest that airway inflammation is locally heterogeneous, and that this phenomenon is directly involved in determining the heterogeneity of regional airway constriction and the local emergence of ventilation defects following allergen challenge.

P109

Activation of sterol response element binding proteins (SREBP) in alveolar type II cells enhances lipogenesis causing pulmonary lipotoxicity

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Introduction: Pulmonary inflammation is associated with altered lipid synthesis and clearance related to diabetes, obesity, and various inherited metabolic disorders. In many tissues, lipogenesis is regulated at the transcriptional level by the activity of Sterol Response Element Binding Proteins (SREBP).

Methods: The role of SREBP activation in the regulation of lipid metabolism in the lung was assessed in mice in which both *Insig1* and *Insig2*, proteins that bind and inhibit SREBPs in the endoplasmic reticulum, were deleted in alveolar type 2 cells.

Results: While deletion of either *Insig1* or *Insig2* did not alter SREBP activity or lipid homeostasis, deletion of both *Insig* proteins ($Insig1/2^{\Delta/\Delta}$ mice) activated SREBP1, causing marked accumulation of lipids that consisted primarily of cholesterol esters and triglycerides in type 2 epithelial cells and alveolar macrophages. Neutral lipids accumulated in type 2 cells in association with the increase in mRNAs regulating fatty acid and cholesterol synthesis. While bronchoalveolar lavage fluid (BALF) saturated phosphatidylcholine (SatPC) was modestly decreased, lung phospholipid content and lung function were maintained. $Insig1/2^{\Delta/\Delta}$ mice developed lung inflammation and airspace abnormalities associated with the accumulation of lipids in alveolar type 2 cells, alveolar macrophages and within alveolar spaces.

Conclusion: Deletion of *Insig1/2* activated SREBP and lipogenesis in respiratory epithelial cells resulting in lipotoxicity related lung inflammation and tissue remodeling.

P111

Feasibility of K-edge subtraction (KES) synchrotron imaging for the measurement of regional aerosol deposition, lung ventilation and airway morphology in rabbit

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Rationale: Currently, no single imaging modality allows the quantitative measurement of aerosol deposition heterogeneity, simultaneous assessment of regional lung ventilation, and the anatomic configuration of the airways. The goal of this study was to assess the feasibility of KES imaging to this end.

Methods: The experiments were performed in six healthy anesthetized, paralyzed, and mechanically ventilated New-Zealand White rabbits (Wt = 2.9 ± 0.1 kg) in upright position. We used KES imaging, a technique that uses synchrotron radiation [AJRCCM, 2009;180:296–303] to quantitatively measure regional lung ventilation, and the regional deposition of iodine-containing aerosol particles (88 mg/mL in NaCl 0.9%), delivered using an ultrasonic nebulizer with a MMAD of 2.6 ± 0.1 μm by laser diffraction (Malvern Mastersizer). Regional specific ventilation images were obtained in four axial slices during inhalation of 70% Xe in O_2. The regional iodine deposition images were obtained in 45 contiguous slices after 0, 5, 10, 15 min of nebulization.

Results: Average regional distribution of iodine deposition was quantified and represented along the apical-caudal axis in six rabbits. Total deposited elemental iodine increased as a function of nebulization time: 0.50 ± 0.38, 1.07 ± 0.71 and 1.66 ± 1.12 mg at 5, 10 and 15 min of nebulization, respectively. Aerosol deposition showed significant spatial heterogeneity and spatial correlation in normal lung.

Conclusions: These data demonstrate the feasibility of KES imaging for the quantitative measurement of regional aerosol deposition, regional lung ventilation and central airway structure *in vivo*. With this aerosol particle size distribution, aerosol transport was not primarliy determined by regional ventilation distribution. This technique will be useful for studying regional aerosol delivery in preclinical models of lung diseases in small animals.

P112

Blood conductance of NO and CO, a tricky problem in the field of lung diffusion

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The ratio k of NO and CO conductance is a determinant of the calculation of membrane conductance (Dm) and capillary lung volume (Vc). A k value of 7.5 can be derived from Carlsen and Comroe 1958 work as Borland et al (2010) in vivo result suggested a k value of 8.7. When k is slightly greater than TLNO/TLCO, the derived value of DmCO is very high and not acceptable. As CO conductance increases in hypoxia while NO conductance change is meaningless, k value in hypoxia would decrease as well as TLNO/TLCO. The specific aim of the study were: (i) to compare the impact of k value on Dm and Vc values, (ii) to describe the relationship between k and TLNO/TLCO at altitude

Methods: The effect of changing k normoxic (kn) value from 7.5 to 8.7 was tested in eight subjects during rest and three levels of maximal aerobic power at 4300 m altitude after acclimatisation. DmCO, Vc, k values and TLNO/TLCO were calculated (Hyper'compact, Medisoft).

Results: The increase in kn value from 7.5 to 8.7 decreased DmCO from 182 to 155 mL/(min mmHg) at rest and 215–186 during exercise and slightly increase Vc. k value at altitude decreased from 7.5 to 6.4 at rest and from 7.5 to 6.5 during maximal exercise as DLNO/DLCO decreased less from 4.95 to 4.4 at rest and 4.53 to 4.3 during exercise. For kn 8.7, at rest k fell from 8.7 at sea level to 7.45 at altitude, during exercise from 8.7 to 7.55. Hypoxia decreased much more k value than TLNO/TLCO ratio.

Conclusion: $k = 7.5$ gave acceptable DmCO values in this experiment inasmuch they were in the range of morphometric values. The definitive acceptability of this k value is nevertheless questionable as DLNO/DLCO values close to k value have been published in healthy or diseased humans as well as in animal experiments.

P113

Longitudinal study of lung Function in the first year of infants with cystic fibrosis diagnosed by newborn screening

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Objective: To measure early lung function in infants with CF diagnosed by newborn screening and to evaluate with a longitudinal study the predicted value of the first evaluation. Infants with CF are longitudinally evaluated for lung function at 10 ± 2 weeks (Visit 1), 7 ± 1 months (visit 2) and 13 ± 1 months of age (Visit 3). Tidal breathing parameters, passive mechanics of the respiratory system, plethysmographic volume and airway resistance, forced expiratory flows and vital capacity using the raised volume rapid thoracic compression (RVRTC) technique were measured in all subjects. To date 18 infants have been included in the study, and have completed the three visits. At visit 1, breathing pattern, plethysmographic functional residual capacity and passive respiratory mechanics were in the normal range (1). Forced expiratory volume (FEV0.5 = 135 ± 15 mL), forced expiratory flow at 50% of vital capacity were decreased compared to the normal range (1). Forced volume capacity (FVC = 165 ± 15 mL) was decreased in the same proportion in so that the ratio VEM0.5/FVC was not decreased (0.8 ± 0.2).

Results at visit 2 and 3 show similar findings. Moreover no improvement was found at visit 2 and 3 in infants with initially decreased forced expiratory parameters. These results with longitudinal evaluation show an early altered airway function in C.F. patients diagnosed by newborn screening. These lung function abnormalities seem to be persistent during the first year of age. In this way first LF evaluation could be useful to predict LF evolution and could be contributive for a better respiratory management of these infants.

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P114

Modifications of sleep quality and cardiac variability during initiation of domiciliary noninvasive ventilation in patients with chronic respiratory failure

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Objective: To assess the evolution of sleep parameters and cardiac variability when initiating long-term noninvasive ventilation (NIV) for patients with chronic respiratory failure at stable state.

Patients and methods: We prospectively included 12 patients with neuromuscular disease ($n = 4$), chronic obstructive pulmonary disease ($n = 6$) and thoracic deformation ($n = 2$) with a recent diagnosis of chronic respiratory failure and being indicated to long-term NIV. A first polysomnography (PSG) under spontaneous breathing (SB) was performed at night 1 (N_1), followed by a PSG under NIV at night 2 (N_2), and another PSG under NIV at the 15th night (N_{15}). The sleep quality was assessed using fragmentation entropy S_f and efficiency entropy S_{eff} computed from hypnograms, both of them having been validated with respect to micro-arousals indices and sleep efficiency, respectively. The cardiac variability was assessed using an entropy S_{VC} computed from DRR and the ratio between positive and negative DRR (asymmetry index). These two indices have been previously used to discriminate healthy subjects from patients with atrial fibrillation and from those with congestive heart failure.

Results: The sleep quality immediately improved during NIV initiation ($S_f = 0.7 \pm 0.2$ at N1 vs. 0.6 ± 0.2 at N2 and 0.7 ± 0.1 at N15, $P = 0.05$), reflecting a significant decrease of the micro-arousals index. The fragmentation entropy did not vary ($S_{eff} = 1.1 \pm 0.3$ vs. 1.1 ± 0.4 and 1.1 ± 0.3, $P = 0.687$) nor the sleep efficiency. Both the cardiac variability ($S_{VC} = 0.29 \pm 0.25$ vs. 0.24 ± 0.21 vs. 0.21 ± 0.19, $P = 0.03$) and the arrhythmias rate ($r_{ar} = 7.1 \pm 8.8$ vs. 3.5 ± 4.5 vs. 3.5 ± 4.0, $P < 0.05$) were significantly reduced under NIV.

Conclusion: PSG recordings during initiation of long-term NIV in patients with chronic respiratory failure display objective and fast improvements of the sleep quality associated with a significant decrease of the cardiac variability and the rate of arrhythmias.

P115

The blood lactate level 3 min after a graded exercise test: maximal criteria in patients with chronic obstructive pulmonary disease

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Objective: The purpose of this study was to determine if a blood lactate level (at the exercise end: $[La^-_{max}]$ and/or 3 min after the exercise end: $[La^-_{3\ min}]$) may be used to attest the exhaustion during a graded exercise test (GXT) in patients with chronic obstructive pulmonary disease (COPD).

Subjets and method: Thirty six patients with COPD (age: 64.4 ± 9 years; body mass: 85.3 ± 23.6 kg; height: 170 ± 7 cm; forced expiratory volume: 1.654 ± 0.662 L; peak oxygen uptake: 15.0 ± 3.4 mL/kg/min) performed a GXT until exhaustion on a cycle ergometer. During the GXT, cardio-respiratory parameters were measured. Moreover, $[La^-_{max}]$ and $[La^-_{3\ min}]$ were determined. Exhaustion was verified by following criteria: (i) maximal work rate ≥80% predicted maximal work rate, (ii) respiratory exchange ratio ≥1.15, (iii) maximal heart rate ≥90% predicted maximal heart rate, (iv) ventilatory reserve ≤10%, and (v) subjective exhaustion. When at least three of these criteria were met, GXT was considered as exhaustive. Inversely, GXT was considered as sub-maximal when less three criteria were noticed. The receiver operating characteristic (ROC) curve method was used to determine a blood lactate threshold level ($[La^-_{max}]$ and/or $[La^-_{3\ min}]$) from which GXT is maximal.

Results: For $[La^-_{max}]$, the area under the curve of ROC was closed to the area under the diagonal ($P = 0.16$). For $[La^-_{3\ min}]$, the identified blood lactate threshold level was: 5.8 mM. At this blood lactate threshold level, the sensitivity and the specificity were equal to 0.92 and 0.56, respectively. The area under the curve of ROC was: 0.76. Moreover, a significant difference was noticed between the area under the curve of ROC and area under the diagonal ($P = 0.01$).

Discussion: The present study shows that it is not possible to use $[La^-_{max}]$ in order to confirm the exhaustion in our population. However, $[La^-_{3\ min}] < 5.8$ mM may help to confirm the non-exhaustion during GXT in patients with COPD. Indeed, below this blood lactate threshold level, 93% patients have less three maximal criteria.

P116

Respiratory disease and urban automobile pollution in Dakar

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Introduction: The prevalence of the respiratory disease increased considerably in the countries in the process of development. The increase in these affections is in relation to urban pollution especially related to the automobile traffic. In Senegal, two thirds of the automobile park of the country is concentrated in the town of Dakar, arousing an intense traffic. So the car remains there an essential atmospheric harmful effect because of topography unfavorable to the dilution of the pollutants. The aim of this study is to evaluate the effects of the automobile pollution in regard to pulmonaru fonction and respiratory affections in tradesmen from the Sandaga market in Dakar.

Materials and methods: It is about a pilot study which has interested 76 tradesmen (63 men and 13 women) of the market, located right in the middle of the town of Dakar. Measurements of the expiratory pick flow were made using an apparatus Minis-Wright in position upright. The subject was to blow as quickly as possible after a maximum inspiration. Measurement was repeated three times. The best result obtained is retained.

Results: We observed that 82.90% of the subjects of the population of study were men. The respiratory affections following the exposure to the air pollution concerned 50 patients (65%). In respiratory affections, cold was most frequent (88%). Bronchitis is classified in second position (29%). Occurred of the respiratory affections in the tradesmen who work in the site is strongly influenced by the presence of the vehicles at the market the cigarette smoke with a relative risk (RR) respectively 2.76 and 1.90. No significant association on the other hand was noted between the use of incense or aerosols of environment and the respiratory symptoms (RR = 0.76). The exploration of the pulmonary function by the maximal expiratory flow was within the limits of the normal for the majority of the subjects.

Conclusion: It comes out from this study that the pollution of the automobile atmosphere of origin of the Sandaga market is quite real. It appears during the analysis of the Senegalese statistics which it has been in constant increase for several years.

P117

Pituitary adenylate cyclase activating polypeptide reverses lung lesions induced by vanadium inhalation in rats

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Vanadium compounds, particularly vanadate, have been identified as a source of occupational bronchial asthma and bronchitis. Vanadate acts first directly on bronchial smooth muscle, promoting the release of Ca^{2+} from an intracellular store and secondly via reactive oxygen species production. Pituitary adenylate cyclase activationg peptide (PACAP) is a neuropeptide exercising recognized effects on bronchodilation, inflammation and oxidative stress. We thus hypothesized that PACAP may regulate airway responsiveness through these effects and examined the effects of exogenously applied PACAP-38 on lung physiopathologic effects induced by ammonium vanadate exposure. Exposure of rats to vanadate aerosols, during 15 min, induced an increase of bronchial resistance, inflammation as assessed by histological observations and an *ex-vivo* study on rat alveolar macrophages. Vanadium also induced an oxidative stress status. Importantly, PACAP alleviated all the deleterious effects of vanadium treatment as assessed by a decrease of bronchial resistance, an increase of anti-inflammatory cytokines and an improvement of the anti-oxydant status. It is concluded that PACAP may reverse physiopathologic effects induced by vanadate inhalation on rat lungs.

P118

Comparison between an automatic algorithm and experts for the detection of onsets of alea nasi and thoracic inspiratory muscles

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Introduction: In the analysis of temporal organization of the human brainstem respiratory network, an assumption is to consider the onsets of respiratory muscles activity as an image of the sequence of the activation of respiratory centers. The development of an automatic method will be useful to face the amount of onset detections needed in the different experimental conditions modifying the respiratory centers behaviour.

The aim of the present study is to compare the performance of an automatic algorithm for the detection of muscles activity onsets with an expert reference. The algorithm used is based on an automatic processing of the electromyograms activity.

Methods: Surface electromyograms (sEMG) of the alae nasi (AN) and the thoracic inspiratory muscles (TH) have been recorded during 5 min in three awake seated healthy subjects at rest. For each subject and each breath ($n = 147$), activity onsets of alae nasi (AN_onset) and thoracic inspiratory muscles (TH_onset) were detected by two experts in double-blind mode on sEMG signals. An automatic algorithm based on the dynamic cumulative sum approach was applied on the same sEMG signals. Differences between onsets detected by expert (E) and by automatic algorithm (A) were calculated for AN ($D\_E\text{-}A_{AN}$) and TH ($D\_E\text{-}A_{TH}$). The delay between AN_onset and TH_onset ($D_{AN\text{-}TH}$ = TH_onset-AN_onset) was calculated for each breath and each subject from the expert and automatic detections.

Results: The result of expert and automatic onset detections are not significantly different ($D\_E\text{-}A_{AN} = -0.17 \pm 0.27$ s and $D\_E\text{-}A_{TH} = -0.16 \pm 0.28$ s). The delays between AN_onset and TH_onset calculated from expert and automatic detections ($D_{AN\text{-}TH}E$ and $D_{AN\text{-}TH}A$ respectively) are significantly correlated ($P < 0.05$).

Conclusion: These preliminary results prove that an automatic algorithm may be used for the analysis of temporal organization of respiratory muscles activity. This tool will allow the efficient analysis of the numerous data needed to deepen the understanding of the respiratory centers interactions.

P127

Intermittent hypoxia induces neuroinflammation, with astro- and microglial alterations

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Introduction: Obstructive sleep apnea (OSA) syndrome is a highly prevalent disease. It is characterized by repetitive upper airway collapses during sleep resulting in nighttime intermittent hypoxia (IH) and daytime cognitive impairment. In the animal model of sleep apnea, rodents chronically exposed to IH also develop cognitive impairment, with oxidative injury and increased neuronal apoptosis in brain regions involved in learning and memory, such as the hippocampus. Recently, astroglial alterations were described in the parietal cortex and hippo-

campus. We postulated that cognitive deficits in OSA patients could result from neuroinflammatory processes involving microglial cells. We aimed at studying the neuro-inflammatory changes in the hippocampus using the murine model of IH.
Methods: Nine week-old male C57BL/6J mice were exposed to 1 day, 6 or 24 weeks of IH (cyclic 21–5% FiO_2, 60 s cycle, 8 h/day) or normoxia (similar cycles with air only), and were sacrificed for brain study. The density and morphology of microglial cells and astrocytes in the dorsal hippocampus were respectively studied by Iba1 and GFAP immunolabeling (n = 4/group). mRNA levels of astrocytic (GFAP) and microglial (Iba1) cell markers, and of pro- and anti-inflammatory cytokines (IL-6, IL-1B, TNFa and IL-10) were measured by real-time quantitative PCR in micropunches of dorsal hippocampus (n = 6/group).
Results: One day of IH increased IL-1B expression while IL-6, TNFa and IL-10 mRNA expression remained unchanged. Chronic IH for 24 weeks increased the number of microglial cells (Iba1 positive cells) without modification of Iba1 and cytokine mRNA levels. Iba1-positive cells exhibited morphological characteristics of primed microglia. IH for 1 day, 6 and 24 weeks increased GFAP mRNA expression but did not seem to affect the number of astrocytes (GFAP-positive cells).
Conclusion: Beside neurons, IH affect astro-and microglia which may impact on the neuron-glia metabolic coupling and plasticity, therefore contributing to the cognitive impairments. The microglial activation with transient alterations in inflammatory cytokines suggests a possible low-grade inflammation-mediated neurodegeneration.

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Environmental enrichment improves episodic-like memory and modulates task-evoked brain activation in adult mice

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Objective: Environmental enrichment constitutes an interesting model to elicit brain plasticity in animals. Indeed, it enhances learning and memory performances in rodents and is able to reduce several memory deficits, such as those occurring during aging. However, the neurobiological bases of these behavioural effects are still not fully understood. The aim of this study is to explore the mechanisms underlying the beneficial effects of enriched conditions (EC) on episodic-like memory in rodents. To do that, we examined the effects of EC on the neuronal network activated during memory recall.
Methods: Episodic-like memory was assessed through an object-recognition task in a Y-maze apparatus. A first set of experiment was conducted to assess the effects of increasing inter-sessions interval (ISI: 2, 4, 6 and 24 h) on memory performances in adult mice housed in standard conditions (SC). In a second set of experiments, episodic-like memory performances of mice maintained in SC or EC (n = 10 per group) during 3-weeks were assessed with a 24 h-ISI. Ninety minutes after the recall session, animals were euthanized and the brains rapidly removed. Task-evoked neuronal activation in brain regions was measured through c-Fos immunohistochemistry.
Results: A significant discrimination of the novel object for the 2 and 4 h-ISI but not for longer ISI was observed in SC mice. At the 24 h-ISI, a delay for which SC mice failed to recognize the novel object, mice housed in EC conditions were able to discriminate the novel object vs. the familiar one. Preliminary results indicated that beneficial effects of EC were associated with a modified task-related neuronal activation profile. Indeed, a more elevated hippocampal c-Fos expression was found in EC mice compared to SC one (n = 5 per group).
Discussion: We document here, for the first time, a temporal decline of episodic-like memory performances with a novel paradigm of object recognition task performed in a Y-maze in mice. Interestingly, EC significantly extends the episodic-like memory performances to a delay of 24-h. Preliminary data suggest that this behavioural effect is associated with a modulation of the neuronal activation profile in brain regions such as the hippocampus.

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Investigation of olfactory disorders: a new method based on eyes responses

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Olfactory disorders occurs in many pathologies and could be a valuable clue for the diagnosis of depression, Alzheimer's disease, Parkinson's disease, schizophrenia and autism. The current tools for detection of olfactory disorders involve active participation of the subject who has to select the correct name matching the odor in a list. But verbal ability may be impaired or unlearned in many patients and an alternative method is required. Based on the principle of multimodal convergence of primary sensory inputs through commons structures as the orbitofrontal cortex (involved in orientation of visual attention and in olfactory perception) and knowing that odor recognition is facilitated by research of visual clues, we propose to develop an objective test for evaluating odor recognition. Via an eye tracking method, we have quantify ocular behavior (orientation of visual attention and pupilary dilatation) during presentation of olfactory stimulation simultaneously to visual stimulus in 10 healthy male and female adults aged from 18 to 35 years. Odor presentation induced an increase of pupil diameter and an increase of time spent to look the target. Our preliminary results show the validity of this method. The pupil dilation is considered as an indicator of physiological response to olfactory stimulation. Increase of time spent to look the 'odor image' suggests that odor stimulation conducts visual attention towards the target. This result allows us to validate the assumptions made in favor of the objectivity of this method. The validation of this method in large healthy population and in pathological population (depression, Alzheimer disease and autistic disorder) would assist diagnosis of many pathology and would also allow an earlier medical treatment.
Keywords: olfactory disorders, eye tracking, pupillary response, visual attention, depression, Alzheimer's disease, Parkinson's disease, schizophrenia, autism

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Region-dependent modulation of L-DOPA-induced dopamine release by 5-HT4 receptors and noradrenergic transporters in the hemiparkinsonian brain

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Introduction: The therapeutic benefit of L-DOPA is commonly attributed to the restoration of dopamine (DA) extracellular levels in the striatum of Parkinsonian patients. The increase in DA release induced by L-DOPA is mediated by serotonergic neurons and therefore occurs in extrastriatal brain regions, which may also participate in the therapeutic benefit of L-DOPA. Some therapeutic strategies, aimed at controlling the release of the false neurotransmitter DA, target the activity of serotonergic neurons. Noradrenergic terminals may also be involved in the heterologous regulation of L-DOPA-induced DA release in the Parkinsonian brain. We tested the hypothesis that the stimulation of $5\text{-}HT_4$ receptors and the blockade of noradrenergic transporters facilitate the efficacy of L-DOPA on extrastriatal DA release.
Methods: In 6-hydroxydopamine-lesioned rats, we used multi-site intracerebral microdialysis coupled to high performance liquid chromatography to test the sensitivity of DA release induced by an acute intraperitoneal (ip) administration of L-DOPA (12 mg/kg 20 min after 15 mg/kg benserazide ip) to the $5\text{-}HT_4$ receptor agonist prucalopride (5 mg/kg, ip) and the noradrenergic reuptake inhibitors desipramine (10 mg/kg, ip) and reboxetine (3 mg/kg, ip) simultaneously in the striatum, substantia nigra pars reticulata (SNr), hippocampus and prefrontal cortex (PFC). **Results:**
L-DOPA induced a stronger increase in DA release in the striatum compared to the SNr, PFC and hippocampus. While L-DOPA-induced DA release in the striatum and hippocampus was not altered by prucalopride administration (20 min before L-DOPA), it was markedly enhanced in the SNr (+297%) and PFC (+116%). Both the administration of desipramine and reboxetine (20 min before L-DOPA) potentiated L-DOPA-induced DA release in the SNr (+107% and +139% respectively), PFC (+150% and +170%), hippocampus (+139% and 565%) but not in the striatum.
Conclusion: These data show that both the activity of serotonergic neurons and the heterologous reuptake of DA by noradrenergic transporters participate in the heterogeneity of L-DOPA-induced DA release in the Parkinsonian brain. It is expected that these therapeutic strategies, by potentiating extrastriatal DA release via distinct facets of the mechanism of action of L-DOPA, may improve the motor benefit of L-DOPA while dampening the emergence of long-term side effects.

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Cortical electrophysiological markers of language abilities in children fitted with hearing aids

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Objective: Usingcortical auditory evoked potentials (CAEP) to investigate electrophysiological markers in language abilities of children with moderate deafness treated with hearing aids.
Patients and method: Eleven children aged 8–12 years (mean age: 10.9 years) with bilateral moderate sensorineural hearing loss (HL) treated with hearing aids who were age and gender-matched with 11 children with normal hearing and language participated in this study. They were divided into two subgroups according to their spoken language and literacy skills assessed using a set of seven standardized computerized language tests from a battery of oral language tests (BILO). Six children scored well (HL+) while five fell into the fair score category (HL-). The two subgroups did not differ significantly in term of hearing level or duration of hearing loss. CAEP to tone stimuli (1100 Hz, 50 ms duration, 70 dB SPL) delivered via loud speakers with four different stimulus rates (700, 1100, 1500, 3000 ms) were recorded from 28 Ag-AgCl cup electrodes referenced to the nose. The peak latency and amplitude of each deflection culminating at fronto-central and temporal sites were analysed.
Results: CAEP deflections (fronto-centralN1b, P2, N250; temporal N1a) in both HL- and HL+ children displayed eithersimilar or greater amplitudes than responses in children with normal hearingand language. Therefore, HL+ and HL- children mainly differed on the temporal N1c wave, which was absent in HL- children and present in HL+ children although of smaller amplitude than in controls.
Conclusion: Most of the CAEP responses were similar or greater than in controls, indicating the
hearing aids allow correct activation of auditory pathways and cortical areas in children with moderate hearing loss. The temporal N1c was the only response which differentiated the two subgroups. This therefore appears to be a good marker of language impairment in HL children as previously shown in language impaired children with normal hearing. Further research is needed to demonstrate whether this electrophysiological marker is an early predictive indicator of later language impairment in children with hearing loss treated with hearing aids.

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Evaluation of the chemical model of vestibular lesions induced by arsanilate in rats

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Several animal models of vestibular deficits that mimic the the human pathology phenotype have previously been developed to correlate the degree of vestibular injury to cognate vestibular deficits in a time-dependent manner. Sodium arsanilate, is one of the most commonly used substances for chemical vestibular lesioning, but it is not well described in the literature. In the present study, we used histological and functional approaches to conduct a detailed exploration of the model of vestibular lesions induced by transtympanic injection of sodium arsanilate in rats. The arsanilate-induced damage was restricted to the vestibular sensory organs without affecting the external ear, the oropharynx, or Scarpa's ganglion. This finding strongly supports the absence of diffusion of arsanilate into the external

ear or Eustachian tubes, or through the eighth cranial nerve sheath leading to the brainstem. One of the striking observations of the present study is the complete restructuring of the sensory epithelia into a non sensory epithelial monolayer observed at 3 months after arsanilate application. This atrophy resembles the monolayer epithelia observed postmortem in the vestibular epithelia of patients with a history of lesioned vestibular deficits such as labyrinthectomy, antibiotic treatment, vestibular neuritis, or Ménière's disease. In cases of Ménière's disease, aminoglycosides, and platinum-based chemotherapy, vestibular hair cells are destroyed, regardless ot the physiopathological process, as reproduced with the arsanilate model of vestibular lesion. These observations, together with those presented in this study of arsanilate vestibular toxicity, suggest that this atrophy process relies on a common mechanism of degeneration of the sensory epithelia.

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Hippocampal NMDA receptors modulation in vestibulo-lesioned rat

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Hippocampus is a cerebral structure strongly involved in integration of numerous sense information required in spatial memory performances through long-term potentiation of NMDA receptors. It has been established that the loss of vestibular sense organ decreased spatial memory performances in human and rodent, and was associated to hippocampal atrophy in human and to biochemical changes of NMDA receptors in rodent. Our objective was to evaluate the time-course of modulation of functionnal NMDA receptors and their NR1 and NR2 sub-types within hippocampus after bilateral vestibular lesion in rodent.

Right and left hippocampus were removed at day 7 and 30 from two groups of Sprague-Dawley male rats, respectively transtympanically injected with Arsanilate or a saline solution. Density of membrane NMDA receptors were quantified by autoradiography after incubation with the [^{3}H]MK-801 (beta-imager), while NR1 and NR2A sub-types were semi-quantified by Western Blot. Density of NMDA receptors was increased by 54.5% and 46.9% within the whole of hippocampus respectively at day 7 and 30. The increase was stronger within the dorsal part of hippocampus by 93% and 86.8% at day 7 and 30 respectively. The NR1 subunit was increased by 95.2% at day 7 while NR2A subunit was increased by 43.3% at day 30. We have confirmed that functional NMDA receptors were increased (Besnard et al. *Hippocampus* 2011) after vestibular lesion mainly at the dorsal part of hippocampus known to receive sensorial information. The vestibular system modulated plasticity of glutamatergic system at the membrane level within hippocampus and might be related to spatial memory impairments after the loss of vestibular information. The time-related modulation of NR1 and NR2A subunit remains unclear but might be related to a change of long-term potentiation targets (Köhr et al. 2006).

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Influence of anxiety in spatial memory impairments related to the loss of vestibular function in rat

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Vestibular information plays an important role in spatial memory [1]. In humans bilateral vestibular lesions permanently impair spatial memory performances associated with hippocampal atrophy [2]. In rodent, bilateral vestibular lesions selectively impair spatial memory [3]. Although vestibular lesion induces anxiety symptoms [4]. in patients that remains controversial and difficult to demonstrate in rodent [5] due to permanent estibular symptoms. The aim of this study was to evaluate the anxiety-like behavior in our original model of compensated vestibular lesion and to test by pharmacological manipulation the influence of anxiety on spatial memory impairment using a complex behavioral task (radial arm maze with four arms baited). This study is the first to clearly demonstrate that 6 weeks after the irreversible loss of vestibular function, rats exhibited anxiety-like behavior in the black and white box test. Chronic treatment with diazepam at 0.5 mg/kg tended to decrease anxiety-like behavior in the vestibular-lesioned group and significantly decreased it in the control group without sedative and amnesic effects. Diazepam did not modify spatial memory performance neither the BVL group nor

the control group. Results showed that in our compensated model, anxiety-like behavior did not participate in spatial memory impairment after the loss of vestibular input.

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P135

Oxidative stress during postoperative pain in man and in experimental animals

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Introduction: The aim of our study was to assess the changes of free radicals and other biochemical parameters after nociceptive stimulation in different experimental animal models. In patiens we detected whether preemptive analgesia had a positive effect on acute postoperative pain.

Material and methods: We implemented large experimental studies using mechanical, inflammatory and visceral nociception and we found out in particular that nitroxid and hydroxyl free radicals and singlet oxygen increased significantly after nociceptive stimulation- especially in the models of mechanical and imflamatory pain and visceral pain (laparotomy). This increase can be suppressed by antioxidants (vitamin A, C, D and Selenium). At the same time, we showed that some parameters of metabolism of lipids, carbohydrates and proteins have also been changed. In particular, we measured the free hydroxyl radicals and singlet oxygen by EPR method in the tail of living and anesthetized rats. This method is absolutely a priority and has never been used before. Earlier experimental results were partially clinically verified using different types of acute and chronic pain in humans (acute pancreatitis, fractures, cholecystitis, the pain of visceral origin and low back pain).

Results: In man we studied postoperative analgesia after the herniotomy and the effects of various types, including preoperative and postoperative medication and its objective evaluation. We focused on an objective assessment of pain using different biochemical parameters, especially the free radicals that are significantly changed during acute pain. It was shown that morphine is the best indication in preemptive analgesia prior to surgery with an expected moderate pain. Morphine in comparison with Pethidine and the group without premedication significantly reduced levels of free radicals 24 h after surgery.

Conclusions: This study combines experimental approach to deal with the changes due to the different types of pain and clinical applications of the impact of preemptive analgesia on postoperative pain course.

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Larger brain oxygenation changes during downhill than uphill walking

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Introduction Evidence suggests that locomotion is influenced by central control mechanisms (Suzuki et al., 2004). Despite evidence that different nervous system control strategies may exist for human concentric and eccentric muscle contractions (e.g. Fang et al., 2001), no data are available to indicate that the brain signal differs for eccentric vs. concentric muscle actions during locomotion task. Using near-infrared spectroscopy (NIRS), the current study was designed to evaluate whether increased activation in the prefrontal and sensorimotor cortex were detected in downhill walking compared with level and uphill walking.

Methods Twelve healthy subjects (males, 26 ± 9 years) participated in the study. Using NIRS, we measured the changes in the cortical oxygenated haemoglobin (OxyHb) while the subjects walked on a treadmill at low and moderate speeds (2 and 5 km/h) with three different slopes (−5%, flat, +5%). NIRS signals overlying sensorimotor and prefrontal areas were collected at 10 Hz with a CW NIRS system (Oxymon MK III, Artinis, Netherlands) along with the heart rate (Polar, Finland). Rate of perceived exertion (RPE) was also measured after each trial.

Results Although heart rate and RPE did not change significantly across all locomotion conditions (except for heart rate during downhill at 5 km/h, $P < 0.05$), the amplitude of OxyHb changes was higher during eccentric than concentric locomotion tasks ($P < 0.05$) regardless the speed.

Discussion This study provided the first evidence that oxygenation levels are influenced in the prefrontal and sensorimotor cortical areas during various mode of walking. Magnitude of changes in OxyHb was larger during downhill walking suggesting that the brain may increase its activity in the prefrontal and sensorimotor areas to cope with more attention-demanding locomotion tasks (i.e. larger amount of sensory information, additional reflex-induced cortical activity from lengthening the muscles).

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Modulating the excitability of the diaphragmatic primary motor cortex using tDCS: preliminary data

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Objective: tDCS (transcranial Direct Current Stimulation) is a non-invasive and painless technique, able to modulate the excitability of motor cortex (Nitsche and Paulus, 2000 and 2001), as shown by an increase of motor evoked potentials (MEP) amplitude from upper and lower limbs muscles after anodal pacing and decrease with the cathodale one (Lang et al. 2004, Nitsche et al. 2005). The main goal of this study was to investigate whether anodal tDCS has a comparable effect on motor cortex zone dedicated to diaphragm.

Materials and methods: Six healthy right handed subjects have been tested with double blind procedures at three different days receiving 10 min of anodal, cathodale or placebo treatments over the left motor cortex. Before, immediately after and 10 min after application of tDCS treatment, MEPs from right and left hemidiaphragms were recorded by mean of surface EMG electrodes over 8th intercostals' spaces. Recruitment curves were obtained by increasing TMS intensity from resting motor threshold up to maximum. ANOVA was performed by using treatments and pre/post periods as influencing factors mainly.

Results: Surprisingly, anodal treatment significantly ($P < 0.05$) decreased right hemidiaphragm MEP amplitude immediately after tDCS challenge, but this effect vanished 10 min later. There was also a slight but insignificant trend to decrease for MEPs under Cathodal tDCS, whereas placebo did not change any parameters. These effects were not found for left hemidiaphragm.

Discussion: This study demonstrated an inhibitory effect of anodal polarity tDCS on diaphragm's MEP which seems paradoxical compared to previous published data concerning skeletal muscle groups. As a respiratory muscle, diaphragmatic contraction is both under cortex and bulbar motor control in response to chemo reflexes as well as complex motor task involvement. This could explain at least partly that change in motor cortex excitability would not lead to an expected increase excitability. Moreover, diaphragm muscle receptors are mainly Golgi organs innervated by Ib fibers, and an increase motor cortex excitability under anodal tDCS would probably favoured a Golgi mediated inhibitory effect at the level of motoneuron pool. Thus, neuromodulation of diaphragmatic function would be an inhibition, with potential application in patient assisted ventilation strategies.

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Estimation of peak oxygen uptake from ratings of perceived exertion elicited during a graded exercise test in obese women with type 2 diabetes

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Objective: The purpose was to assess the validity of predicting VO_2peak (i.e., the peak oxygen uptake) from ratings of perceived exertion (RPE) ≤15, during a graded exercise test (GXT), in obese women with type 2 diabetes.

Subjets and method: Seventeen obese women with type 2 diabetes (age: 54.6 ± 5.7 years, body mass: 103.6 ± 15.8 kg, height: 159 ± 7 cm, body mass index: 41.3 ± 8.3 kg/m^2, body fat: 46.2 ± 4.1%, glycosylated hemoglobin: 7.1 ± 1.2%) performed GXT. GXT was carried out on a cycle ergometer, with an initial resistance set at 10 W and increments of 10 W/min, until volitional exhaustion. During GXT, oxygen uptake (VO_2) and RPE were measured. Individual linear regressions between VO_2 and RPE ≤ 15 were extrapolated to RPE 20 in order to predict VO_2peak.

Results: Actual and predicted VO_2peak were not significantly different (12.7 ± 3.6 vs. 13.1 ± 3.7 mL/kg/min, respectively; $P = 0.32$). The Pearson product moment correlation between actual and predicted VO_2peak was high ($r = 0.89$; $P < 0.001$). The 95% limits of agreement analysis on these values (bias ± 2 standard deviations) was −0.4 ± 3.4 mL/kg/min.

Discussion: The results suggested that RPE ≤ 15 elicited during a sub-maximal GXT provides accurate VO_2peak prediction. Therefore, it is not necessary to perform GXT to voluntary exhaustion to determine VO_2peak in obese women with type 2 diabetes. This result has a dual purpose. Firstly, cessation of the GXT at RPE 15 allows the GXT to be completed safely, and limits the risk of cardiovascular complications. Secondly, it avoids a negative affect during the highest exercise intensities. Indeed, the negative affect may decrease the enjoyment and intrinsic motivation for physical activity, reducing adherence to a rehabilitation program.

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Prediction of maximal oxygen uptake from ratings of perceived exertion elicited during sub-maximal tests in competitive cyclists

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Objective: The purpose of this study was to test the validity and the accuracy of estimating maximal oxygen uptake (VO_2max) from ratings of perceived exertion (RPE), during sub-maximal tests in competitive cyclists.

Subjets and method: Twelve competitive cyclists performed a graded exercise test (GXT) and a test with randomised workloads (TRW) on a cycle ergometer. During GXT, the initial power output was set at 150 W for 4 min then increased by 50 W every 4 min, until 300 W. After this stage, an increment of 25 W was administered every 2 min until exhaustion (in order to determine the actual VO_2max). The TRW consisted of alternating 4-min pedalling periods with 8-min passive recovery periods. During the pedalling periods, the workloads were 150, 200, 250 and 300 W, in a randomised order. During the both tests, oxygen uptake (VO_2) and RPE were measured at 150, 200, 250 and 300 W. Individual linear regressions between VO_2 and RPE were extrapolated to RPE 19 in order to estimate VO_2max from each test.

Results: Actual and estimated VO_2max from GXT and TRW were not significantly different (65.0 ± 6.9, 68.3 ± 8.8 and 73.1 ± 12.0 mL/kg/min, respectively; $P > 0.05$). The estimated VO_2max were significantly correlated to actual VO_2max whatever the test ($P \leq 0.05$; $r \geq 0.57$). The bias and the 95% limits of agreement analysis represented −3.3 ± 14.5 and −8.1 ± 17.7 mL/kg/min for GXT and TRW, respectively.

Discussion: These results suggested that the RPE elicited during a sub-maximal GXT provided a valid prediction of VO_2max in competitive male cyclists. Consequently, it is not necessary to perform the test to voluntary exhaustion in order to determine VO_2max. However, the accuracy of estimated VO_2max may be sometimes insufficient for competitive cyclists.

P146

Treatment of asthma and doping: kinetic of salbutamol in urines among asthmatic sportmen, after inhalation of an usual dose to prevent asthma and after a bout of high-intensity exercise

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Introduction: Using salbutamol is unauthorized in sport practice when the urinary concentration of salbutamol exceeds the high level value of the normal range that is defined according to the results of scientific and published studies. However, none of those studies concerned subjects depicted as asthmatic and athletic at the same time, and none of those studies concerned an usual and therapeutic dose of salbutamol inhaled to treat or in prevention of moderate asthma before a high-intensity exercise bout. The aim of our study is to compensate for those lacks.

Method: Our project is supported by a grant from the French Anti-Doping Agency. We planned to include 33 asthmatic and 18–40 years old athletic subjects but we only report the first results of this study for six subjects. Salbutamol was inhaled, at an usual dose for moderate asthma (in prevention or to treat a low grade crisis), according to a daily recommended schedule (3 × 200 µg each day) and during five consecutive days. The 4th and 5th days, subjects came in our laboratory from 8 a.m. to 2 p.m. During each of those experimental sessions, urinary salbutamol concentration ($Csalb_U$) was measured (from 8 a.m to 2 p.m), either at rest (resting session) or after a 90 min cycling bout (exercise session) at 80% of the maximal aerobic power output. $Csalb_U$ was determined using mass spectrometry coupled with solid-liquid and hydrophilic separation method gas chromatrophy.

Results: Either for the resting or the exercise session, the maximal $Csalb_U$ value at the beginning of those experimental sessions was 288 µg/L. During the resting session, $Csalb_U$ decreased over the 6 h. During the immediate recovery period after the exercise bout (exercise session), $Csalb_U$ reached a maximal value of 422.7 µg/L and decreased over the four next hours of recovery.

Conclusion: Among asthmatic sportmen, inhalation of an usual dose of salbutamol to treat moderate asthma (in prevention or to treat a low grade crisis) and according to a validated schedule seems to lead to a maximal $Csalb_U$ under 423 µg/L. This value is clearly lower than the higher value, 1600 µg/L, accepted for anti-doping control.

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Effects of diet-induced obesity and aerobic training on ErbBs receptor expression

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Neuregulin was initially described as a neurotrophic factor involved in the formation of the neuromuscular junction in skeletal muscle. More recently, neuregulin has been reported to be a myokine that exerts relevant effects on muscle glucose metabolism. Thus, neuregulin has been recognized to stimulate glucose transport in a similar and additive manner to insulin. From a fundamental and clinical point of view, it seems interesting to establish whether a neuregulin pathway dysfunction may be linked to hyperglycaemia observed in metabolic disorders as diabetes, obesity or in the metabolic syndrome. Moreover, neuregulin being released from muscle contraction, a regular physical training could activate neuregulin pathway. Thus, neuregulin has been shown to induce similar effects to those induced by physical training on muscle energetic metabolism.

From this data, the aim of this study was first to establish if the expression of neuregulin receptors (ErbBs) is altered in diet-induced obese rats and, secondly, if a 2-month aerobic physical training may improve these parameters in obese rats. To this end, 32 male Wistar rats, aged 11 months, were fed with a control diet (C, $n = 8$) or a high sucrose-high fat diet (HSF, $n = 24$) for 16 weeks. In a first step, eight animals in each group (C and HSF) were submitted to an oral glucose tolerance test (OGTT) and sacrificed to assess ErbBs receptors expression in gastrocnemius muscle. In a second step, 16 rats from the HSF group were randomly divided into two groups, a sedentary control group (NEHSF, $n = 8$) and a trained group (EHSF, $n = 8$) (treadmill running, 5 day/week during 8 weeks, high sucrose-high fat diet). Western-blot of ErbBs receptors were then performed in NEHSF and HSF.

As expected, our results indicated an impaired glucose tolerance in diet-induced obese rats as compared to C. As neuregulin receptors are concerned, results showed that high fat diet did not alter ErbBs expression. In contrast ErbBs expression was upregulated by aerobic training. So, neuregulin pathway could contribute to muscle and metabolic improvement observed after aerobic training in diet-induced obesity.

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Protective effect of Eucalyptus globulus against acétaminophéne induced kidney damage in male rat

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Under our experimental conditions, acetaminophen poisoning resulted in an oxidative stress evidenced by a significant increase of lipid peroxidation level in renal tissue. Previous administration of *Eucalyptus globulus* extract was found to alleviate this acetaminophen induced damages.

Experimental design: Rats were divided into four batches: (C) was the control group; (P) was a group of rats treated with acetaminophen (300 mg/kg); (P) was a group of rats given *Eucalyptus globulus* extract (20 g/100 mL) during 4 weeks; (PE) was a group treated by *Eucalyptus globulus* then injected by acetaminophen (300 mg/kg). After 4 weeks, animals from each group were rapidly sacrificed.

Biochemical assays: Level of lipids peroxidation was measured as thiobarbituric acid reactive substances (TBARS), according to (Powers and Lennon, 1999) The total (Cu-Zn and Mn) superoxide-dismutase (SOD) activity was determined by

measuring its ability to inhibit the photoreduction of nitroblue tetrazolium (NBT). One unit of SOD represents the amount inhibiting the photoreduction of NBT by 50%. The activity was expressed as units/mg protein, at 25°C. (Fontaine et al.2002) Glutathione-peroxidase (GPX) activity was expressed as µmoles of GSH oxidized/min/g protein.

Results: TBARS levels in renal tissue were increased in acetaminophen treated rats as compared to controls. Administration of *Eucalyptus globulus* significantly reduced these TBARS levels.

Activities of enzymes which protect against oxidative stresses, SOD, CAT and GPX were found to be respectively reduced in kidney of acetaminophen treated rats, as compared to controls. These changes, revealing a failing defence against an oxidative stress, were largely corrected in animals treated by *Eucalyptus globulus* extract.

References:

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2. Fontaine E, Barnoud D, Schwebel C, Leverve X, Place des anti-oxydants dans la nutrition du patient septique. Réanimation 2002; 11: 411–420.

P149

Effects of intermittent exercises on the obesity class

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Objective: The aim of the present study was to examine the possible influence of obesity class (moderate obesity: 30≤ body mass index (BMI) < 35 kg/m^2; severe obesity: 35≤ BMI < 40 kg/m^2; and morbid obesity: BMI ≥ 40 kg/m^2) on physiological adaptations after an intermittent exercises program.

Subjects and method: Thirty-one obese women (moderate obesity: n = 4; severe obesity: n = 14; and morbid obesity: n = 13) integrated an intermittent exercises program, whereas 22 others obese women (moderate obesity: n = 5; severe obesity: n = 9; and morbid obesity: n = 8) were untrained (i.e., control group). During the intermittent exercises the women alternated 80% and 120% ventilator threshold every 2 min for 32 min on a cycle ergometer (three sessions by week during 10 weeks). Before and after the intervention period, body mass, BMI, body fat, waist and hip circumferences, waist-to-hip ratio, resting heart rate, resting systolic and diastolic blood pressures, and limit distance during a 6-min walking test were measured.

Results: The body mass (103.9 ± 14.6 vs. 101.1 ± 14.4 kg), the percentage of body fat (47.4 ± 3.1 vs. 45.9 ± 4.5%), the hip circumference (122 ± 11 vs. 119 ± 9 cm), the resting heart rate (75 ± 8 vs. 70 ± 7 bpm), and the resting systolic blood pressure (135 ± 13 vs. 130 ± 10 mmHg) decreased only after the training program in the trained group (whatever the obesity class; $P < 0.001$). Moreover, these women increased their limit distance during a 6-min walking test (389 ± 69 vs. 436 ± 61 m; $P < 0.001$).

The women with morbid obesity have a higher percentage of body fat compared to two others groups (43.8 ± 3.1, 45.5 ± 3.1, 49.4 ± 2.8% in women with moderate, severe and morbid obesity, respectively). Moreover, the obesity class influenced the waist (105 ± 7, 115 ± 9, 125 ± 10 cm in women with moderate, severe and morbid obesity, respectively) and hip (111 ± 6, 120 ± 7, 128 ± 12 cm in women with moderate, severe and morbid obesity, respectively) circumferences ($P < 0.05$).

Discussion: These results suggest that an intermittent exercises program produces beneficial effects on obesity, regardless of obesity class.

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Continuous vs. intermittent exercise: the best exercise modality in obese women

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Objective: The purpose of this study was to determine the better exercise modality (i.e., continuous vs. intermittent exercise) in obese women.

Subjects and method: Twenty eight obese women (age: 51.5 ± 8.1 years, body mass: 101.1 ± 17.3 kg, height: 162 ± 7 cm, body mass index: 38.5 ± 5.6 kg/m, body fat: 45.5 ± 4.5%) performed a graded exercise test (in order to determine the ventilatory threshold: VT), and two sub-maximal exercises (i.e., continuous and intermittent exercises) on a cycle ergometer. The continuous exercise consisted of pedaling continually at 100% VT for 32 min, whereas during the intermittent exercise the subjects alternated 80% and 120% VT every 2 min for 32 min. Thus, an identical relative workload (i.e., 100% VT) and time duration (i.e., 32 min) were proposed, whatever the exercise modality. During the both exercises, the heart rate (HR) and the feeling (from feeling scale) were measured at the end of each 2-min period.

Results: The scores on the feeling scale were higher during the intermittent exercise compared to the continuous exercise (1.0 ± 1.5 and 0.3 ± 1.8, respectively; $P = 0.02$), whereas the HR was not significantly different between the tests (113 ± 16 and 115 ± 14 bpm during the intermittent and continuous exercises, respectively;
$P = 0.21$).

Moreover, the individual results show that the scores on the feeling scale were higher during the intermittent exercise in 18 women (i.e., 64.3%), whereas higher scores on the feeling scale were noticed during the continuous exercise in seven women (i.e., 25.0%). Finally, a same score of feeling was observed during the both exercises in three women (i.e., 10.7%).

Discussion: These results suggest that, in the present study, the obese women felt the intermittent exercise as being better than the continuous exercise. Moreover, cardiac adaptations similar were noticed whatever the exercise modality (in regard to the HR). Consequently, this study permits us to suppose that, for the same cardiac adaptation, intermittent exercises prescription rather than continuous exercises could probably increase the patients' adherence to training programs.

P151

Kinetics of glycemia in type 2 diabetes through an incremental exercise

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Objective: To describe the time course of glycemia in type 2 diabetic patients through an incremental exercise and in the short-term recovery.

Method: Seven subjects (four women) whose diabetes was discovered between 6 months and 1 year ago participated to the study. Their treatment was based on oral antidiabetic medications and/or diet alone. The test started approximately 1 h after their breakfast by 2 min of zero Watt cycling followed by a progressive exercise intensity (20 W/min) until the patient decided to stop the exercise. We did not take account of the diabetes balance state. The finger capillary glycemia was measured at rest (G_{Rest}), at the end effort (G_{Pmax}), and at 5th, 10th, 20th, 30th and 60th min of recovery (G_{R5}, G_{R10}, etc). None of the subjects took drug 24 h before the test.

Results: The average age was 41.71 ± 3.2 years (38–46 years). One subject was obese and two in overweight. The mean of maximal power reached was 123 ± 39 W (80–180 W). The glycemia had significantly fallen at the end of exercise, going from 2.13 ± 0.49 g/L at rest to 1.8 ± 0.57 g/L ($P = 0.009$). We noted a rise from the 5th min of recovery with a progressive increase until the 20th min post-exercise, followed by a new fall at 30th and 60th min. The values obtained during the recovery time were intermediate between G_{Repos} and G_{Pmax} and on no account, significantly different to these ones. There was no correlation between glycemic variation at the end of exercise and the maximal power.

Conclusion: In relatively moderate maximal powers and high starting level of glycemia during incremental exercise, type 2 diabetic glycemia can show a significant fall without reaching hypoglycemia threshold in the first hour of recovery.

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The factors correlated to the maximal fat oxidation rate in competitive cyclists

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Objective: The purpose of the present study was to determine the factors correlated to the maximal fat oxidation rate (MFOR) in competitive cyclists.

Subjets and method: Twenty four competitive cyclists (age = 23.0 ± 5.3 years; body mass = 69.6 ± 5.6 kg; height = 178 ± 6 cm; body fat = 12.6 ± 3.2%; maximal oxygen uptake = 65.6 ± 6.2 mL/kg/min; experience in cycling = 9.9 ± 4.6 - years; training time = 11.4 ± 3.6 h/sem) performed a graded exercise calorimetry test in order to determine the MFOR on a cycle ergometer.

To establish the list of factors (age, body mass, body mass index, percentage of body mass, maximal oxygen uptake, experience, and training time) linked to MFOR, simple and multiple linear regressions were performed.

Results: The simple linear regression showed that the MFOR was not correlated to the body mass, the body mass index, the percentage of body mass, the maximal oxygen uptake, and the training time ($P > 0.05$). From the multiple linear regression, the factors correlated to MFOR were the age ($P = 0.007$; $r = 0.50$) and the experience ($P = 0.001$; $r = 0.59$). However, the multiple linear regression showed that only the experience was linked to MFOR. Indeed, the age was a redundant data when the experience was integrated in the model.

Discussion: These results suggest that the MFOR is linked to experience (i.e., the number of years in practice) in competitive cyclists. It means that more a cyclist is experienced, more he is able to oxidize an important quantity of lipids.

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Synthesis and cytotoxic properties of novel acridine-based platinum (II) complexes: structure-activity relationships between ester and amide functionalities as a potential powerful linker

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Introduction: In clinic, anticancer drugs, such as cisplatin and oxaliplatin, are used in protocols to treat various types of solid tumors including colorectal cancers (CRC). However, platinum (Pt) complexes efficacy could be restricted by high toxicity and the development of cellular resistance. Such limits have stimulated researchers to develop novel platinum-based agents. We reported the synthesis, characterization and cytotoxic activity of novel acridine-based tethered (ethane-1,2-diamine) platinum(II) complexes connected by a polymethylene chain to a ester or amide function at the 9-position of the acridine moiety.

Methods: For the synthesis process, acridine-9-carboxylic acid was coupled selectively with appropriate aminoalcohol or diol. The resulting alcohols were converted to diamine ligands. Pt complexes were obtained by addition of K_2PtCl_4. Characterization included ^{1}H, ^{13}C and ^{195}Pt NMR analyses, as well as accurate mass measurements. Cytotoxicity was assessed on human colon cancer cell lines

HCT116, SW480 and HT29. Subconfluent cells were treated for 72 h by increasing concentrations of the newly synthetized compounds or by the reference molecules cisplatin or oxaliplatin. After incubation, cell viability was measured by crystal violet staining.
Results: The two series of new acridine-based platinum(II) complexes were prepared in a three step synthesis, and further characterization confirmed Pt coordination and the proposed empirical formulas. Pt compounds containing the acridine-9-carboxamide chromophore exhibited low or no cytotoxic effect. Interestingly, series of acridine-9-carboxylate complexes displayed higher cytotoxic effect than cisplatin or oxaliplatin in HCT116, SW480 and HT29. Moreover, the length of the polymethylene linker in platinated complexes could modulate the intensity of the cytotoxic effect.
In conclusion, our study demonstrated that newly synthetized acridine-9-substituted chromophore exhibited potentially interesting cytotoxic properties, especially with acridine-9-carboxylate functionality, and that a relationship between cytotoxicity and polymethylene chain length in platinum compounds could also be described. Future experiments will be performed to assess whether cytotoxic effect could be related to a dual mode of action, including platination and intercalation.

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Oral chemotherapy: a regional project to improve the outpatient's adherence and safety

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Introduction: Oral chemotherapy represents an increasing proportion of cancer therapies. However, they are causing many side effects not properly identified and managed by patients and/or local health professionals (general practitioners (GPs), community pharmacists, community nurses). Regional information on the proper use of these therapies, their iatrogenic effects, their adverse events' prevention, the safe handling of excreta has been developed to improve patient adherence to treatment and experience at home.
Materials/Methods: Supported by the Regional Health Observatory and the Regional Oncologists Group network, a multidisciplinary committee has been established, comprised of hospital pharmacists, oncologists, community pharmacists, GPs, community nurses and members of different Order Regional Councils. Documents are available at a free access website.
Results: Two types of drug leaflets (for professionals and patients) were created for each oral chemotherapy.
This contains rules of prescription and dispensation, standard posology, as well as advice on the occurrence of any side effects, and basic instructions to patients.
For health professionals a list of various oral chemotherapy (with therapeutic class, mechanism of drug action, indications and rules of dispensation) is available.
A patient diary for treatment's follow-up was developed to record, on a weekly basis, the administration, the adverse effects and the comments of patient, pharmacist, GP and community nurse. The diary, is presented by the patient to the oncologist at each consultation. This should permit the adherence evaluation of treatment (notably with the date of delivery recorded by the pharmacist).
Training on different classes of oral chemotherapy (pharmacodynamic properties, adverse effects), health professional awareness of non-adherence risks and practical use of drug leaflets was carried out. Four hundred and sixteen primary care professionals in the geographical region (97 GPs, 262 community pharmacists and 57 nurses) took part to this training.
A leaflet with guidelines for the safe handling of chemotherapy's excreta was also developed by the Regional Health Observatory to ensure an optimal safety for the patient.
Conclusion: This multidisciplinary project addresses a need for training nearby health professionals and should contribute to improve patient co-ordination of therapy, link oncologist and patients at home. For patients, better interface of all health practitioners should increase successful adherence to treatment and the odds of optimal treatment.

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Inhibition of monounsaturated fatty acid synthesis induces apoptosis in colon cancer cells: involvement of ER stress

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Background: Cancer cells present a sustained de novo fatty acid synthesis with an increase of saturated and monounsaturated fatty acid (MUFA) production. This change in fatty acid metabolism is associated with overexpression of Stearoyl-CoA desaturase 1 (Scd1), which catalyses the transformation of saturated fatty acids into monounsaturated fatty acids (e.g. oleic acid). Several reports demonstrated that inhibition of Scd1 led to the inhibition of proliferation and induction of apoptosis in cancer cells. Nevertheless, mechanisms of cell death induced by abolition of MUFA synthesis remain to be better understood.
Material and methods: In order to answer this question, we abrogated de novo MUFA synthesis in colon cancer cells (SW480 and HCT116) by three different approaches, Scd1 extinction by siRNA, inhibition of Scd1 activity by a pharmacological inhibitor (CVT-11127)[1] and by treatment with trans-10,cis-12 conjugated linoleic acid (t10,c12 CLA). Scd1 activity in cells was determined as [^{14}C]stearic acid conversion into [^{14}C]oleic acid by HPLC analysis. We evaluated global cell death by PI staining and apoptosis by annexin V staining, caspase 3 activity or PARP Cleavage expression. ER stress hallmarks have been identified by RT-PCR and by western blotting.
Principal findings: We showed that inhibition of Scd1 by siRNA, pharmacological inhibitor or t10,c12 CLA led to an efficient repression of de novo MUFA synthesis and to a modification of 16:0/16:1 and 18:0/18:1 ratio in colon cancer cells. Furthermore, the modification of Scd1 activity triggered colon cancer cell death by apoptosis and induction of several endoplasmic reticulum (ER) stress hallmarks (sXbp1, p-eIF2α, CHOP). Moreover, we evidenced that increase of CHOP expression participated to the induction of apoptosis in colon cancer cells in which MUFA synthesis was prevented.
Conclusion: Altogether these results suggest that inhibition of de novo MUFA synthesis by Scd1 extinction could be a promising anticancer target by inducing cell death through ER stress and CHOP activation.
Reference:
1. A generous gift from Dr. Jeff Chisholm, Gilead Sciences Inc., Palo Alto, California, USA

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Mathematical modeling of child T-cell lymphoblastic lymphoma for anticancer drug evaluation: preliminary results

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Rare diseases are defined based on their low incidence, <1 in 2000. The aim of the CRESim ('Child-Rare-Euro-Simulation') project is to develop a platform performing trial modelling and simulation in order to identify optimal trial designs in children for the evaluation of (orphan) drugs tailored to different types of rare diseases.
Objective: To report the preliminary results of the discursive and mathematical models of child T-cell lymphoblastic lymphoma (T-LBL) and to identify the most critical (sensitive) parameters of the model.
Methods: Published models have been searched for (i) cell cycle of primitive haematopoiesis in bone marrow; (ii) dynamics of thymus colonization; (iii) residual disease measured in blood. Equations of the model were solved using fixed-step methods after implementation in Matlab (R2011a version). Sensitivity to parameter values was assessed using numerical simulations.
Results: The global model involves an age-structured cell population model with control of death (apoptosis) and proliferation rates. Its mathematical formulation includes a system of 16 partial differential equations and 40 parameters. We have identified crucial values for: two parameters which characterize probability of shunting G0 phase; seven parameters which characterize transition ratios of cells between cycle phases; 12 parameters which characterize death ratios.
Conclusion: This model integrates basic knowledge of the role of cell cycles in T-LBL development, and represents the first step (i.e. the input-output submodel) in the global CRESim approach applied in the field of haemato-oncology. Its further links with (PB)-PK-PD drug models and execution submodels (i.e. various designs of clinical trials) will help to identify the optimal trial designs for the evaluation of (orphan) drugs in T-LBL.

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Assessment of nutritional status and dietary management of outpatients in oncology

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In oncology, malnutrition is a risk factor for worsening of side effects of chemotherapy by increasing the free fraction of drug and also to slow healing after surgery [3]. Maintaining a good nutritional status is essential in cancer patients. The main objective of this study was to assess the prevalence of malnutrition in ambulatory patients with cancer. Secondary objectives are: assessment of the level of patient information and patient involvement in their nutritional care and promotion of dietary consultations. In August 2011, a prospective study was performed in an Outpatient Medical Oncology. The assessment of nutritional status was performed by determining the Body Mass Index (BMI) and the use of self-administered questionnaire extracted from Patient-Generated Subjective Global Assessment (PG-SGA) [Bauer J et al. Eur J Clin Nutr 2002]. Finally, semi-structured interviews were conducted with patients. Fifty-four patients with a mean age 57.7 years were included. The prevalence of malnutrition was estimated at 24%: 84.5% of moderate and 15.5% of severely malnourished. After analysis of the PG-SGA, 33% of patients reported eating less than usual vs. 15% reported eating more. The main symptoms were prevented from eating enough: appetite loss and faster fullness. According to interview data, 44.4% of patients were aware of the dietary consultations. 35.2% of patients had used the nutritionnal oral complements (NOC). They were predominantly prescribed by their oncologist. Only 26% of patients have respected the doses of NOC. The prevalence of malnutrition is important in this study but is less than the literature [Hébuterne X et al. Nutr Clin Metab, 2006]. The use of biological criteria such as albumin in addition to the anthropometric criteria could increase the cases of malnutrition. PG-SGA, only validated tool in oncology, was used to assess nutritional status. The decrease in food intake was due to two major symptoms and dietary advices are available to overcome these symptoms. The poor compliance with NOC must be taken into account of comprehensive care's cancer patients. It is important to integrate the dietary management strategy in cancer treatment to improve the quality of life of patients and their tolerance to treatment.

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Vitamin D intake and light chain-IgG monoclonal antibodies in patients with multiple myeloma

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Objectives: To estimate the average daily vitamin D intake and to determine the type and levels of light chain-IgG monoclonal antibodies in patients with multiple myeloma (MM).

Patients and methods: A total of 52 subjects divided into three groups (Groups 1; $n = 19$ patients with MM [10 males, nine females], Group 2; $n = 13$ relative controls [nine males, four females], Group 3; $n = 20$ unrelated controls [11 males, nine females]) were recruited at the Hematology Department of Tlemcen University Medical Center (Algeria). Nutritional survey was conducted over a period of 1 month through a diet history interview, based on the 24-h recall. The characterization of monoclonal antibodies was performed by serum immunofixation test on Hydragel IF K20 (Sebia, France).
Results: The vitamin D intake was slightly lower in patients as compared to unrelated controls and was associated with risk of developing MM (OR = 2.08). Additionally, the frequency of IgG-kappa band was significantly higher than that of IgG lambda-band.
Discussion: Vitamin D could be considered as an effective prognostic factor and treatment of MM, especially in patients with monoclonal IgG kappa immunoglobulin.

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Microbial reduction of Aflatoxin M1 in milk

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Object: The present study was aimed to analyze the presence and levels of Aflatoxin M1 (AFM1), 'considered as a proved carcinogen' [1], in the raw and the recombined milk, in one hand, and to study the kinetics of its degradation due to the lactic acid bacterias throughout the process of the production of Leben (whey = fermented milk, product of wide consumption in the Maghreb) in the other hand.
Materials and methods: Physicochemical and microbiological characterization of the raw milk, the recombined one, water and the used ferments in the production of whey.
Immunochemical assay of the rate of AFM1 in the raw and recombined milk intended for the manufacturing of Leben (whey);
Evaluation of the degradation, of this toxin, by the lactic acid bacteria, by using the the competitive ELISA method.
Results: AFM1 rate determined by competitive ELISA in raw milk and recombined one, gave similar rates, of 119 057. 10^{-3} µg/kg, which are 2.38 times higher than the maximum recommended by the regulatory.
An evident correlation is revealed between the evolution of the AFM1 rate and the acidity whether it is for Leben with raw milk or that with recombined milk. Indeed after 18 h of maturation of Leben with raw milk, the AFM1 and acidity rates are, respectively, 32 991. 10^{-3} µg/kg and 73 °D, with a degradation rate of 88.29%. Also for Leben with recombined milk, these same rates are, in the respective order, 29 405. 10^{-3} µg/kg and 78°D, with a degradation rate of 89.36%.
Discussion: Aflatoxins are highly toxic, immunosuppressive, mutagenic, teratogenic, and carcinogenic compounds. The main target organ for their toxicity and carcinogenicity is the liver. For this reason, AFM1 in milk and dairy products should be controlled. Animal feed and food products are strictly inspected for AF contamination
We finally held (retained) that the consumption of Leben, contrary to milk, is more reassuring and present few risks of poisoning due to the AFM1
Reference:
1. CIRC. Aflatoxins. IARC Monograph on the Evaluation of Carcinogenic Risks to Humans. WHO, Lyon, France, 2002; 82: p 171

P166

Neurohormonal determinants of early changes in cardiovascular structure and function in subjects with abdominal obesity

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Objectives: We investigated the association between aldosterone (ALDO) and renin (REN) plasma concentrations with early changes in cardiovascular structure and function involved in the progression to heart failure (HF) in subjects with abdominal obesity (AO) (1).
Background: The contribution of obesity to the incidence of HF is increasing substantially (2, 3). This condition is associated with an activation of the renin angiotensin aldosterone system, which may be involved in the progression to HF (4).
Methods: Subjects with AO and age and sex matched healthy volunteers (HV) underwent ALDO and REN measurements as well as cardiac and arterial phenotyping to explore early remodeling (trans-thoracic echocardiography, cardiac magnetic resonance imaging, intima-media thickness measurement, pulse wave velocity and fibrosis biomarkers).
Results: We enrolled 116 AO subjects (BMI: 31.7 ± 3.4 kg/m^2) and 53 HV (BMI: 22.4 ± 2.0 kg/m^2). Subjects with AO had higher ALDO [mean (range) 59 (33–106) vs. 34 (18–65) pg/mL, $P < 0.0001$], left ventricular mass (LVM) [mean ± standard deviation (SD): 97 ± 25 vs. 84 ± 21 g, $P = 0.003$], and cardiac remodeling index (CRI = LVM/LV end diastolic volume) (mean ± SD: 0.69 ± 0.16 vs. 0.60 ± 0.10 g/mL, $P = 0.004$). In multivariate analyses, REN was significantly associated with LVM ($P = 0.036$) and CRI ($P = 0.009$). PIIINP, a marker of collagen synthesis, was independently associated with diastolic dysfunction ($P = 0.043$).
Conclusions: Early cardiac remodeling is detectable in subjects with abdominal obesity. These may involve renin, which could represent a target for the prevention of progression to HF.
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P167

Cyclooxygenase pathway activation in sleep apnea syndrome

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Background: Patients with obstructive sleep apnea syndrome (OSA) exhibit an early vascular remodelling and alterations of acid arachidonic (AA) pathway. Thromboxane A_2 (TXA_2) is a cyclooxygenase (COX)-derived metabolite of AA involved in vascular remodelling.
Objectives: (i) To characterize COX-pathway in apolipoprotein E-deficient ($ApoE^{-/-}$) mice exposed to chronic intermittent hypoxia (CIH, cyclic 21–5% FiO_2, 60s cycle, 8 h/day) and in OSA patients in comparison to controls; (ii) To establish the specific role of COX pathway activation in OSA-associated atherogenesis.
Methods: Forty male $ApoE^{-/-}$ mice, 14-week-old, were submitted to CIH or normoxia (N) for 8 weeks. Atherosclerosis lesions were determined in aortic roots. Expression of COX-pathway genes were investigated on aortas. Fifty lean OSA patients free of known cardiovascular risk factor matched with 25 healthy volunteers (HV) for body mass index and age, were included, as well as 56 OSA patients with cardiovascular co-morbidities. Urinary excretion of 11-dTXB$_2$ was measured by liquid chromatography-tandem mass spectrometry. Carotid ultrasonography to assess intima media thickness (IMT) was performed.
Results: Exposure of mice to CIH induced an increase of atherosclerotic plaque size in aortic roots ($P = 0.008$). This was associated with an increase of COX-1 ($P = 0.05$) and thromboxane synthase (TXBS) ($P = 0.03$) mRNA levels in aortas. Atherosclerotic plaque size significantly correlated to mRNA levels of COX-1 ($r = 0.654$, $P = 0.0003$), COX-2 ($r = 0.576$, $P = 0.002$), TXBS ($r = 0.693$ and $P \leq 0.0001$) and prostacyclin synthase ($r = 0.447$, $P = 0.02$). In patients, urinary excretion of 11-dTXB$_2$ did not differ between OSA patients free of cardiovascular risk and healthy volunteers, but was increased in OSA patients with comorbidities compared to OSA patients without (694.0 (425.9–1235.6) vs. 616.0 (354.3–838.2) pg/mg creatinine respectively; $P = 0.007$). Finally, urinary 11-dTXB$_2$ was increased by 30% in OSA patients with carotid hypertrophy (IMT>0.8 mm) compared to OSA patients without carotid hypertrophy (783.0 (582.8–938.0) vs. 592.9 (278.9–782.5) pg/mg creatinine, respectively; $P = 0.02$).
Conclusion: COX-pathway is activated in $ApoE^{-/-}$ mice exposed to CIH and in OSA patients with associated cardiovascular risk factors. This activation seems to be involved in the atherosclerotic process. Therefore, inhibition of COX-pathway could be of potential interest in prevention of cardiovascular morbidity in OSA patients.

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Alteration of NO production in type-2 diabetes by dietary intervention

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Numerous studies have indicated that the insulin resistance associated with type-2 diabetes contribute to endothelial dysfunction, with notably development of hypertension and/or atherosclerosis. This dysfunction is characterized by a reduced bioavailability of nitric oxide (NO). Diet intervention may improve some of vascular complications associated with insulin resistance.
Our aim was to evaluate the effect of 4 weeks of High-Fat (HF) vs. Low-Fat (LF) diets compared to normal chow diet (N) on aortic NO production in normal Wistar and Goto-Kakazaki rats, a non-obese type-2 diabetic animal model. Twenty-five weeks old Male Goto-Kakazaki (GK = 17 divided into three groups N, HF and LF) and Wistar ($n = 4$) rats (N), were anesthetized and thoracic aortic segments harvested. Using electron paramagnetic resonance spectroscopy associated with NO trapping by iron–diethylthiocarbamate (Fe-DETC) complexes, we measured aortic NO production. Our data show increase NO production in GK rats (wistar 100 ± 8 vs. GK 215 ± 10%; $P = 0.0001$) fed normal chow. Further, ACh (3 µM) increased aortic NO levels in both wistar and GK rats, although the relative increase was significantly higher in GK (388 ± 21% vs. 207 ± 23, $P = 0.002$) indicating an increased NO order to overcome loss of NO by peroxynitrite ($ONOO^-$) formation. LF diet reduced basal and Ach-stimulated NO-production (61 ± 12%, $P = 0.0001$ and 66 ± 08%, $P = 0.0001$, respectively vs. GK (N)), but did not modify NO bioavailability. In contrast, HF diet did not alter basal NO production and significantly reduced NO bioavailability (Normal 108 ± 11%, HF 63 ± 16%, $P = 0.03$). In conclusion, the vascular dysfunction observed in type-2 diabetes is initially associated with an increased NO production. Four weeks of LF diet reduces this high rate of NO basal production independently of the blood glucose level, while a HF diet was unable to reduce basal NO-production. The result was with HF diet a reduction in NO bioavailability vs. N diet.

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Skin microdialysis coupled with laser speckle contrast imaging to assess microvascular reactivity

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Objective: Laser Speckle Contrast Imaging (LSCI) can be used to assess real-time responses of skin microcirculation to pharmacological interventions. The main objective of this study was to determine whether intradermal or subdermal microdialysis fiber insertion, coupled with skin flux recording using LSCI, can be used to assess baseline cutaneous flux and the post-occlusive reactive hyperemic response. The microdialysis sites were compared to control area without microdialysis fibers.

Methods: One dermal and two subdermal microdialysis fibers were randomly inserted in the right forearm skin of six healthy volunteers. We performed consecutively tests of post-occlusive hyperemia, infusion of 29 mM sodium nitroprusside (SNP), local thermal hyperemia at 43°C and a second 29 mM SNP infusion at the end of the experiment.
Results: Two hours after fiber insertion, cutaneous vascular conductances (CVC) at the subdermal fiber sites were not different from their respective control regions of interest, while at the dermal site CVC remained higher (0.48 ± 0.15 vs. 0.37 ± 0.1 PU/mmHg, $P = 0.003$). The peak CVC and area under the curve observed during post-occlusive reactive hyperemia were similar at all fiber sites and their respective controls. We observed a similar increase in CVC using 29 mM SNP infusion, 40 min local heating at 43°C, and their combination. Finally, physiological and pharmacological responses of the subdermal sites were reproducible in terms of amplitude, whether expressed as raw CVC or as %CVCmax.
Conclusions: We showed that studying skin microvascular physiological or pharmacological responses using inserted subdermal microdialysis fibers coupled with LSCI is feasible and reproducible, and provides two-dimensional information. This technique will be useful for future mechanistic studies of skin microcirculation.

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Retrospective analysis of surgery vs. endovascular intervention in takayasu arteritis: a multicenter experience

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Background: With recent advances in endovascular treatment, percutaneous endoluminal angioplasty has become particularly attractive for arterial lesions of Takayasu arteritis (TA). However, data provided from cases reports or small series and the long term outcome has not been reported. The incidence of potential vascular complications after surgery or endovascular treatment is still to be determined.
Methods and results: Retrospective multicenter study analysing the results and outcome of 79 consecutive patients with TA [median (IQR) age 39 (25–50) years, with 63 (79.7%) females)] who underwent 166 vascular procedures [104 (62.7%) surgery and 62 (37.3%) endovascular] for the management of arterial complications.
After a time of follow-up of 6.5 (2.2–11.5) years, 70 complications were observed including restenosis ($n = 53$), thrombosis ($n = 7$), bleeding ($n = 6$), and stroke ($n = 4$). The overall 1-, 3-, 5- and 10-years arterial complication free survival rates were of 78% (69–88), 67% (57–78), 56% (46–70) and 45% (34–60), respectively. In multivariate analysis, biological inflammation [Odds ratio 7.48 (95% confidence interval, 1.42–39.39; $P = 0.04$] was independently associated with the occurrence of arterial complications after the vascular procedure. Patients who experienced complications had higher erythrocyte sedimentation rate ($P < 0.001$), C-reactive protein ($P < 0.001$) and fibrinogen ($P < 0.005$) serum levels compared to those without complications.
Conclusion: Our study suggests that biological inflammation at the time of revascularisation increased the likelihood of complications in patients with TA.

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Synergistic action of AT1 receptor blockade and PPAR-gamma activation on diameter restoration of pial arterioles in SHR

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Aim Some angiotensin II (Ang II) receptor blockers (ARBs) activate peroxysome proliferator activated receptors (PPAR)-gamma [1]. Both ARBs and PPAR-gamma activation may change vascular structure [2, 3], and modify AT_2/AT_1 equilibrium [4], which is important for cerebral circulation [5]. We hypothesized that short-term combination of ARBs and PPAR-gamma activation exerts a synergistic action on pial arteriolar diameter, *via* structural remodeling and/or changes in AT_2/AT_1 receptors function.
Methods Five-month-old male SHR received water, ARB without PPAR-gamma activity (candesartan cilexetil, CANDE, 10 mg/kg/day), PPAR-gamma agonist (pioglitazone, PIO, 2.5 mg/kg/day) or CANDE+PIO for 10 days. Telmisartan, an ARB with intrinsic PPAR-gamma activity, served as positive control (TELMI, 2 mg/kg/day). We measured internal diameter of pial arterioles (ID, cranial window) at baseline and following suffusion with Ang II (10^{-6} M). Passive ID (ID_{EDTA}) was evaluated following deactivation (EDTA) of smooth muscle cells.
Results For similar antihypertensive effects, only CANDE+PIO, but not CANDE nor PIO alone, significantly (two-way ANOVA + Bonferonni) increased baseline (+43% vs. SHR) and passive (+30% vs. SHR) ID. CANDE+PIO, but not CANDE (despite similar plasmatic concentrations) nor PIO alone, abolished the Ang II-vasoconstriction (CANDE+PIO −69% vs. SHR). The impact of TELMI on baseline ID, ID_{EDTA} and Ang II-vasoconstriction was not different from that of CANDE+PIO.
Discussion Following 10 days treatment, Ang II receptor blockade and PPAR-gamma activity exert a synergistic action to restore hypertension-induced narrowing of pial arterioles better than ARBs alone. This effect goes beyond the blood pressure lowering action, through changes in structure and vasoreactivity of pial arterioles.
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P172

Influence of ischemia and kinetics of myocardial reperfusion on the structure and function of mitochondria in pigs

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Introduction: The prolonged interruption of coronary flow induces severe cardiac lesions involving the production of ROS, alterations of Na^+/H^+ and Na^+/Ca^{2+} exchangers, structural and functional changes of mitochondria, reduction of cardiac contractility energy and death by ventricular fibrillation Desobstruction of the coronary artery is then required, but can paradoxically result in more severe lesions during reperfusion. The objective of this study was to evaluate electrophysiological and hemodynamic changes together with structural and functional alterations of mitochondria in an ischemia/reperfusion model in pigs.
Material and methods: Anesthetized and artificially ventilated pigs were assigned to five groups ($n = 6$) including GI: controls without ischemia; GII: 45-min myocardial ischemia; GIII, GIV and GV: 45-min ischemia followed by 1-, 10- or 40-min reperfusion, respectively. Sinus heart rate, the duration of monophasic action potentials (dMAP), and left ventricle dP/dt_{max} were measured after 1, 5, 10, 20, 30 and 45 min of ischemia.
Results: Compared to controls, ischemia alone as well as reperfusion were found to induce tachycardia, reduced dMAP and left ventricle dP/dtmax, ultrastructural alterations of mitochondria and mitochondrial function changes including decreased O_2 consumption, increased production of ROS, and reduced calcium retention.
Discussion: Overall, electrophysiological and hemodynamic changes together with ultrastructural and functional alterations of mitochondria during ischemia alone were found to be aggravated during the first seconds of reperfusion. The main mechanism seems to be the lack of O_2 supply during ischemia and the massive O_2 supply during reperfusion resulting in the marked overproduction of ROS leading to calcium overload and mPTP opening. These changes were more marked after 1 min of reperfusion than after 10 or 45 min. The observed improvement was correlated with the conservation of the structure and function of mitochondria evidenced by the moderate decrease in O_2 consumption and the moderate increase in the production of ROS, associated with enhanced mitochondrial calcium retention. These results support the use of pharmacological agents (beta-blockers, anti-ischemic agents) that are able to prevent ischemia/reperfusion lesions by improving mitochondrial functions before reperfusion of ischemic myocardium.

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Short-term angiotensin 2 receptor blockade reverses cerebral arteriolar but not arterial remodeling in the spontaneously hypertensive rat

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Aim: Short term treatment with angiotensin 2 receptor blockers (ARBs) normalizes cerebrovascular function [1]. As different levels of the cerebral circulation and cerebral vessel structure participate in cerebrovascular function, we examined whether a short term treatment with an ARB may induce structural changes in cerebral arteries and arterioles.
Methods: We treated male young adult spontaneously hypertensive rats (SHR) for 10 days with the ARB telmisartan (TEL, 2 mg/kg/day, po). Untreated SHR and age-matched normotensive WKY rats served as controls. Two sets of experiments were conducted. In the first experiment, cerebral arteriolar internal diameter was measured at baseline (ID_{CA}) and following deactivation of smooth muscle cells (EDTA ID_{CA}, EDTA, 67 mM, 30–35 mmHg arteriolar pressure) through a cranial window technique ($n = 14$–16 per group). In the second experiment, middle cerebral arteries (MCA) were mounted and pressurized (60% of mean arterial pressure, MAP) in a small vessel arteriograph to measure arterial baseline (ID_{MCA}) and passive (EDTA ID_{MCA}, EDTA, 2 mM) internal diameter ($n = 9$–13 per group). In both experiments, elastic modulus was calculated from stress-strain relationships obtained in deactivated vessels. Values are mean±sem; * $P < 0.05$ vs. WKY; $ $P < 0.05$ vs. SHR (One-way ANOVA, Bonferroni post-test).
Results: TEL significantly reduced MAP in SHR (WKY: 115 ± 2; SHR: 168 ± 3*; SHR-TEL: 129 ± 3 mmHg*$). Baseline and passive IDs were significantly reduced in both cerebral arterioles (ID_{CA}: 36 ± 2*; EDTA ID_{CA}: 77 ± 6* μm) and MCA of SHR (ID_{MCA}: 161 ± 13*; EDTA ID_{MCA}: 205 ± 7* μm) when compared to WKY (ID_{CA}: 50 ± 2; EDTA ID_{CA}: 98 ± 3; ID_{MCA}: 217 ± 15; EDTA ID_{MCA}: 284 ± 6 μm). TEL restored baseline and passive ID in cerebral arterioles (ID_{CA}: 49 ± 2$; EDTA ID_{CA}: 89 ± 4 μm) but not in MCA (ID_{MCA}: 157 ± 9*; EDTA ID_{MCA}: 209 ± 8* μm). TEL did not significantly change elastic moduli.
Discussion: Short-term treatment with TEL reverses hypertension-induced narrowing of cerebral arterioles, through a structural effect. This is not the case at the arterial level. A longer treatment period could be necessary to improve cerebral arterial structure.
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Deep hypothermic cardiac arrest treated by extracorporeal life support in a porcine model, does the rewarming rate matter?

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Introduction: Accidental hypothermia is a frequent cause of death under certain circumstances or precipitant factors. Extracorporeal life support (ECLS) rewarming is the reference technique of active internal rewarming. Neurologic injuries and pulmonary edema are the most frequent rewarming complications associated with ECLS. The current experiment was designed to study the impact of different rates of re-warming on pulmonary lesions after deep hypothermic cardiac arrest (DHCA), in a porcine model of accidental hypothermia.

Methods: Twenty four pigs were cannulated for ECLS, cooled until cardiac arrest occurred and subjected to 30 min of DHCA. During the rewarming phase, we compared a low blood flow rate of 1.5 L/min vs. a high flow rate of 3 L/min as well as two rewarming strategies: a temperature in the extra corporeal circuit adjusted to 5°C above the central core temperature vs. a temperature of 38°C maintained all along the rewarming phase. Hemodynamics and pulmonary function parameters were evaluated at specific points during the cooling and rewarming phase and 30 min after 35°C was reached. Baseline and endpoints biomarkers analysis were compared (IL1-beta, IL6, TNF-alpha, RAGE, NSE).

Results: Several significant differences were observed between baseline (*i.e.* before hypothermic cardiac arrest) and the endpoint of the experiment (*i.e.* after resuscitation). Overall results showed an impairement in PaO2/fiO2 ratio (269 ± 30 vs. 400 ± 30, $P < 0.001$) and an increase in total pulmonary vascular resistance (9.9 ± 1.8 vs. 3.7 ± 0.5, $P = 0.002$) after resuscitation with no differences between groups. The mean cardiac output was also reduced (2.65 ± 1.7 vs. 4.38 ± 1.2 L/min, $P < 0.001$) after resuscitation and was lower in the low flow rate vs. the high flow rate group (1.96 ± 1.4 vs. 3.34 ± 1.7, $P = 0.05$). Among biomarkers analysis, an increase of the RAGE was found in the 38°C temperature group vs. the controlled temperature group (6.2 ± 1.1 vs. 4.75 ± 1.1, $P = 0.047$)

Discussion: We developed a porcine model of deep hypothermic cardiac arrest treated by ECLS and were able to test and evaluate four different strategies of rewarming and their impact on cardiovascular and pulmonary lesions. Our data suggest that a high flow rate strategy and a temperature adjusted to 5°C above the central core temperature could improve cardiac and lung function.

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Endothelial gamma-glutamyltransferase contributes to the vasorelaxant effect of S-nitrosoglutathione in rat aorta

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Aim: *S*-nitrosoglutathione (GSNO) is involved in storage and transport of nitric oxide ($^{\bullet}NO$), exhibits higher stability than $^{\bullet}NO$, mediates protein *S*-nitrosation, and plays an important role in vascular homeostasis. GSNO is metabolized by gamma-glutamyltransferase (GGT), which promotes $^{\bullet}NO$ release. We investigated whether vascular GGT influences the vasorelaxant effect of GSNO in isolated rat aorta.

Methods: Aortic rings were isolated from male Wistar rats (480 ± 32 g). Measurement of GGT activity and its histochemical localization were performed by using chromogenic substrates in aorta homogenates and sections, respectively. The role of GGT in GSNO metabolism was evaluated by measuring GSNO consumption rate (absorbance decay at 334 nm), $^{\bullet}NO$ release (Griess method) and protein nitrosation (Griess-Saville method) in aorta homogenates ($n = 3$). The vasorelaxant effect of GSNO was assayed in an *ex vivo* model using phenylephrine (10^{-6} M)-precontracted rat aortic rings (in the presence or absence of endothelium, $n = 6$–12). In each experiment, the role of GGT was assessed using: the exogenous gamma-glutamyl acceptor, glycylglycine, which increases GGT activity, and the competitive reversible inhibitor of GGT, serine borate complex (SBC).

Results: Specific GGT activity was measurable in rat aorta homogenates, and was localized in the endothelium. Consumption of exogenous GSNO by aorta homogenates decreased after inhibition of GGT with SBC, and increased after GGT stimulation with glycylglycine. Release of free $^{\bullet}NO$ and *S*-nitrosation of proteins was either decreased with SBC or increased with glycylglycine. GSNO concentration-response curves in endothelium-intact aortic rings gave a half maximal effective concentration (EC_{50}) of $3.2 \pm 0.5.10^{-7}$ M. This value was increased in the presence of SBC ($1.6 \pm 0.2.10^{-6}$ M) and decreased in the presence of glycylglycine ($4.7 \pm 0.9.10^{-8}$ M). In endothelium-denuded aorta, EC_{50} for GSNO alone increased to $2.3 \pm 0.3.10^{-6}$ M ($P < 0.05$ vs. GSNO with endothelium-intact), with no change in the presence of SBC ($1.4 \pm 0.2.10^{-6}$ M).

Discussion: These data suggest that endothelial GGT activity has an important role in mediating the vasorelaxant effect of GSNO. This should be taken into account in a pharmacological perspective, when considering the development of new therapeutics based on GSNO analogues.

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Erythropoietin protects newborn rat against sevoflurane-induced neurotoxicity

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Introduction: Recent data obtained in newborn animals exposed to anesthetics have raised serious safety concerns regarding current anesthesia practice in young children (1). Indeed, studies in rodents have demonstrated a widespread increase in brain apoptosis shortly after exposure to sevoflurane followed by a long-term neurologic impairment (2). In this context, we aim to evaluate the protective effect of rhEPO, a potent neuroprotective agent, in rat pups exposed to sevoflurane.

Material and methods: Sixty-nine post natal day 7 rats pups were allocated into three groups: SEVO+EPO ($n = 21$): exposed to sevoflurane 2 vol% (0.5 CAM) for 6 h in a mixture air/O_2 (60/40) + 5000 UI/kg rhEPO IP; SEVO ($n = 21$): exposed to sevoflurane + vehicle IP and CONTROL ($n = 21$) exposed to the mixture without sevoflurane + vehicle IP (IP: Intra Péritoneal). Three days after anesthesia (D10), quantification of apoptosis was performed on brain extract with TUNEL method and caspase3, NGF and BDNF expression was determined by Western blotting. Rats reaching adulthood (D100) were evaluated in terms of exploration capacities (object exploration duration) together with spatial and object learning (Water maze and Novel object test).

Results: Postnatal sevoflurane exposure impaired normal behaviour in adult rats by reducing the exploratory capacities during the novel object test and impaired both spatial and objects learning capacities in adult rats. Rh-EPO reduced sevoflurane-induced behavior and learning troubles in adult rats (Water Maze; Place trial: Ratio time to find platform 3rd trial/1st trial: 0.3 ± 0.1 vs. 1.1 ± 0.2; $n = 9$, SEVO + EPO vs. SEVO; $P = 0.01$). Three days after anesthesia, EPO prevented sevoflurane-induced brain apoptosis (5 ± 3 vs. 35 ± 6 apoptotic cells/mm^2; $n = 9$, SEVO+EPO vs. SEVO; $P = 0.01$) and elevation of caspase 3 level and significantly increased the brain expression of BDNF and NGF.

Conclusion: A single administration of rh-EPO immediately after postnatal exposure to sevoflurane reduced both early activation of apoptotic phenomenons and late onset of neurological disorders. To our knowledge this is the first report of the neuroprotective effect of rh-EPO after post natal sevoflurane exposure.

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Keywords: Neurotoxicity; Neuroprotection; Anesthesia; Newborn

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Medication use in the patients of the French multiple system atrophy (MSA) reference center

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Objective: MSA is associated with the degeneration of nerve cells in specific areas of the brain. This cell degeneration causes problems with movement, balance and other autonomic functions of the body such as bladder control or blood pressure regulation. The cause of MSA is unknown and no specific risk factors have been identified. Our aim is to describe medication use in MSA patients and to relate it with different characteristics of the disease.

Methods: Patients were assessed at the French MSA reference Center. The following variables were collected: MSA diagnosis ('probable' vs. 'possible' according to Gilman criteria), disease duration, autonomic dysfunction (SCOPA-Aut), disease severity (UMSARS I+II), clinical subtype (MSA-P vs. MSA-C), and any medication use (coded by ATC). Data were analyzed by chi-square test, only significant differences are reported.

Results: One hundred and forty-seven MSA patients were recruited (mean age 65.3 ± 0.7, 50% males, 61% MSA-P, 82% 'probable' MSA, mean UMSARS-score 48.9 ± 1.3, mean disease duration 5.1 ± 0.2). Overall, MSA patients received 8.2 ± 0.4 medications. Seventy-three % patients received at least one antiparkinsonian (mainly levodopa: 67%), 33% midodrine and 10% fludrocortisone. More severely affected patients (UMSARS>47) received antithrombotics (27% vs. 14%, $P < 0.05$) antidepressants (61% vs. 38% $P < 0.01$,) or drugs for bowel disorders (30% vs. 10%, $P < 0.01$). More patients with MSA-P (vs. -C) received antiparkinsonian (90% vs. 46%, $P < 0.01$), antihypertensive (28% vs. 12%, $P < 0.05$) or analgesic (36% vs. 11%, $P < 0.01$) medications. More patients with 'probable' MSA (vs. 'possible') received midodrine (39% vs. 7%, $P < 0.01$) and less alpha-blockers (5% vs. 19%, $P < 0.02$). Patients with SCOPA-Aut score > 22 were more frequently on fludrocortisone (18% vs. 3%, $P < 0.01$) or antidepressants (57% vs. 41%, $P < 0.04$). Patients with disease duration>5 years were more frequently on alpha-adrenergics blockers for urinary problems (14% vs. 3%, $P < 0.01$).

Discussion: In MSA, medication use significantly differs according to disease characteristics.

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Medication use in patients with Multiple System Atrophy (MSA) or Parkinson's Disease (PD) compared to a group of patients consulting a general practitioner (GP)

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Background and objective: Multiple System Atrophy (MSA) is a rare and severe orphan neurodegenerative disorder characterized by the combination of parkinsonism poorly responding to levodopa, cerebellar ataxia, dysautonomia and pyramidal symptoms. Our aim was to compared medication use in MSA, PD or GP patients.

Methods: One hundred and forty-seven MSA patients (according to Gilman criteria) were assessed at the Midi-Pyrénées (France) reference Center between 2008 and 2011. Six hundred and fifty-three PD patients (according to UKPDSBB criteria) and 98 patients assisting to a GP for reasons not related to PD or MSA were recruited from the same geographical area. Data were analyzed by chi-square test followed by pairwise comparisons by bonferroni-adjusted z-test for proportions.

Results: MSA patients were younger as compared to PD or GP patients (65 ± 1 vs. 68 ± 1 or 71 ± 1 years $P < 0.001$). Proportion of males was similar in the three groups (MSA: 50% vs. PD: 49% or 46%, $P = 0.9$). MSA patients were more

frequently treated with drugs for bowel disorders (19% vs. 6% or 10%, $P < 0.001$). Similarly, MSA patients were more frequently treated with midodrine or fludrocortisone (42% vs. 3% vs. 0%, $P < 0.001$). Additionally, patients with MSA were more frequently treated with urinary antispasmodics (18% vs. 2% or 1%, $P < 0.001$). Antihypertensives were more frequently administered to patients assisting to a GP (58%) compared to PD (40%, $P < 0.05$) or MSA patients (22%, $P < 0.05$ vs. GP or PD). MSA patients were less frequently on antiparkinsonian as compared to PD (73% vs. 88% $P < 0.05$). Finally, MSA patients were more frequently on antidepressants (48% vs. 18% or 10% $P < 0.001$).
Conclusions: Medication use patterns differed patients with MSA, PD or those assisting to a GP.

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Misuse of Clonazepam remained very frequent in France before the recent limitation of prescription

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Objective: A French national survey, performed by the CEIP (Evaluation and information center about drug-dependence) from 1998 to 2006, highlighted a very increased number of patients with drug-dependence and a very frequent misuse of oral presentations of Clonazepam. In fact Clonazepam is very often prescribed to treat insomnia, pain, behavioral troubles or to prevent epilepsy crisis. So, from November 2010 to February 2011, we performed a prospective study in the University Hospitals of Poitiers and Rouen in order to evaluate the importance of this misuse.
Methods: We evaluated the prescriptions of Clonazepam to inpatients hospitalized in 12 medical units of each hospital where prescriptions of Clonazepam were frequent: psychiatry, neurology, geriatrics, pneumology, palliative care, addictology.
Results: During 4 months, the prescriptions of Clonazepam to 313 inpatients (15–94 years old) were recorded. The psychiatry units where the main units where Clonazepam was prescribed. Only 6.7% of patients received Clonazepam for a curative treatment of epilepsy. So 93.3% of prescriptions were off-label. A percentage of 88.3% of prescriptions of Clonazepam was associated with other psychotropic drugs. Among the inductions of treatment with Clonazepam made in these medical units (44.6% of the 313 prescriptions), 20% followed the recommendations of the SCP. The drop presentation was the more frequent presentation used for off-label prescriptions of Clonazepam (75.9% of the prescriptions). The parental presentation (2.6%) was mainly used for treating epilepsy crisis. The duration of treatment was more than 3 months for about half patients and it was between 1 and 3 months for 16.8% of patients.
Discussion: Prescriptions of Clonazepam are very often off-label and inappropriate, with an insufficient re-evaluation. The frequent prescription of clonazepam in association with other psychotropic drugs, notably in patients with psychiatric disorders and/or aged patients exposes to drug-drug interactions which are probably often under-estimated. The recent measures decided by the AFSSAPS should decrease inappropriate prescriptions, adverse events and pharmaco-dependence.

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Long-term effects of amantadine in parkinsonian

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Background: Amantadine is the only drug commonly recommended for the treatment of Levodopa-Induced Dyskinesia (LID) in Parkinson's disease (PD). However, the duration of its efficacy remains a matter of debate, and some authors claimed that this effect did not last for more than few months.
Objective: To assess if the interruption of a chronic treatment with amantadin in PD patients with LIDs was responsible of a rebound of LIDs.
Patients and methods: This was a French multicenter placebo-controlled parallel-group randomized clinical trial conducted in dsykinetic PD patients on amantadine therapy for >6 months for LIDs. Patients were switched in a double-blind manner to amantadine (unchanged dosage) or placebo and followed-up for 3 months. Dyskinesia duration and severity were assessed using UPDRS IV items 32 + 33 (primary outcome) and patients'diaries. Other exploratory outcomes included AIMS scale, quality of life (PDQ 39), UPDRS, number of premature terminations, proportion of 'responders' (with a worsening of at least one unit of dyskinesia score of the sum of items 32 and 33 of UPDRS IV) and various scales assessing motor and non-motor symptoms (frozen gait, apathy, fatigue).
Results: Fifty-five patients were included (mean age 64 ± 7.2 years, mean PD duration 13.6 ± 6.7 years, mean L-DOPA equivalent dose 1096.2 ± 475.5 mg/day, mean duration of amantadine therapy 3.5 ± 4.1 years, mean amantadine dose 250.9 ± 57.3 mg/day, mean UPDRS 32 + 33 score 3.2 ± 1.6). The mean change in UPDRS IV items 32 + 33 was significantly greater in patients switched to placebo (2.1 ± 1.9) as compared with those remaining on amantadine (0.2 ± 1.5; $P = 0.0002$, ITT analysis). Twenty-eight patients discontinued prematurely: 23 in the placebo group (worsening of dyskinesia: 18) and five in the amantadine group (worsening of dyskinesia: 3; $P < 0.001$). Patients in the placebo group also had a greater increase in the duration of the total daily ON time with troublesome dyskinesia ($P < 0.01$).
Discussion: The present results show that amantadine remains efficacious in controlling LIDs in patients with PD even after 6 months of treatment.
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Case report: management of a severe carbamazepine intoxication

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Introduction: Carbamazepine (Tegretol®) is an anticonvulsant drug and is also used widely used for treatment of simple or complex partial seizures, trigeminal neuralgia, phantom limb pain, and bipolar affective disorder. We describe a case report involving carbamazepine overdose, treated by activated charcoal administrations.
Observation(s): A 45-year-old woman with bipolar disorders was admitted in emergencies having ingested an unknown quantity of carbamazepine. She was unconscious, not responding to the noxious stimuli (Glasgow coma scale = 3) and alcoholised (1.26 g/L at admission). Because of the critical condition and the respiratory distress, she was intubated and placed on mechanical ventilation. Carbamazepine level 2 h after the time of the admission was 43.20 µg/mL (therapeutic range: 8–12 µg/mL). The patient received gastric lavage, activated charcoal, and Augmentin® (to prevent inhalation pneumopathy). Nine hours after admission, her serum carbamazepine level was 52.20 µg/mL, and it was only after 3 days that carbamazepine level fell up to the therapeutic concentrations. The patient was discharged without after-effects. After a psychiatric evaluation, the doctor holds the indication of hospitalization in specialized environment to rebalance the treatment and to optimize psychiatric care.
Discussion: For the treatment of carbamazepine overdose, contradicting results about the efficiency of plasmapheresis, hemodialysis, or hemoperfusion have been reported. Despite the fact that activated charcoal is often underutilized or used in insufficient dosages, our data suggest that multiple doses activated charcoal may be optimal treatment of toxic level (ingestion of carbamazépine. Therefore, clinical monitoring is essential in order to prevent complications, and the use of others treatments (plasmapheresis, hemodialysis…) has to be discussed according to the neurological status improvement.

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Drugs commonly related to orthostatic hypotension in multiple system atrophy (MSA) patients

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Background and objective: Multiple System Atrophy (MSA) is a rare and severe orphan neurodegenerative disorder characterized by the combination of parkinsonism poorly responding to levodopa, cerebellar ataxia, dysautonomia and pyramidal symptoms. The objectives were to explore the potential hypotensive effects of exposure to drugs commonly associated with orthostatic hypotension (OH) in a large sample of MSA patients.
Methods: Patients were assessed at the French MSA reference Centers between 2008 and 2011. The following variables were collected prospectively: MSA diagnosis ('probable' vs. 'possible' according to Gilman criteria), disease duration, disease severity (UMSARS II), clinical subtype (MSA-P vs. MSA-C) and medication use. Blood pressure (BP) was measured 5 min after lying down and every min during 10 min after standing up. According to Gilman's criteria, OH was defined as systolic/diastolic BP fall ≥30/15 mmHg during the first 3 min after standing. Exposure to drugs commonly associated with OH, such as insulin, antihypertensives of any class, peripheral vasodilators, drugs used for heart-disease treatment (including antiarrhytmics), alpha1-adrenergic receptor antagonists, levodopa, dopamine agonists, monoaminooxidase-B or catechol-o-methyl transferase inhibitors and antidepressants of any class, was recorded. Data was analyzed by chi-square test followed by logistic regression.
Results: One hundred and forty-one MSA patients were included in the study, but only 131 provided complete data (age 64.7 ± 0.7, 50% males, 61% MSA-P, 83% MSA 'probable', UMSARS II score 25.3 ± 0.7, disease duration 5.0 ± 0.2). OH was detected in 76 (58%) patients. Patients with OH were less frequently exposed to antihypertensives (16% vs. 31%, OR [95%CI]= 0.38 [0.16–0.92], $P < 0.01$), levodopa (58% vs. 78%, OR = 0.38 [0.17–0.84], $P < 0.01$) or dopamine agonists (9% vs. 36%, OR = 0.18 [0.07–0.46]). Results remained significant after adjusting for demographic or disease-related factors by logistic regression analysis.
Conclusion: Our study suggests that drug exposure may not be a major factor connected with OH in MSA. Patients with OH were less frequently exposed to antihypertensives, levodopa or dopamine agonists. It is possible that treating physicians may have avoided exposing MSA patients to these drugs as they are known to induce or aggravate OH, which is a hallmark of MSA.

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A protective role of wormwood extract in aluminum induced brain rat alteration

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Background: Aluminum (Al) poisoning is a potential factor in brain damage, neurochemical dysfunction and severe behavioral problems. Considering this effect, our study was carried out to investigate the effects of wormwood extract to restore enzymes activities, lipid peroxidation, carbonyl and behavioral changes induced by Al.
Methods: Thirty Wistar rats were divided into four groups: three groups exposed to 100 mg/kg b.w of Al in the drinking water for 5 weeks and one groups as control. Aqueous wormwood extract (Al+A.Ab) (200 mg/kg body weight) was

administrated to intoxicated (Al+A.Ab) and control groups (A.AB) for four supplemental weeks. Activities of lactate deshydrogenase (LDH), Catalase (Cat), thiobarbituric acid-reactive substances (TBARS) and carbonyl level were determined in the whole brain of female rats and the grooming and locomotors activity were defined in all groups.
Results: The intoxicated group (Al) has a significantly increased TBARS and carbonyl value compared with the control ($P < 0.05$) and, after treatment with the wormwood extract, a significant reduction was noted. The enzyme activity decreased significantly ($P < 0.05$) in the Al group compared with the control, by −39% in LHD activity and are significativelly increased in catalase activity by +196%. After wormwood extract administration, LHD and Cat activity were significantly modified compared to Al group ($P < 0.05$). The behavioral test (locomotors and grooming test) indicates a significant hyperactivity in the Al group compared with the control group. After treatment with wormwood extract, the Al+A.Ab indicates a lower activity compared with control but remain unchanged compared to Al group.
Conclusion: These data suggest that wormwood extract may play a very useful role to reduce neurotoxicological damage induced by Aluminum.

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Intravenous misuse of methylphenidate (Ritalin®) for recreational purposes? Two case reports

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Misuse of methylphenidate is a well known clinical fact, although few data are available on its misuse by addicts. We would like to emphasize the misuse of methylphenidate by intravenous injections for recreational purposes. To our knowledge, side effects, addictive potential and the existence of a withdrawal syndrome are unknown when it's taken intravenously.
Patient 1: A 31-year-old man, who injected daily 120 mg of methylphenidate intravenously for 8 weeks. He had injected from 10 to 30 mg every 4 h. For 45 min he felt an amphetamine-like 'up', with euphoria, anxiolysis, and feelings of increased focus and intellectual abilities. The 'down' lasted 4 h and the patient used flunitrazepam and zolpidem to minimize discomfort. Injected doses increased rapidly, with a notable tolerance effect. Psychotic-like symptoms (verbal-acoustic delusions and paranoia) appeared after 4 weeks of use, linked with the increased doses.
Patient 2: A 42-year-old man, who injected daily 80 mg of methylphenidate intravenously for 9 months. At this point he had lost 20% of his initial weight. Most of his time was spent looking for the drug, which motivated his request for assistance. While quitting, a withdrawal syndrome appeared, with anxiety, cravings, sleep disruption, and dysphoria. These were not relieved by prescribing benzodiazepine. Physical withdrawal symptoms lasted 7 days and included abdominal and lumbar pains, sweating, shivering, nausea and vomiting.
These two cases illustrate intravenous injection use of methylphenidate. Used for a recreational purpose, at doses >80 mg/day, there seems to be a potential for addiction and a withdrawal syndrome. Long-term consequences, particularly cardiovascular, are unknown. However, daily use exposes subjects to local and general infectious complications. In both cases, HIV and HCV antibody tests were negative, and subjects were given information on reducing risks.
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Drug tests for opiate addicted patients' treatment in ambulatory care

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Purpose: According to French guidelines concerning opiate maintenance treatment, urine drug tests should be performed during opiate substitutive treatment (OST), and are compulsory for initiating methadone. Few studies have evaluated their effectiveness for OST management in ambulatory practice. The aim of this study was to investigate if drug tests in the context of ambulatory care modify OST retention.
Materials and method: We performed an observational study from the French regional Health Insurance System Database. Data from January 2009 to December 2010 were extracted. All patients with a first prescription of buprenorphine or methadone from July, 1 2009 to June 2009, 30 were included and followed-up to December 2010, 31. Two groups of patients were defined: testing group (all patients with at least one prescription of drug test throughout the follow-up) and control group (patients without any drug test prescription). The assessment criterion was OST retention. We performed a survival analysis by multivariate Cox proportional hazards model adjusted on several potential confounding factors: age, gender, exposure to drugs used in chronic diseases (infectious, psychiatric, and cardiovascular) and to drugs with abuse liability, 'doctor shopping', prescriber speciality, hospitalizations, health insurance coverage... Exposition to a drug test was considered as a time-dependent covariate with unique change.
Results: The cohort included 1507 newly treated patients (72% by buprenorphine) with a median age of 32 [Q1Q3 = 19–45], and with 75% of men. Retention rate at 6 months was 35.3% [95%CI: 32.9–37.8%]. Median OST retention was 88 days for control group and 243 days for testing group ($P < 0.001$). In the Cox proportional hazards model, drug testing improved OST retention (RR = 0.37 [95%CI: 0.21–0.65] as well as 'doctor shopping' (RR = 0.33 [0.27–0.41]; whereas hospitalization, prescription of drugs use in alcohol dependence and of morphine decreased OST retention (respectively RR = 1.15 [1.01–1.30], RR = 1.15 [1.19–1.92] and RR = 1.67 [1.24–2.32]).
Discussion: Only few patients had drug test during OST. Drug testing in ambulatory practice should be associated with better OST retention.

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Advantage of using psychotropic drugs urine test strips in maintenance treatments

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Introduction: Under current regulations, a urine test is mandatory prior to treating a patient with methadone, and recommended during the course of treatment. Regulations are more flexible for buprenorphine. What psychotropic drugs urine test strips could bring in the control of maintenance treatment (MT) is unexplored and subject to controversy. This work aims at evaluating MT prescription changes brought by the use of urine strips for patients requiring a MT renewal.
Equipment and method: Data collection: questionnaire to evaluate MT prescriptions (dosage, prescription duration, pace of delivery) before and after using a strip. Participating doctors: doctors belonging to all MT prescribing structures: drug centres, general medicine and drug centres for prisoners.
Duration of the study: 1 month Inclusion criteria: all patients calling for a MT renewal and accepting the urine strip test during the period were included.
Each call went through a two step process: at first, the doctor had to write his MT prescription prior to performing the strip test. Then, the test was done, and the doctor had to write a second MT prescription taking into account the new information brought by the strip reading.
Evaluation criteria: any change in the MT prescription (dosage, prescription duration, pace of delivery) between step 1 and step 2 of the consultation.
Results: Out of 429 valid clinical analysis, 122 (28.4%) had one change in the MT prescription following the strip reading. In 31 cases there was more than one change. The 159 changes corresponding to the 122 patients broke down into 65 (40.9%) dosage changes, 44 (27.7%) prescription duration changes and 50 (31.4%) pace of delivery changes.
Discussion: Using the urine test strip allows a decision change for a MT prescription for one patient out of three. This method complements laboratory tests, and has the further advantage of supplying the doctor with an immediate answer allowing him to optimize his prescription. Psychotropic drugs urine test strips should be generalized.
Keywords: maintenance treatment, urine test strips

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Cutaneous complications of buprenorphine intravenous drug abuse

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Introduction: France is one of the only european countries to have a specific system dedicated to drugs abuse and dependence potential evaluation (Center for Evaluation and Information on Pharmacodependence, CEIP Network). Records of dependence or abuse cases are notified by health professionals. This system is regularly faced to injection drug abuse situations, particularly injection of pharmaceutical tablets crushed and mixed with water. Injection drug abuse is a world-wide problem responsible for numerous minor and fatal complications.
Since March 2011, Nantes CEIP has been alerted by reports of severe harms resulting of injection of pharmaceutical tablets by drug users. This work reviews 16 cutaneous complications of intravenous drug abuse cases.
Observations: All subjects developed acute complications occurring within a few hours to 48 h after injection of buprenorphine. Fifteen patients had injected buprenorphine generic, and one Subutex®. Most of the subjects had used sterile supplies, skin antisepsis and had filtered his solution with cotton wool ball or filter. In the majority of the situations, immediately after injection, patients have felt intense pain and burning. Within a few hours a purple spot appeared followed for half of the patients by a necrosis in the territory of the injection point. Treatments with antibiotic or chirurgical intervention were necessary. Diagnosis was vascular thrombosis or livedo.
Discussion: the spectrum of cutaneous manifestations secondary to intravenous drug use is increasing as much as the number of drugs available for abuse increases. With buprenorphine, a large majority of problems described were abscesses, thrombophlebitis, oedema, cellulitis. Cutaneous necrosis is less reported. Modality of injection (use of syringe filters) and tablet fillers can be involved (specially insoluble fillers like talc used in the preparation of buprenorphine generic tablets). More investigations would be necessary in order to understand the mechanism of these harmful consequences.

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Chronic abuse of motor gasoline: about a case report

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Introduction: Questions and notifications on inhalant abuse or pharmacodependence are rare in the CEIP-Addictovigilance of Nantes. Experiments of volatile solvent inhalations are common in adolescents but repeated uses remain

uncommon. We present the case of a 30-year-old man hospitalized to his request for care in a context of chronic abuse of motor gasoline associated with suicidal ideations.
Case report: Inhalations of gasoline began in adolescence at the same time of other psychoactive substances consumptions (tobacco, alcohol and cannabis). Since he was 16, he displayed periods of massive inhalations alternating with periods without use. Nevertheless, taking advantage of a stable social and professional environment, he has not consumed any inhalant in the past 3 years.
Following a recent separation, he started again his excessive consumption of gasoline. For several weeks, he daily practiced some inhalations, several times a day. He slept only 4 h a day, the remaining time was spent to use and recover the effects of gasoline inhalations. The inhalations were performed by 'sniffing' and 'huffing'. He stated gasoline use in order to 'go crazy', and in search of self destruction and alterations of perceptions.
During the first days of hospitalization, he displayed signs of psychological withdrawal. There were no signs of physical withdrawal. Therefore physical and biological examinations showed no evidence of complications of chronic intoxication.
He leaves the hospital after 4 weeks of hospitalization without craving for gasoline, or more suicidal thoughts. However, he showed some persecution ideas associated with psychomotor agitation for which a mood stabilizer and a major tranquilizer were introduced.
Discussion: Inhalations of volatile solvents are frequently and early tested by adolescents in France, but the practice is most often transient. Development of a true addiction is rare, especially with gasoline. Repeated exposure to volatile solvents can cause various physical and psychological damages which can be severe. Inhalant abuse can be related to an underlying psychiatric disorder and/or other substance-related disorders.

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Management of pain in patients with substance-related disorders or opioid maintenance therapy: from the inventory towards improving quality of care

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Introduction: The CEIP-Addictovigilance of Nantes is frequently requested by health professionals about use of analgesics in patients with substance-related disorders or with opioid maintenance therapy, to which he answers in each case. There are no clinical practice guidelines to help clinicians to manage this specific caring. To fill this lack, the CEIP was surrounded by a multidisciplinary workgroup at the University Hospital of Nantes. The aim of this study was to provide an overview of health professionals knowledges and difficulties in the management of these patients, in order to develop appropriate support documents.
Methods: All clinicians of departments of the University Hospital of Nantes and some targeted departments of Hospital Center of Saint-Nazaire were asked to participate in this study. An ad hoc questionnaire was developed and was consisted of three parts: (i) frequency of substance-related disorders and perceived problems, (ii) screening for psychoactive substances consumptions and (iii) management of pain. The questionnaire was sent to clinicians along with a briefing note explaining the study and with a 1 month follow-up in order to increase the response rate. A specific database has been developed. The data were analyzed at the end of data collection.
Results: One hundred and thirty-one clinicians from both hospitals participated in the survey. Almost all faced at least once a patient with a substance-related disorder. Illicit drugs most often found were cannabis, opiates and cocaine. Opiates and cocaine were the substances perceived as the most problematic. Less than half of respondents systematically asked their patients about their psychoactive substances consumption. The therapeutic management of pain in these patients, especially those with opiate dependence or with an opioid maintenance therapy, seemed to be not always appropriate. The pharmacology of illicit substances and opioid maintenance therapies was not fully known and there were difficulties in the drug management of pain in these patients.
Discussion: Clinicians are facing difficulties in the management of pain in patients with a substance-related disorder or with opioid maintenance therapy. Protocols have been developed to provide information and to guide health professionals in the therapeutic management of these patients. Training actions have been organized in several hospital departments.

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Online notification: a real progress for pharmacodependence's cases

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Objective: France is one of the only european countries that has a specific system dedicated to drugs abuse and dependence potential evaluation (Centre for Evaluation and Information on Pharmacodependence, CEIP Network). Records of dependence or abuse cases are notified by health professionals. This system suffers from an under-notification especially by the hospital professionals. To increase and facilitate the collection of notifications, it has been decided to work on a computer tool in Nantes University Hospital.
This work aims at presenting the different steps of the development of a software for pharmacodependence's notifications in the hospital.
Methods: Call for tender: a company has been chosen to develop a software for the notifications. The software had to be clear, simple, attractive and intuitive for the users.
Adaptation of the software: many workshops have been planned to tailor the actual system of notification to the software. It has been adapted to the dependence criteria.
Choose of test departments: among the departments that notified most, four have been chosen to test the software.
Training: staffs have been trained to declare dependence's cases with this new tool.
Launch in the entire hospital
Results: The software is installed in every computer of the hospital and operational is the four test departments. In each test department, almost one person masters the software and is able to notify and to explain to the staff.
The date for the launch in the entire hospital and a training campaign are planned.
Discussion: This new tool facilitates spontaneous pharmacodependence's notifications in Nantes University Hospital. An impact assessment in a few months will evaluate the increase of spontaneous notification since the software's launch.
This online system should also increase exchanges between the hospitals departments and the CEIP and could be a tool to send pharmacodependence's messages or alerts.
According to the results it would be interesting to adapt this software for pharmacists and general practitioners.

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Hypnotic drugs use and quality of life: longitudinal analysis from the VISAT cohort

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Introduction: Throughout the world, hypnotic drugs do not have the indication for chronic insomnia. This is based on the tolerance to their hypnotic effects and on the risk of pharmacodependence. In addition to pharmacodependence, the reason of high prevalence chronic use of hypnotic drugs could be related to a positive effect on the quality of life (QoL) of users.
Objective: To evaluate the association between chronic use of hypnotic drugs and the evolution of QoL, in a population of workers of three areas of Southern France.
Methods: This study included 1257 French workers from the VISAT[1] *(Ageing, Health and Work)* cohort. The evolution of QoL scores (using the Nottingham Health Profile) after a 5 years follow-up (2001–2006) was investigated among two categories of hypnotic drugs users: 'non-consumers' (no consumption at the two data collection times) and 'chronic consumers' (consumption of at least 1 year declared in 2006) with logistic polytomic regression models adjusted for several potential confounders. The drugs studied were all benzodiazepines (used for hypnotic effect) and derivatives (zolpidem and zopiclone), carbamates and H1-antihistamines (doxylamine).
Results: In 2006, 38 (3.0%) 'chronic consumers' have been compared to the 1146 (91.2%) 'non consumers' of hypnotics. We found no significant difference in QoL evolution (no significant Odds-Ratio for each improvement or deterioration compared to non-evolution) between these two groups for all dimensions studied: 'Sleep', 'Emotional reactions', 'Energy' and 'Social isolation'.
Conclusion: This study was performed in a worker population, which is a younger population than the older patients most frequently included in hypnotic drug use studies. After adjustment for many confounding factors, and despite the limits of our study, our results suggest that chronic treatment with hypnotics does not seem to be justified by an improvement of the QoL of a population of chronic users.
Reference:
1. Marquié, JC, Jansou P, Baracat B, Martinaud C, Gonon O et al. Ageing, health and work: overview and methodology of the VISAT prospective study. Le Travail Humain. 2002;65 (3): 243–260.

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Which psychoactive substance are simultaneously consumed with alcohol ? oppidum and opema 2010 surveys data

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Objective: This study aims to describe which prescription or illicit drugs are the most consumed with a simultaneous alcohol use by subjects suffering a substance use disorder.
Methods: OPPIDUM and OPEMA are two pharmacoepidemiological programs developed by the French CEIP-A network, including subjects suffering a substance use disorder (except for tobacco and alcohol) or under opiate maintenance treatment. OPPIDUM 2010 survey has been led in october in specialized care centers, and OPEMA's in november in GPs surgeries. For each patient, the investigators collect in particular data about all the psychoactive substance currently consumed, and their consumptions modalities –among them, a concomitant alcohol use (CAU).
Results: In 2010, 11 887 valid descriptions of substance consumption have been collected in OPPIDUM (n = 9735) and OPEMA (n = 2152) surveys. Seven illicit drugs and 32 prescription drugs get more than 25 CAU valid observations.
Among illicit drugs, ecstasy is the illicit drug with the highest rate of CAU (70%), followed by amphetamine (57%) and crack (49%). Cannabis is simultaneously less used with alcohol (31%) than cocaine (40%). Heroin (21%) get the lowest rate.
Among prescription drugs, nine substances get more than 30% of CAU, methylphenidate (40%) and codeine (39%) getting the higher rates. The others are five benzodiazepines (oxazepam, diazepam, lorazepam, flunitrazepam, clonazepam), alimemazine and amisulpride.
Among maintenance treatments, methadone rate is lower than buprenorphine's (17% vs. 21%, $P < 0.001$).
Antidepressants and antipsychotics get the same global rate (19.5%).

Between 15 and 25 CAU valid observations, two substances get CAU high rates: meprobamate (44%) and ketamine (59%).
Discussion: Concomitant alohol use is a relevant indicator for abuse liability. Those data are specific to subjects attending for a substance use disorder so those results can't be extrapolated to the whole French population. This study might may deserve further investigations, speciallyabout the reasons of CAU.

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What are the differences for drug consumptions depending on age among subjects attending in specialized care centers?

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Objective: To assess the way consumptions and behavior evolve from age classes for pharmacodependent subjects.
Methods: OPPIDUM program constits of cross sectional surveys performed every year by the French CEIP network in specialized centers for drug users. People suffering a substance use disorder or under opiate maintenance treatment are included. Recorded data are about their socioeconomic situation (including their age) and their drug consumptions. For this study, four age classes have been computed from 2010 survey data: <18, 18–29, 30–39 and more than 40 years old. Main data have been compared for those classes.
Results: Five thousand one hundred and six people have been included: $n = 94$ (2%) were <18 (class1), $n = 1819$ (36%) were from 18 to 29 (class2), $n = 1819$ (36%) were 30–39 (class3), and $n = 1374$ (27%) were more than 40 (class4).
Most consumed products are, for class 1, cannabis ($n = 90$), heroin ($n = 3$) methadone ($n = 3$) and buprenorphine ($n = 2$); cannabis is the only consumed drug for 85 subjects, 27 subjects use it with a concomitant alcohol use, and 12 subjects suffer an alcohol dependence. For classes 2 and 3, methadone ranks first, followed by cannabis and buprenorphine, and for class4, methadone, buprenorphine and cannabis.
The rate of people consuming prescription drugs is growing from class 1 to 4, as for people benefiting a maintenance treatments (5%, 71%, 82%, 83%), benzodiazepines (2%, 13%, 23%, 28%), antidepressants (0%, 4%, 8%, 12%), and people suffering an alcohol dependence (13%, 15%, 23%, 28%).
Illegal acquisition for prescription drugs is higher for classes 2 and 3.
For illicit drugs, heroin highest consumption prevalence is for class 2 (3%, 24%, 16%, 10%), class 3 for cocaine (1%, 11%, 11%, 9%), and cannabis consumption prevalence is decreasing as the age grows (96%, 45%, 36%, 28%). Higher rates for use of intravenous and nasal routes are for class2 (10% and 25%).
Discussion: The more age is growing, the more prescription drugs are consumed. For illicit drugs, peaks are for subjects aged from 18 to 39.

P207

Is pregabalin abused in France?

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Introduction Pregabalin is a gamma-aminobutyric acid (GABA) analogue available in France since 2006 for the treatment of neuropathic pain, partial-onset seizures and generalized anxiety disorder. A recent case of abuse in Southern France raises the question of his abuse liability.
Observation In February 2011, a 47-year-old male who had taken over a period of 24 h in a recreative context 100 mg of tramadol (Contramal®) prescribed for pain) and 1600 mg of pregabalin (Lyrica® prescribed to his girl friend for polynevritis) called a general practitioner for a visit. He presented agitation with logorrhea, mydriasis, tachycardia with normal blood pressure and reported euphoria. The patient, who presented a history of drug and alcohol abuse, reported that his first pregabalin experience was similar to that with ecstasy. The regional poison centre recommended hospitalization for monitoring and the patient was finally discharged a few hours later after full recovery.
Discussion Following this warning, we investigate a potential signal of abuse for pregabalin among the different French addictovigilance programs. In OPPIDUM (Observation of Illegal Drugs and Misuse of Psychotropic Medications), 12 subjects have reported pregabalin consumption including four in the context of abuse/dependence. In OPEMA (Observation of Pharmacodependences in Ambulatory Medicine) four subjects have reported pregabalin consumption including one in the context of abuse/dependence. In OSIAP, nine reports of pregabalin obtained by falsified prescriptions were identified (which six in 2010).
Other recent cases concerning pregabalin abuse and dependence have been reported in literature especially in Scandinavian countries. Recently a data-mining algorithm was applied to reports of possible drug abuse or addiction in the Swedish national register (SWEDIS), and of 198 reports, 16 concerned pregabalin (Schwan 2010). In another recent study, accounts of pregabalin misuse were found among 32 websites in which recreational drug use was discussed (Schifano 2011). A meta-analysis of pregabalin adverse effects, have reported that euphoria is the second adverse event significantly associated with pregabalin treatment (Zaccara 2011).
There is some evidence for addictive potential of pregabaline and health practitioners must be aware especially for patients with a history of drug and/or alcohol abuse.

P212

Harm reduction centers (CAARUD) vs. ambulatory care centers (CSAPA)

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Objective: The purpose of this study is to compare dependent subjects attending two kinds of specialized centers, considering their situation, behaviors and consumptions.
Methods: OPPIDUM program consists of cross sectional surveys performed every year by the French CEIP-A network in specialized centers for drug users. Subjects suffering a substance use disorder or under opiate maintenance treatment are included. Data about their socioeconomic situation and their drug consumptions are recorded. For this study, 2010 data have been restricted at two different kinds of centers (CAARUD and CSAPA), and to metropolitan France.
Results: During the 2010 OPPIDUM survey, 156 subjects have been included in CAARUD and 3549 in CSAPA. Those subjects have described 7619 drug consumptions.
CAARUD's subjects are younger (32.0 ± 8.2 vs. 34.2 ± 8.2, $P < 0.01$). Their socioeconomic situation is more precarious. Their first dependence substance are more often prescription drugs (12.2% vs. 5.8%, $P < 0.01$). In CAARUD there is a higher rate of subjects indicating an abuse or a dependency (28%, vs.14%, $P < 0.001$).
CAARUD subjects use significantly more often the intravenous and the nasal routes, and report significantly more illegal acquisitions for prescription drugs.
There are less subjects under maintenance treatment in CAARUD (65% vs. 82%, $P < 0.001$), consuming more buprenorphine and less methadone. Some prescription drugs known to be diverted are more consumed in CAARUD: morphine, clonazepam.
CAARUD's subjects consume less antidepressant and antipsychotics.
All the illicit drugs are also more consumed in CAARUD, as for heroin (40% vs. 15%, $P < 0.001$) or cocaine (27% vs. 8%, $P < 0.001$).
Some drugs presenting a emerging signal of abuse, as methylphenidate, or told to be marginally consumed, as methamphetamine, are also proportionately much more consumed in CAARUD.
Discussion: For dependence assessment in a surveillance system, CAARUD are strategically crucial to identify more precociously emerging phenomenons.
Such a study should deserve more investigations and be led in more CAARUDs (only seven have participated, whereas there are more than a hundred in France).

P213

What differences between Buprenorphine generic and princeps? About a study on modalities of use and patients' perceptions?

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Objective: Since 2006, generic buprenorphine has been marketed. In 2008, the national market penetration rate of generic buprenorphine is only 32% whereas it is 82% for other prescription drugs. Which factors may explain the lower use of buprenorphine generic?
Methods: A study has been conducted among patients followed in addiction care centers. All patients have consumed at least one time buprenorphine generic and princeps. Different informations are collected by a questionnaire such as history of maintenance opiate treatment (MOT), buprenorphine consumption and patients' perception between generic and princeps.
Results: Forty-eight patients have been included. Informations concerning history of MOT have shown that a lot of patients have MOT since a long time, they have stopped and changed it several times and some of them have obtained it on black market before prescription. Concerning modalities of use of generic vs. princeps, when the patients have consumed generic, 23% have used intravenous route (vs. 29%, $P < 0.01$) and 19% nasal route (vs. 33%, $P < 0.01$), 4% have obtained buprenorphine by deal (vs. 23%, $P < 0.001$) and in both cases, half patients have fractionated their treatment. Ten percent of patients leave melt buprenorphine under the tongue <5 min and half of patients take a drink in the same time. Concerning patients' perception between generic vs. princeps according various criteria (more, less or identical), the study highlights that 65% of subjects have had an *a priori* concerning generic, 51% of patient have reported that galenic form of generic is less practical (too small, difficult to cut…) (28% more practical), 68% that generic melts faster (13% slower), 36% that the taste is less pleasant (34% more pleasant), 55% that generic is less effectiveness (11% more), 45% that effects are less intense (15% more), and 38% a duration of action slower (13% faster).
Discussion: This study highlights that patients have a long history with MOT and princeps is more misused than generic. Some patients seem not taking appropriately buprenorphine by sublingual route. Finally, patients seem to have different perception between buprenorphine generic and princeps according different criteria.

P214

Identification of regional available antidotes and emergency drugs to optimize the management of poisoned patients

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Introduction: Poisoning treatment requires faster access to antidote or other supportive medications. However, there are no guidelines about the storage of these drugs in hospital. To optimize their management, the OMEDIT of Upper Normandy (a Regional Health Observatory) made a directory of antidotes and emergency drugs and performed a regional inventory of these drugs in the hospital pharmacies (HP).

Matérials/Methods: This directory has been established from the data of Toxicology Centers, experts' recommendations and literature. For each medication, information about packaging, indications, dosages and routes of administration were recorded. Quantities of antidotes needed to treat a patient of 70 kg for 24 h and time available before treatment (as suggested by the International Program on Chemical Safety) were proposed. An inventory was associated with the directory, in order to identify the antidotes, their quantities in hospital pharmacies, the phone number available 24 h a day and the possibility of borrowing from another hospital.
Results: Thirty-nine drugs (28 antidotes and 11 emergency drugs) have been listed in the document, nine of them had a 'temporary use authorization'. Among these 39 drugs, 20 had to be administered immediately or within 30 min after the poisoning, suggesting that they must be readily available 24 h/day in hospital pharmacy or stored in the emergency department. Sixteen other antidotes or emergency drugs had to be available as soon as possible or within 2–6 h after intoxication. The regional inventory of antidotes and emergency drugs highlighted different availability of these drugs, due to the hospital's activity and antidote's specificity: Flumazenil, *N*-acetylcysteine, Naloxone and Glucagen are stored in almost all hospitals, while dexrazoxane, indicated in the urgent care of anthracycline extravasation, is only available in two hospital pharmacy, among the 15 hospitals administering chemotherapy in the region.
Conclusion: This directory, written to improve the management of poisoned patients, has to be adapted to each hospital according to its activities and proximity to other hospital. Some antidotes with significant economic impact (for a hypothetical use) are subject to a regional reflection about pooling these drugs over a health territory, while respecting the time available to treat.

P215

Study on intravenous use of methylphenidate and patterns on its abuse and diversion in two French administrative Areas (PACA, Corse)

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Objective: Methylphenidate is approved for the treatment of attention-deficit hyperactivity disorder (ADHD) for children aged over 6 years; and for narcolepsy after inefficacy of modafinil (only for Ritalin 10 mg®). As part of its mission, the CEIP-Addictovigilance network has collected reports of methylphenidate abuse, especially in PACA area. In this context, Marseille CEIP-Addictovigilance has set up a specific study in its area in order to assess the magnitude of this signal and to describe methylphenidate abuse patterns.
Methods: This study has three components: a pharmacoepidemiological component based on drug reimbursement databases using different indicators (clustering method, doctor-shopping indicator and indictors usually used by the regional direction of health insurance); a health component including information from health professionals and workers on specialized centers dedicated to drug dependence (42 questionnaires from pharmacists were analyzed and 36 interviews were conducted with specialized centers (CSAPA and CAARUD)); and a patient component by collecting directly methylphenidate informations from drug users by semi-interviewed questionnaires (during a 1 month period, 64 methylphenidate drug users have described their consumption).
Results: Data from drug reimbursement databases have shown a 116% increase of the number of patients who had a dispensing for methylphenidate the first quarter of the year (between 2005 and 2009). According 2009 data, the proportion of subjects with a deviant behaviour is 1.3% and the doctor-shopping indicator is 3.2%. According data from pharmacists have shown that the conditions of prescription and dispensing are not always respected (during prescription refill only 13 of 31 pharmacies have always the presentation of the initial hospital prescription). The study has shown that methylphenidate abuse is restricted actually to the Bouches-du-Rhone and Alpes-Maritimes. The subjects are usually consumers of stimulants, have already use intravenous route, have precarious situation and few subjects has a history of ADHD. Methylphenidate is often used by intravenous route and in megadose. Some CNS and non CNS effects such as insomnia, depression, anxiety, cardiovascular effects, abcess, weight loss are reported.
Conclusion: Due to a growing misuse of this drug, it is important to inform health professionals and to recall the conditions of prescription and dispensing.

P216

Study of diverted consumption of psychoactive substances by intravenous, nasal and inhaled routes from the 2009 OPPIDUM survey

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Objective: What are the psychoactive substances (PAS) consumed by another route than the oral one by the subjects under opiate maintenance treatment (OMT) and/or drug abusers? And what is these users 'profile?
Methods OPPIDUM program consists of cross sectional surveys performed every year by the French CEIP-A network in specialized centers for drug users. Subjects suffering a substance use disorder or under OMT are included. Data about socioeconomic situation and drug consumptions are recorded. For each psychoactive substance consumed the week before inclusion, route(s) of administration used is filled (oral/sublingual, nasal, inhaled, intravenous (IV) or other). For each route, a summary of reported substances was made. For each subject, from indicated route(s), a profile is determined (oral exclusive, nasal, IV, inhaled users or several routes except oral). For this study, 2009 data have been used, except the cannabis consumption data.
Results: Four thousand two hundred and fifty-four subjects were included and they described 7669 product consumptions. Among these, 6.5% were consumed by IV, 14.6% nasally, 4.2% and inhaled.
Whatever is the used route (IV or nasal or inhaled), the three most used PAS by non oral routes are heroin, cocaine (or crack) and buprenorphine. The other PSA: ketamine (83% by nasal route), morphine (62% by IV route), methylphenidate ($n = 2$ injections) and few benzodiazepines are injected or sniffed.
IV users are more precarious than nasal users.
Discussion/conclusion: Knowledge of substances that present the highest risk of diversion is helpful in terms of addictovigilance but also in public health (better targeting of support and care).

P217

Intravenous route use among dependent subjects followed by general practitioners (GP): results from the OPEMA program

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Objective: To describe patterns for sociodemographic situation, products consumption and modalities of use for included subjects depending on their intravenous route (IR) use.
Methods: OPEMA is a program based on annual cross-sectional surveys performed by the CEIP network, including subjects aged under 60, suffering a substance use disorder or under opiate maintenance treatment (OMT). For this work, 2010 data are used, concerning subjects' socioeconomic situation, state of health and consumptions. Three subgroups are distinguished, with subjects reporting:
No IR use during life time ('non users')
An IR use, but not during past month ('past users')
An IR use during past month ('current users')
Results: In OPEMA 2010, 96 GP have recruited 632 IR non users (53%), 467 past users (39%) and 87 current users (7%).
IR users vs. non-users: IR users are older** (36.8 ± 8.3 vs. 34.4 ± 8.6). IR users have less stable accommodation** (80% vs. 90%), and are less employed** (48% vs. 61%). Their state of health is worse (more HIV** and HCV** infections, more alcoholic dependence**, worse dental health**). Their ages for first consumption** and dependence** are lower and substances implicated in first consumption** and dependence* are less often a prescription drug (PD). IR users consume more cannabis*, cocaine**, flunitrazepam** and clonazépam*. OMT** are significantly more prescribed to IR users (97% vs. 85%). IR get PD more often by illegal ways*. Current IR users vs. past users: Current users have less stable accommodation**. There are no other significant differences concerning their situation, and neither concerning their state of health, but there are some for their consumptions: current users consume significantly more cocaine** (15% vs. 6%) and heroin** (18% vs. 6%), less cannabis* (8% vs. 18), more buprénorphine* (62% vs. 49%) and less methadone* (34% vs. 47%). They are more often nasal route users** (22% vs. 10%)** and get PD more often by illegal ways**(22% vs. 5%). $^{*}P < 0.05$; $^{**}P < 0.01$
Discussion: Worse socioeconomic situation and state of health are closely related to IR use and may still concern past users, but stopping IR use changes consumptions and modalities.

P218

Setting up a system to improve pharmacists notification on suspect requests of products of the pharmacopea: a pilot study in PACA-Corse

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Objective: the CEIP-Addictovigilance French network assesses drug abuse liability based on the different post marketing surveillance systems. Some of them are based on reports from health Professional, like pharmacists. In this context, since several years, the CEIP-A coordinates a specific network of community pharmacies, called PSSP (Pharmaciens Sentinelles de Santé Publique). This PSSP network participates to the OSIAP survey (Ordonnances Suspectes, Indicatrices d'Abus et de Pharmacodépendance) (Lapeyre-Mestre, 1997) included especially on prescription drugs obtained with suspect prescription. Nevertheless, pharmacists may deliver other products within the pharmaceutical monopoly, which can be delivered without prescription. Some of these products can have psychoactive effects or may be used in order to manufacture or adulterate drugs. Following a recent report by pharmacy concerning important requests of caffeine, we decided to collect more systematically data from pharmacists, using a specific form.
Methods: Since January 2011, we have added on the OSIAP form a specific part on the 'monitoring on products and chemical substances sensibles'. This part is divided in:
Chemical substances or veterinary product, over the counter drug (OTC),
Advice product, dietary supplements,
Medicinal plants, essential oils, solvents etc…
Each month this form is sent to the PSSP network (193 pharmacies in PACA-Corse area).
Results: Since January 2011, 56 reports have been collected including 41 OSIAP and 15 other Reports (26%) related to the following situations:
Request of prescription drugs without prescription (meprobamate, clonazepam, methadone)
Request of prescription drug without prescription for a off-label use (Lidocaine/Prilocaine, Steroids)
Request of important quantity of medications or OTC drug (Ephedrine, Codeine, Metopimazine),
Requet of chemical substances like Potassium Nitrate, or essential oils forbidden in France (Sassafras, green anise, Ruta graveolens, Wall Germander), request of preparation by the pharmacist (such as a preparation including melatonine).
Discussion: This system allows improving the notifications by the pharmacists using a specific form and expanding the scope of the notification to all psychoactive substances or other 'sensible' products of the pharmacopea.

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Regional survey on diversion of medicines containing codeine

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Objective: to assess the potential diversion of medicines containing codeine, particularly those that are over-the-counter (OTC) drugs.

Methods: We conducted a survey within a regional network of 151 community pharmacies. For each patient requesting a medicine containing codeine, pharmacists were asked to fill in a questionnaire intended to describe the characteristics of the patient, the medicines requested and to assess if pharmacists can identify diversion.

Results: Eighteen pharmacies participated to the study and 116 patients were included. There were 68 women (59%) and 48 men (41%). Mean age was 51 years (range: 14–90 years). In more than 70% of cases, the patient was known by the pharmacist who had access to the pharmaceutical file in 30% of cases. In 8% of cases (n = 9), the pharmacist registered the OTC medicines in the pharmaceutical file of the patient.

The most frequently requested medicines were two OTC brand names (Neocodion® and Codoliprane®) and one prescription medicine containing a combination of paracetamol and codeine (Dafalgan codeine®). In 52% of cases (n = 60), a medical prescription form was presented; in 41% of cases (n = 48), the patient asked spontaneously the medicine and in 7% of cases, the medicine was delivered on advice of the pharmacist. When known, the indication was pain in 63% of cases (n = 52) and cough in 37% (n = 31).

Pharmacists suspected a diversion in 29% of cases (n = 34 patients): Neocodion® (62%, n = 21) and Codoliprane® (26%, n = 9) were the most frequent medicines requested. In those cases, the patient never requested the pharmacist's advice. In most of these cases, the indication was unknown.

Discussion: Pharmacists are on the front line to identify diversion of OTC medicines containing codeine, but this detection may be difficult. The registration of OTC drugs in the pharmaceutical files could be a good means to trace 'pharmaceutical shopping'; however, subjects diverting medicines often refuse a pharmaceutical file.

P220

A recent evaluation of trihexyphenidyl hydrochloride (Artane®) abuse and misuse

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Trihexyphenidyl (THP) is a synthetic anticholinergic used in psychiatric patient for the relief of neuroleptic-induced extrapyramidal symptoms and in Parkinson's disease. THP is liable to an abuse linked to its hallucinogenic and euphoric effects by schizophrenic patients and/or drug addicts. An update of THP misuses among the French population has been considered.

Methodology: It includes literature and internet investigations, analysis of data collected by CEIP-A from NotS (Spontaneous notifications) and OSIAP (suspicious prescriptions indicating possible abuse) banks, OPPIDUM (Observation of Illegal Drugs and Misuse of Psychotropic Medications) program, and examination of sale data.

Results: The NotS, OSIAP and OPPPIDUM notified from 2001 to 2011 represented <2%, 1% and 0.5% of the total number of reported cases in each bank respectively. The analysis of these data resulted in the following overall trends: population was mainly represented by male subjects (75%); patients and drug abusers were equally represented; THP was mainly taken orally with some cases of intravenous injection; a part of subjects did not abuse but high doses might be observed (60 tablets in a single intake; 30 tablets/day in chronic consumption);THP was often associated to substitution treatment, one or more benzodiazepine, neuroleptics, alcohol or illicit drugs; when the drug was stopped, withdrawal symptoms occurred; the drug was obtained by medical prescription but also by deal and false prescriptions.

Data from the health reimbursement system confirmed the abuse and dependence potential associated with THP.

Data provided by the Manufacturers showed a regular decrease of the THP sales from 10 years excepted in Reunion Island where they remained stable or increased. Numerous traffics are regularly dismantled between Madagascar and Reunion Island and confirm a misuse by the local population.

Conclusion: A constant but low misuse of THP is observed in French metropolis, without change during the recent years, with nevertheless a more acute alarm in the big towns such as Paris, Marseille, Lyon. In Reunion island, THP misuse remains an acute health problem that could induce a new evaluation of the benefit/risk of the drug.

P221

Acute coronary syndromes in the young after cannabis intake: to what extent?

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Aim: Recent data suggest that cannabis is associated with acute coronary syndromes (ACS), and even identified as a triggering factor. This study aimed to investigate deeper ACS occurring in patients using cannabis by describing this population and the clinical characteristics of outcomes in comparison with a population of non-exposed patients.

Methods: All patients under 50 years old admitted in the cardiology intensive care unit of Toulouse university hospital between December 2010 and April 2011 for ACS associated with a subnormal level of troponin were prospectively included. Assessment of cannabis exposure was based on questioning and semi-quantitative urine analysis for cannabinoids derivatives. Assessment was more deeply explored in patients who had acknowledged cannabis use. The 'exposed' and 'non-exposed' groups were statistically described and compared.

Results: Out of the 63 patients included, 15 (23.8%) were recent cannabis users. The exposed and non-exposed groups were similar in age (41 years old SD 6 vs. 42 SD 7, respectively. P = 0.4) and gender (93.3% of males vs. 79.2%. P = 0.2). Common risk factors for ACS (values of serum cholesterol and triglycerides, existence of hypertension or diabetes, history of ACS) were similarly distributed in the two groups. Interestingly, these differed in the type of outcome: ST elevated myocardial infarctions (STEMI) were more frequently observed than non STEMI in the non-exposed group (70.8% vs. 29.2%) whereas it was the opposite in the exposed group, with a higher rate of non STEMI (66.7% vs. 33.3%). Time between cannabis use and outcome of ACS was shorter than 3 h in 11 subjects (73.3%).

Discussion: The part of cannabis users in this cohort of patients with ACS far exceeds that observed in the general population. The short time between intake and outcome reinforces the hypothesis according to which cannabis should act as a triggering factor for cardiovascular complications. The greater magnitude of non STEMI in patients exposed to cannabis may underlie many disregarded cases of thoracic pain in patients with moderate cardiovascular risk, and particularly in young people. These results underline the need to investigate cannabis exposure in young patients with more or less typical acute coronary syndromes.

P222

Spontaneous addictovigilance notifications (NotS) and hospital medical information system (PMSI) database: two complementary methods of detection of signals?

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Background: Spontaneous reports of suspected drug abuse or dependence seem to be largely under-notified. In this context, PMSI database, in which reasons for admissions to hospitals are recorded, could be interesting.

Objectives: The objective of this study was to assess reports obtained via the PMSI in regard to those obtained by the spontaneous reporting system (NotS) in the same university hospital.

Methods: PMSI database was retrospectively analyzed during the period 2008–2010. ICD-10 codes (F11-F19) were used to extract patients' admissions for drug abuse or dependence. For each case, medical records were used to describe demographic conditions, clinical situation, severity and drug consumption. During the same time, NotS declared by healthcare hospital were collected.

Results: During the study period, 211 reports were received by the NotS system (59 in 2008, 68 in 2009, 84 in 2010) and 120 patients hospitalized were extracted from PMSI database (43 in 2008, 40 in 2009, 37 in 2010). The mean age of patients was 39 and 22 years-old from the NotS and the PMSI, respectively. Reports were severe in 75% of PMSI cases (NotS 47%), concerned licit substances in 63% of PMSI cases (NotS 83%), illicit substances in 26% of PMSI cases (NotS 9%) and an association of both in 11% of PMSI cases (NotS 8%). Adverse-effects due to drug abuse and/or dependence was described for 41% of cases of PMSI (NotS 36%), a drug withdrawal for 12% of PMSI cases (NotS 41%), a neonatal abstinence syndrome for 35% of PMSI cases (NotS 0.5%). Involvement in the spontaneous notification seems to be different according to medical unit (ie: Emergency unit PMSI cases 33% vs. NotS cases 6%).

Conclusion: Notifications from PMSI database seem to be more related to illicit substances consumption, and their effects (clinical damages), than drugs diversion, these latter reports being mainly obtained by NotS. Moreover, in our university hospital, PMSI allows to collect cases of drug abuse and/or dependence, not notified by the NotS, requiring the implementation of information and the formation of the healthcare professionals.

P228

The Moroccan population's perception of drugs

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Introduction: The aim of this study is to assess the perception of drugs by Moroccan people and to analyze the fluctuation of their degree of familiarity and behavior with respect to economic and socio-demographic profiles.

Population and methods: A cross-sectional prospective study of intramural population of Casablanca, Morocco was conducted from December 2008 until May 2009. This study was carried out using the quota method and a questionnaire to be filled by the target population with the help of the investigators.

Results: One thousand interviews were conducted. The response average rate was 88%. The mean age was 36.6 years with a sex ratio (M/F) 0.98. 39.9% of interviewers were illiterate and 11.5% had a higher level of study.

Assessment of drug familiarity showed that 67.6% of the sample thought that a good prescription is the one which contains one or two drugs, 79.5% thought that expensive drugs are the most effective and 71.6% knew that drugs could induce side effects, mainly women (P = 0.047).

Assessment of attitudes toward drugs showed that 59% prefer to see a physician and 49.7% prefer to buy all prescribed drugs when they are sick. Self-medication is occasionally chosen (75.6%), whatever their status is and whether they benefit from health insurance or not (P = 0.084). In addition, non insured subjects buy fewer drugs recommended by their pharmacist compared to insured ones (P < 0.001).

Conclusion: The study of drug perception by population will enable health professionals and concerned authorities to provide citizens with information on efficient and optimal use of medicines and change aberrant practices which will lead to serious consequences on the health and the well being of such population.

P229

Benefit of pharmacological intervention on drug-induced mild hyponatremia in elderly: A prospective randomised trial

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Objective: Drug induced hyponatremia occurs more frequently in elderly patients, and mild hyponatremia is usually tolerated by physicians. This study evaluates the biological and clinical impact of therapeutic adaptation in elderly in order to correct mild hyponatremia.

Methods: In a general hospital, elderly with mild hyponatremia detected by a routine biological monitoring, were included in the research for a year.

Patients without any potentially hyponatremiant drug were excluded.

Patients were randomized in two arms: Intervention: 'Changing drug therapy after a pharmacologist's expertise'

No intervention: 'drug therapy was not changed during the 3 months of follow-up'

The effectiveness of medical intervention was evaluated by an objective biological criterion: normalization of serum sodium after 4 weeks. The secondary endpoint assessed the interest of correcting hyponatremia to reduce the number of falls in the mid term (3 months).

Results/discussion: Twenty-seven elderly with mild drug-induced hyponatremia have been included.

Drug therapy could not be changed in four patients due to their clinical conditions. Twenty-three patients were randomized: only 14 were evaluated because nine patients had exclusion criteria between baseline and the primary endpoint. Among the 14 patients, eight underwent intervention. Six of them (75%) normalized their serum sodium after 4 weeks. Six patients in the non-intervention arm have been monitored at 1 month, without any normalization. Interventions were: stopping a proton pump inhibitors (3), adjustment of antihypertensive treatment (2), review of dietary salt restriction (1), decreased of valproic acid treatment (1), decrease of laxatives (1). The evaluation of falls 3 months before and after inclusion, in 12 of 14 evaluable patients, showed an improvement in four patients out of five patients fallers in intervention arm against none in the other arm of four patients fallers.

Conclusions: Research, despite its small size, has shown biological and clinical benefit of drug adjustment to correct mild hyponatremia.

P230

Non Steroidal Anti-Inflammatory Drugs (NSAIDS) and increased risk of hypertension treatment intensification: a population-based cohort study

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Background: Non Steroidal Anti-Inflammatory Drugs (NSAIDs) are known to antagonize the effects of antihypertensive drugs. Their associations can lead to an increase in arterial blood pressure. However, the impact of NSAIDs on hypertension treatment management in large-scale populations remains poorly evaluated. We evaluated if the introduction of NSAIDs could induce an intensification of hypertension treatment (defined as the introduction of a new antihypertensive drug).

Methods: We conducted a cohort study on 5710 hypertensive subjects of the French Health Insurance System Database, treated and stabilized with their antihypertensive therapy and not exposed to any NSAID prescription between 1 April 2005 and 1 April 2006. The maximum follow-up duration was 4 years.

Results: Adjusted Hazard Ratios (HR) for hypertension treatment intensification were 1.34 (95% CI: 1.05–1.71) for NSAIDs in general [and 1.95 (95% CI: 1.13–3.36) for oxicams in particular]. There were significant interactions between NSAIDs and Angiotensin Converting Enzyme Inhibitors (ACEIs, HR = 4.09, 95% CI: 2.02–8.27) or Angiotensin Receptor Blockers (ARBs, HR = 3.62, 95% CI: 1.80–7.31), but not with other antihypertensive drugs.

Conclusions: Exposure to NSAIDs (and to oxicams particularly) leads to an intensification of hypertension treatment, especially in patients treated with ACEIs or ARBs. Renin Angiotensin System blockers should be avoided whenever NSAIDs are prescribed.

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Laboratory monitoring of patients treated with antihypertensive therapy and newly exposed to non steroidal anti-inflammatory drugs

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Background: Drug Interactions between Non Steroidal Anti-Inflammatory Drugs (NSAIDs) and Angiotensin Converting Enzyme Inhibitors (ACEIs), Angiotensin Receptor Blocker (ARBs) or diuretics can lead to renal failure and hyperkalemia. Thus, monitoring of serum creatinine and potassium is recommended when a first dispensing of NSAID occur in patients treated with these antihypertensive drugs.

Objective: To evaluate the proportion of patients treated with ACEI, ARB or diuretic and receiving a first dispensing of NSAID who had relevant serum creatinine and potassium laboratory monitoring.

Methods: We described the first dispensing of NSAID among 3509 patients of a 4-year cohort (6983 antihypertensive-treated patients recorded in the French Health Insurance Reimbursement Database). We analyzed serum creatinine and potassium laboratory monitoring within the 3 weeks after the first NSAID dispensing.

Results: General Practitioners prescribed the majority of NSAIDs (85.6%, 95% CI: 84.4–86.7). The more commonly prescribed NSAIDs were ibuprofen (20%), ketoprofen (15%), diclofenac (15%) and piroxicam (12%). Relevant serum creatinine and potassium monitoring was 10.7% (95% CI: 9.5–11.9) in patients treated by ACEIs, ARBs or diuretics. Overall, monitoring was more frequently performed to patients aged over 70, treated with digoxin or glucose lowering drugs, but not to patients treated with ACEI, ARB or diuretic. Monitoring was more frequent when NSAIDs' prescribers were cardiologists and anesthesiologists.

Conclusion: Monitoring of serum creatinine and potassium of patients treated with ACEI, ARB or diuretic and receiving a first NSAID dispensing remains insufficiently performed and need to be reinforced through specific interventions.

P232

Drug interactions with colchicine: results from a local data mining

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Introduction: Colchicine is a well-known narrow therapeutic index drug, but letal or life threatening situations resulting from drug interaction are still reported to the pharmacovigilance system. Notification background noise appears to be dramatically constant.

Method: So we made an estimation of this interaction burden by data mining within the electronic prescriptions hospital database between January 2007 to September 2011. We extracted patients who received colchicine (name and treatment dates). We did the same for all of the contraindicated or disadvised drugs (according to the AFSSAPS Thesaurus, version before last): ciclosporin, verapamil, pristinamycin, erythromycin, azithromycin, clarithromycin, josamycin, roxithromycin, midecamycin, and spiramycin. We crossed the files and selected all patients who received, at the same time, colchicine and any of these interacting drugs. We used the medical chart to assess symptoms and analyse risk factors such as preexisting renal insufficiency, starting dose, use of prophylactic antidiarrheal drugs.

Results: One thousand and twenty-four patients received Colchicine Opocalcium® (without opium and tiemonium). Among them, 45 (4.4%) also received an interacting drug. Nine (0.9%) were symptomatic: 7 (0.7%) presented colchicine-induced diarrhea, and 2 (0.2%) presented life threatening intoxication leading to resuscitation unit admission. Three patients had renal insufficiency, two had a high starting dose, and four had both renal insufficiency and high starting dose (included the two patients resuscitated patients). Median duration of coprescription was 4 days. Prophylactic antidiarrheal drug use was high 29% (13/45): loperamide (77%), racecadotril (23%) or diosmectite (8%). Within the medical units, prospective drug interaction detection and interception rate was low 11% (5/45).

Conclusion: This study suggests that prescription of drug interaction with colchicine is frequent in a hospital setting. Fortunately, it appears to be asymptomatic in most of the cases. A particularly noteworthy feature of life threatening cases, is the conjunction of risk factors (renal insufficiency, high starting dose), making considering them as easily preventable. So we made an information campaign about colchicine handling, within the concerned medical units. Now, the same kind of study is warranted for ambulatory prescriptions, using data from national health insurance fund, to draw the complete spectrum of the problem.

P233

Relationship between antibacterial agents usage and resistance to quinolones and ceftriaxone in Escherichia coli hospital isolates

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Objective: The relationship between antibiotic consumption in hospitals and resistance of *Escherichia coli* to quinolones and ceftriaxone is poorly understood. Our objective was to assess the relationship between antibacterial agents hospital use and incidence of the resistance to quinolones and to ceftriaxone in *Escherichia coli* hospital isolates.

Methods: Antibacterial agents consumption and resistance data were collected among 36 private and public hospitals of the Pays de la Loire region, France, during year 2009. Antibiotic quantities were converted to defined daily doses (DDD) per 1000 patient-days for each hospital. Antibacterial agents were grouped in 16 classes that were adapted from the ATC classification. The incidence of resistant strains was calculated by ratioeing the number of resistant strains against the number of patient-days. The relationship between antibiotic consumption and incidence of resistant strains was assessed using negative binomial regression. The results were expressed as incident rate ratios (IRR) [95% confidence intervals].

Results: Four classes of antibacterial agents were independently and significantly associated with the incidence of quinolone-resistant strains: tetracyclines (1.1387 [1.0296–1.2593]), quinolones (1.0072 [1.0001–1.0144]), first and second-generation cephalosporins (1.0072 [1.0018–1.0125]) and third-generation cephalosporins (1.0286 [1.0095–1.0482]). Two classes of antibacterial agents were significantly associated with the incidence of resistance to ceftriaxone: quinolones (1.0095 [1.0002–1.0189]) and third-generation cephalosporins (1.0320 [1.0076–1.0571]).

Discussion: For the first time, we report that hospital use of quinolone antibiotics is associated with resistance of *Esc*herichia coli to quinolones. It is biologically plausible that quinolones consumption favors quinolone resistance. Inversely, quinolone resistance may favor consumption of third-generation cephalosporins. Additionaly, consumption of quinolones and third-generation cephalosporins seems to select resistance to ceftriaxone in *E. coli* hospital isolates. These results highlight the influence of hospital consumption of quinolones and third-generation cephalosporins on bacterial resistance. Decreasing the usage of these antibiotic classes may help to control the resistance of *E. coli* to quinolones and to ceftriaxone.

P234

Potentially inappropriate medications and adverse drug effects in patients with Alzheimer's disease or other dementia

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Background: To assess the prevalence of potentially inappropriate medications (PIM) in patients with Alzheimer's disease (AD) or related dementia, and evaluate the prevalence of adverse drug reactions (ADR) related to PIM use.

Methods: A multicentre cross-sectional study was carried out in six French regions. Patients with AD or related dementia and treated with cholinesterase inhibitor and/or on memantine were recruited according to the following repartition: 60% from their various places of residence, 30% from nursing homes and 10% from long-term care units. Data registered were socio-demographic characteristics, type of dementia (diagnostic, treatment), medical history, drugs used and ADR. Drugs were classified using the consensual French PIM list. Two independent pharmacovigilance experts assessed the ADR.

Results: Five hundred and fifty-eight patients aged 84.4 ± 5.0 years [74–99] were recruited; 75.8% suffered from AD. Patients used 6.6 ± 2.8 drugs distributed as follows; central nervous system medications: 29.8%, cardiovascular system: 28.0%, digestive and metabolic system: 20.8% each. The distribution of anti-dementia drugs was: 28.3% memantine, 28.3% donepezil, 17.7% rivastigmine, 12.7% galantamine and 19.0% association. The prevalence of PIM use in patients with dementia was 29.2%. The prevalence of ADR was 18.1% and the prevalence of ADR related to PIM was 1.4%. Anti-dementia medications were involved in 37.5% of the cases, and PIM in 14%.

Conclusion: One third of French patients with dementia used PIM; in a previous population-based study, half of French subjects aged ≥75 years were using PIM. The prevalence of ADR was twice that shown in another study carried out in the French population with dementia (PEIMA study, 5%). PIM are seldom involved in ADR occurrence (1.4%), as we previously showed in another study conducted in the French elderly (6%). The quality of prescription in the elderly with dementia is an important challenge and special attention should be paid when prescribing and overseeing anti-dementia drugs.

P238

Potentially inappropriate medications among patients with Alzheimer disease in REAL.FR: be aware of atropinic and benzodiazepine drugs!

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Context: Quality of prescriptions among elderly people has been often studied, especially with several lists of potentially inappropriate medications (PIM). Few pharmacoepidemiological studies have investigated prescribing practices in Alzheimer Disease (AD) according to PIM lists.

Objectives: To assess the prevalence of PIM use in community-dwelling patients with mild to moderate AD patients and to identify the clinical factors associated with prescription of PIM.

Methods: REAL.FR is a 4-year, prospective, multi-center cohort of AD patients. We analyzed baseline data of AD patients, including demographic characteristics, clinical outcomes [medical and surgical history, physical disability using both the Activities of Daily Living (ADL) scale and Instrumental Activities of Daily Living scale, cognitive function relying on both the MMSE and Alzheimer's Disease Assessment Scale-cognitive components severity of dementia using both the Reisberg GDS scale and Clinical Dementia Rating scale and nutritional status by the Mini Nutritional Assessment (MNA)] and drug intake. We used two PIM lists: the French Laroche and American 2003 Beers ones.

Results: A total of 684 AD patients were included (mean age 77.8 ± 6.8, 486 (71%) females). Most of them (89%) were treated with cholinesterase inhibitors (none with memantine). According to the Laroche list, 320 (46.8% CI 95% [43.0–50.5%]) patients had at least one PIM. Using 2003 Beers list, 173 (25.3% CI 95% [22.0–28.6%]) patients received at least one PIM. Cerebral vasodilators were the more used drugs with 165 (24.0% CI 95% [20.9–27.3%]) prescriptions. Users of atropinic drugs (imipraminics, phenothiazines and some antipsychotics, hypnotics, H1 antihistamines, antispasmodics, memantine, atropine, carbamazepine, paroxetine, atropinics antiparkinsonians…) were 116 (16.96% CI 95% [14.13–19.78%]). Benzodiazepines with long half-life were prescribed to 58 (8.5% CI95% [6.4–10.6%] patients. In multivariate analysis, only female gender and number of drugs were associated with PIM prescription.

Conclusions: This study shows that PIM prescription concerns around one out of two AD patients. Among these drugs, cerebral vasodilators, atropinics and benzodiazepines were the more used.

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Trend in opioids analgesic consumption in Europe from 2002 to 2009

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Introduction: Before 2000, the under-utilisation of opioids in France was pointed out, and led to the implementation of three consecutive national strategic plans to improve pain management since 1998. This work describes longitudinal trends in opioids use in France and in continental Europe since 2002.

Objective: To investigate the trends in opioids consumption in Continental Europe between 2002 and 2009.

Methods: Data were collected for all the continental European Countries. Statistics figures on opioids consumption were researched and extracted from the consumption reports and/or databases of the respective national authorities. Data were expressed in DDDs/1000 inhabitants/day (DID). Total opioids consumption (N02A) and utilization of selected substances (morphine, oxycodone, fentanyl, codeine, dextropropoxyphene and Tramadol) were investigated. Moreover, information on the national opioids prescription and/or delivery regulations and their eligibility for reimbursement was also collected.

Results: Data collected were mainly represented by sales data collected by the national authorities from wholesalers. France was the largest consumer of opioids in 2002–2009 (about 50 DID), but the amounts used were mainly represented by dextropropoxyphene combinations (53.9% of total consumption in 2009 (24.7/44.7 DID) and up to 73.3% (42.6/58.1 DID) in 2005). However, the consumption of dextropropoxyphene decreased in all countries since 2005. During the observed period, the total consumption of opioids (N02A) showed a slight increase. The consumption of morphine was stable or decreased slightly in all countries except in the UK (1.03 DID in 2005 to 1.19 in 2009 (+70%)). The utilization of fentanyl increased in most countries, in particular in Iceland (1.7–2.7), which was the largest user.

Discussion: Levels and profiles in opioids utilisation are varying extensively among European countries. In France, the amount of opioids used was mainly explained by the significant contribution of weak opioids, particularly dextropoxyphene containing medicines. As a consequence of definite withdrawal of dextropropoxyphene in March 2011, substantial changes in opioids consumption patterns are expected. The possibility for a transfer of prescribing to products with higher dependence and/or abuse potential or with unfavorable safety profile need to be closely monitored.

P240

Factors influencing premature end of proton pump inhibitors prescription in chronic NSAIDs users at risk of gastrointestinal complications

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Gastroprotection with a Proton Pump Inhibitor (PPI) is recommended for patients at risk for gastrointestinal (GI) complications receiving a nonsteroidal anti-inflammatory drug (NSAID), especially when it is on a chronic basis. Whereas it is well-known that patient's compliance diminishes during the course of treatment, maintenance of PPI prescription by the physician has been less studied. This study aimed to assess maintenance of PPI prescription in chronic at-risk NSAIDs users.

We used a validated electronic database (LPD, Cegedim) covering a representative panel of general practitioners in France. Included patients received NSAID prescription for at least 2 years from 2007, without more than 180 days-long interruption, associated with an initial concomitant prescription of a PPI. Kaplan Meier curves were used to explore the probability of still being treated by a PPI at 12 and 24 months from the index prescription. Risk factors for PPI disruption were described by univariate and multivariate Cox analyses. The GI complications rates were compared using Student's t-test.

A total of 1856 at-risk patients were included. Patients were mostly female (63.8%) and older than 65 years (74.4%). Median number of NSAID prescriptions during study period was 8 (min 2, max 24). The probability of still being prescribed a PPI concomitantly to NSAID was 77.5% [95%CI 75.6–79.4] and 68.3% [66.1–70.4] 1 and 2 years after study inclusion respectively. Risk factors for PPI disruption were: NSAID molecule switch (OR 2.01, 95% CI: 1.69–2.39, $P < 0.001$), female gender (OR 1.20; 1.01–1.42, $P < 0.05$), no GI side effects (OR 1.26; 1.01–1.58, $P < 0.05$). The number of co-prescribed drugs had a protective effect (OR 0.94; 0.91–0.96, $P < 0.001$). PPI was reintroduced within 6 months following its stop for half the patients. As no specific reason to reintroduce PPI was reported in about 70% of the cases, that interruption was probably unintended. GI complications rate was significantly higher for patients without adequate PPI prescription (17.8% vs. 12.8%, OR 1.48; 1.12–1.98, $P = 0.007$).

Adequate PPI prescription is obtained for only two-thirds of chronically NSAID-treated at-risk patients initially receiving a PPI. It is of real concern as PPI disruption appears to be associated with an increased GI complications rate.

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Risk of venous thromboembolic events on antipsychotic drugs: a meta-analysis of observational studies

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Background: Antipsychotic drugs are widely prescribed for the treatment of various psychiatric disorders such as schizophrenia, mania, dementia, major psychotic depression… An unexpected frequency of thromboembolic complications is observed with the use of antipsychotic drugs, a priori unrelated to psychiatric disease. Several observational studies have tried to quantify the association between antipsychotic drug use and the risk of thromboembolic events, but no synthesis is available.

Methods: We performed an exhaustive meta-analysis of analytic observational studies assessing the existence of a link between antipsychotic drug use and risk of thromboembolic events. The computer-assisted research was conducted via MEDLINE, Cochrane Library and Google Scholar. Congress proceedings have also been consulted. The outcome of interest was the presence of a venous thromboembolic event (deep vein thrombosis and/or pulmonary embolism) objectively confirmed. The exposition was defined as current antipsychotic drugs use or use in the last 3 months before the inclusion. Odds Ratio (OR) and 95% confidence intervals (95%CI) of each study was combined weighting by the inverse of the variance using log(OR) method.

Results: Seven studies were included (five case-controls and two cohorts) including 370 516 patients. When pooling adjusted OR estimated in each study, an increase of 82% of the risk of thromboembolic events was observed in patients taking antipsychotic drugs compared to non-users: OR = 1.82 (95%CI 1.38–2.41), $P < 0.001$. The trend remains the same when pooling crude OR (OR = 2.02 (95%CI 1.51–2.70), $P < 0.001$). The increased risk of venous thromboembolic events was observed regardless of the type of antipsychotic drugs (first generation OR = 2.30; 95%CI 1.29–4.11 or second generation OR = 1.85; 95%CI 1.54–2.21) and regardless of the design of the studies (case-control OR = 2.42; 95%CI 1.70–3.44 or cohort OR = 1.16; 95%CI 1.01–1.34).
Discussion: This meta-analysis, which is the first to quantify the association between antipsychotic drugs and risk of venous thromboembolic events, showed a significantly increased risk of thromboembolic events in patients taking antipsychotic drugs. The pharmacological rational for the association between antipsychotic drugs and risk of venous thromboembolic events remains questionable. In this way, individual data would be necessary to identify all risk factors underlying this association.

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Use of medicines in a rural setting: frequency of subjects at risk of medicines requesting attention (MRA) misuse

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Introduction: The ability of the elderly to use medicines in good conditions is important to limit the occurrence of adverse effects and optimize drug benefit. This may particularly affect medicines for which usage requires special attention (MRA: Medicines Requiring Attention).
Objective: The objective of this study was to estimate the proportion of subjects at risk of MRA misuse.
Method: A cross-sectional pharmacoepidemiological study was conducted from baseline data collected in the AMI cohort, a French prospective cohort that included 1002 elderly subjects (65 years and older) living in rural areas and retired from agriculture, to study age-related pathologies. For each subject, health care reimbursement data were extracted from the French health Insurance database of the '*Mutualité Sociale Agricole*'. After establishment of the MRA list (defined as medicines that in case of misuse may have a particular risk of adverse drug reactions [ADR], compromised benefit, or both), we identified the frequency of subjects at-risk of MRA misuse through a decision-tree considering, for each user, three cognitive status, (dementia with clinical diagnosis, cognitive impairment not dementia [CIND] and no cognitive impairment), social environment, and medical data. This frequency was assessed overall, and considering dementia status.
Results: The majority of subjects of the AMI cohort were consumers of at least one MRA (98.8%). Overall, 3% of subjects were at risk of MRA misuse: this proportion was 2.6% in demented subjects, 4.2% in subjects with CIND, and 2.5% in subjects with normal cognitive status. In this rural population, the main characteristic that led to risk of MRA misuse was the presence of visual impairment. Independently of this factor, the proportion of subjects at-risk would have been 0.3% overall, 0.2% in those demented, 0% in CIND, and 0.1% in normal subjects.
Discussion: Apart from visual impairment, this study shows that care of the elderly is adequate to limit the misuse of MRA drugs in this rural elderly population.

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Impact of main antihypertensive and lipid lowering agents on cognitive decline in a cohort of stroke patients

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Introduction: In literature, post-stroke dementia prevalence varies widely from 6% to 31.8% at 3 months. Influence of antihypertensive and lipid-lowering agents exposure on post-stroke dementia incidence is discussed. The aim of this analysis was to study the impact of main antihypertensive and lipid lowering agents on cognitive decline in a cohort of stroke patients.
Methods: Biostroke, a prospective cohort, was conducted on patients with recent stroke (<48 h), included between June 2005 and April 2009, with a 3 years of follow-up. The primary outcome measure was post-stroke cognitive decline at 3 months defined by MMSE ≤ 24, among non demented subjects at baseline (IQCODE = 78). Drug exposition was defined by lipid lowering agents (including statins and fibrates) or antihypertensive drugs intake (including angiotensin converting enzyme inhibitors, angiotensin receptor antagonists, diuretics, β-blockers and calcium channel blockers) for more than 15 days before the stroke. Multivariate logistic regression models were used to estimate adjusted OR and 95% confidence intervals (95% CI).
Results: Among the 477 patients initially included in the Biostroke cohort, 179 subjects were presenting inclusion criteria for this analysis. Patients included in the analysis had a mean age of 60.8 years (±14.8) and 58.7% were male. Twenty three subjects (12.8%) presented cognitive decline 3 months after the stroke. Univariate analyses showed that patients with cognitive decline were significantly more often sedentary ($P = 0.04$) than the others. In multivariate analysis, angiotensin converting enzyme inhibitors increased significantly post-stroke cognitive decline (OR = 4.07; 95%CI = 1.01–16.43). Statins and β-blockers showed a trend to cognitive protection ($P = 0.17$ and $P = 0.12$) while calcium channel blockers intake was trendy associated to cognitive decline ($P = 0.10$).
Discussion: This study highlights that previous treatment before stroke could influence positively or negatively post-stroke cognitive impairment while this analysis is limited by a lack of power. However, this analysis is made on an observational cohort of patients who reflects the true cognitive evolution and takes into account treatment non-compliance.

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Prescription drugs during post-partum in Midi-Pyrenees: a study in the French Health Insurance database

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Objective: After delivery, mothers are subject to a range of medical conditions such as intrauterine infections, depression or breastfeeding problems that may require drug prescription. The aim of the present study was to analyze reimbursed drugs which have been dispensed to women during post-partum period compared with women who didn't give birth during the last three previous months (controls).
Methods: We conducted a study in a cohort of women from the French Healthcare database at the Midi-Pyrenees regional level. Women who gave birth between April 2009, 1st and March 2010, 31st and randomly 1:1 matched non-pregnant women (same age and same district) were identified. Drugs reimbursements were compared in both groups of women during: (i) the first three postpartum months for postpartum women and (ii) the same 3 months period for controls.
Results: During the postpartum period, 22 165 of the 25 149 pregnant women (88%) were redeemed at least one prescription. The majority of prescriptions during this period were for ATC codes N (central nervous system), G (Genito-urinary system), B (Blood and blood forming organs) and A (Alimentary tract and metabolism). Prescription proportion during postpartum among genitourinary system and blood system drugs was due to bromocriptine (G02CB01) and iron (B03AA07) which were reimbursed to respectively 30% and 37% of the women. A 2-fold to 6-fold difference was similarly observed after a pregnancy with vitamins, mineral supplements, analgesics and sex hormones mainly levonorgestrel. Conversely, purchases of oral corticoids and antihistaminics were 2-fold lower among control women.
Discussion: The high prescription rate of drugs after pregnancy seemed to be most likely caused by medical conditions after delivery. With respect to the post-partum drug use, the most important limitation in this study was the fact that some prescriptions to the mother could be probably intended for the child.

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Prescription of antihypertensive drugs in ambulatory care

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Objective: High blood pressure (HBP) is a major public health problem in Morocco. The objective of this study is to evaluate the prescribing behavior according to the therapeutic recommendations, to analyze the place of different therapeutic classes and to appreciate the using of fixed combination drugs and generic medication.
Materials and methods: This is a prospective study from November 2010 to November 2011, including 742 patients with essential hypertension. Clinical data and treatment were studied. We use the arithmetic mean of three ambulatory measures and analysis of data from the self-measurement and/or Ambulatory Blood Pressure Monitoring (ABPM). We chose the definition and classification of hypertension published in 2009 by the European Society of Cardiology (ESC) and European Society of Hypertension (ESH).
Results: The mean age was 62.1 ± 11.2 years. 65.3% of patients were female. Hypertension was complicated in 58.9% of cases. The HBP goals were achieved in 319 patients (43%). Pharmacological treatment was prescribed in 96.1%. Monotherapy was used in 175 patients (23.6%). The most prescribed therapeutic classes were Calcium Channel Blockers (CCBs; 35.1%), followed by Angiotensin Converting Enzyme inhibitors (ACE inhibitors 23.7%), the Angiotensin II Receptor Blockers (ARBs; 23.7%), beta-blockers (b-blockers; 10.8%) and diuretics (5.9%). Three hundred and twenty-eight of patients (44.2%) receive two-drug combination and 19.1% receive a fixed combination of antihypertensive agents. The most prescribed fixed combinations were ACE inhibitors + diuretic (25.6%) and CCBs + diuretic (18.3%). Triple therapy was prescribed to 210 patients (28.3%), while the quadruple covered only 29 (3.9%) of hypertensive patients. Of a total of 1104 prescribe antihypertensive drugs, 43.5% were generics.
Discussion: The use of diuretics is limited by their potential effects on glucose tolerance, lipid metabolism and uric acid. Since the analysis of Lindholm, b-blockers are no longer a first line treatment for hypertension. Indeed, ACE inhibitors, ARBs and CCBs have proven effective in the treatment of hypertension particularly when combined with other Cardiovascular Risk Factors (CRF). The prevalence of this category of hypertension is high in our population. The lack of need for laboratory tests prompted a wider prescription of CCBs.

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Insulin glargine and risk of cancer: a cohort study in the french national healthcare insurance database

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Objective: A suspected higher risk of cancer in insulin glargine (IG) than in human insulin (HI) users was investigated in the *Echantillon Généraliste de Bénéficiaires* (EGB) database. Since increased mortality might hide an increased risk of cancer, the combined outcome of death or cancer was also studied.

Methods: The EGB is a representative 1/97th permanent random sample of the national healthcare insurance system database that covers approximately 80% of the French population. It includes claims reimbursed since 2003 for approximately 600 000 beneficiaries. The study population was all adults (≥18 years) with at least two dispensations of insulin between 1 January 2003 and 30 June 2010, without diagnosis of cancer at the time of first insulin dispensation, or death in the following month, and with no more than 1 year without claims. Four cohorts were defined according to incident or prevalent use and whether one insulin was used exclusively or predominantly (≥80% use time). Cox proportional hazards time-dependent models stratified on the propensity score quartiles for use of IG vs. HI, and adjusted on insulin, biguanide and sulfonylurea possession rates, were used to assess the risk of cancer and death or cancer.
Results: Only patients with type 2 diabetes were analysed, because there was only one cancer among incident type 1 diabetes. Exposures varied from 2273 to 614 patient-years for incident exclusive IG or HI users respectively, and from 3125 to 2341 patient-years for all predominant IG or HI users. All-type cancer hazard ratios (HR) with IG vs. HI ranged from 0.59 (95% confidence interval, CI: 0.28–1.25) in incident exclusive users to 0.58 (95%CI: 0.34–1.01) in all predominant users. Cancer risk increased with exposure to insulin or sulfonylureas in these patients. Adjusted HR for death or cancer associated with IG compared to HI ranged from 0.58 (95%CI: 0.32–1.06) to 0.56 (95%CI: 0.36–0.87).
Discussion: There was no excess risk of cancer in type 2 diabetic patients on IG alone compared to HI alone. The overall risk of death or cancer in patients on IG was about half that of patients on HI, thereby excluding bias from competing risk of death.

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Secondary prevention drugs following acute myocardial infarction after hospital discharge, 6 and 24 months thereafter: results from the EOLE cohort

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Objective: Use of drugs for secondary prevention after acute myocardial infarction (AMI) is recommended by international cardiology societies. The objective of this analysis was to estimate the use of the drugs recommended following AMI after hospital discharge, at 6 months, and at 24 months of follow-up.
Methods: A cohort study was designed to include 5000 patients with recent AMI (<3 months) recruited by hospital and non-hospital cardiologists. At inclusion, drug exposure was assessed from physician and patient declarations. At 6 months and at 24 months, it was assessed from patient declaration.
Results: Between May 2006 and June 2009, 5538 patients were included. Follow-up drug exposure was available for 3348 patients (60.5%) at 6 months, and 3763 (67.9%) at 24 months. The baseline characteristics of analysed populations were similar to those of included patients. Mean age was 62.1 years, 77.6% were male, 9.6% current smokers, 16.7% had diabetes, 44.6% hypercholesterolemia, 43.6% high blood pressure. For 86.7%, it was the first AMI, 70.5% had all three AMI criteria (symptomatic, electrical, enzymatic), and 8.2% had LVEF<40%. At inclusion, 99.4% of patients were exposed to aspirin or other anti-platelet agents, 95.8% statins, 89.7% beta-blockers, 73.8% angiotensin-converting enzyme inhibitors (ACEi). Exposure to the recommended combination of these four treatments (BASI) was 65.7% at inclusion, 57.1% at 6 months (initiation after inclusion: 4.3%), and 50.3% at 24 months (initiation after inclusion: 5.5%). Persistence of exposure to the BASI treatments from inclusion was 79.3% at 6 months, and 66.8% at 24 months. Omega-3 supplementation, also recommended, was 15.7% at inclusion, 17.5% at 6 months (initiation after inclusion: 3.4%), and 16.0% at 24 months (initiation after inclusion: 4.4%). Omega-3 supplementation persistence was 82.4% at 6 months, and 68.2% at 24 months.
Discussion: Long-term persistence to the BASI treatment combination only concerned two-thirds of patients. The reasons for this remain to be elucidated in an attempt to improve use of this recommended drug regimen. Furthermore, omega-3 supplementation was not frequent.

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Prospective study assessing the risk factors of bleeding in patients taking Vitamin K antagonists (VKA) treatment, admitted to Amiens University Hospital with an INR>5

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Introduction: It is a clearly established fact that an INR > 5 under vitamin K antagonists (VKA) is associated with an excessive bleeding risk. However, in many observations with high INR, there is no apparent bleeding. So, it is implied that other factors should be combined to lead to bleeding.
Methods: This prospective, epidemiological, analytical and descriptive study was carried out from 01/01/2006 to 01/01/2008. In order to assess the main risk factors leading to bleeding, patients taking oral VKA admitted to the Amiens University Hospital with a major bleeding risk situation (INR>5), were studied. Two groups were identified: (i) patients with bleeding and (ii) patients without bleeding. These two dependant variables (bleeding or not) were compared in relation with potentially explanatory variables linked to the patients or linked to the treatment
Results: In this study, were included 906 patients. There was a clinical apparent bleeding in 241 and 80% of them were serious. Bleeding led directly or took part in the death of patient in 17%. Nearly half of these haemorrhages was considered as avoidable or potentially avoidable. Such an high INR was the risk factor that emerged with the greatest power from this study. The other bleeding risk factors, statistically significant were: previous high INR not managed, chronic alcoholism, recent trauma or digestive lesions, prescription outside the indications defined in the SPCs, drug interaction with heparin, recent patient mistake. The risk factors that did not emerge were: age, gender, severe kidney failure, many drug interactions (fluoroquinolones, aspirin or NSAID).
Discussion: This study brings out that a big part of patient had INR sometimes very high and that the close control of the INR is essential for trying to reduce the bleeding risk. It shows also that this critical situation is probably underestimated (a part of the bleedings was avoidable). An action seems to have to be led on two fronts: (i) with patients in order to improve the compliance, (ii) with health care professionals by the respect of SPCs, ensuring the continuity of health care (coordination between health professionals) and the installation of a real therapeutic education.

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Evolution of the consumption of potentially inappropriate medications in elderly people after hospitalisation in a geriatric unit

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Introduction: Elderly people tend to have multiple comorbid conditions and subsequent polypharmacy, which place them at a higher risk of adverse drug events, drug-drug and drug-disease interactions and potentially inappropriate medications (PIM). PIM includes several patterns, such as inappropriate dose or duration, prescribing drugs having significant drug-disease or drug-drug interactions. Hospitalisation in a geriatric unit can provide the opportunity to review medications.
The main purpose of this study was to follow-up during 1 year the prevalence of PIM in patients aged 75 and older after hospitalisation in a geriatric unit.
Methods: All elderly people admitted to a geriatric unit in Limoges University Hospital between January 2008 and July 2009 and registered with the French National Health System were included. Prescribing patterns were established on admission to the Hospital (E-CHU), on admission in Geriatric Unit (E-SSRG), on discharge from Geriatric Unit (S-SSRG), 1 month (M1), 3 months (M3), 6 months (M6), 9 months (M9) and 12 months (M12) after discharge. The French PIM list was used.
Results: One hundred and twenty-nine patients were included. The mean consumption of drug on E-CHU, E-SSRG, S-SSRG, M1-M12 were respectively: 7.3 ± 2.9; 8.6 ± 3.4; 7.9 ± 2.9; 9.9 ± 4.4; 8.1 ± 3.6; 8.1 ± 3.4; 7.9 ± 3.8 and 8.2 ± 3.6.
The prevalence of polymedication was at least 80% whatever the period. The prevalence of PIM decreased from 46.5% on admission in hospital to 26.4% on discharge from geriatric unit and was 31.5% at M1; 28.9% at M3, 27.2% at M6, 30.0% at M9 and 28.3% at M12.
The PIM most often prescribed were long-acting benzodiazepins, anticholinergic antihistamines, cerebral vasodilators and concomitant use of two or more psychotropic drug from the same therapeutic class. On discharge from Geriatic unit, PIM use was reduced by almost 50% with a decrease of 80% in cerebral vasodilators and 70% in anticholinergic antihistamines.
Discussion: Hospitalisation has a long lasting positive effect on PIM consumption. Efforts still have to be done to reduce consumption of drugs and PIM.

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Generic substitution in primary care in 2011: differences according to pharmacological classes?

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Introduction: Generic substitution has been permitted for several years and is promoted in order to reduce health expenditures. However, reluctance concerning use of generic drugs exists for different reasons: suspicions about their efficacy and/or safety, differences in content (excipients) and discussions about bioequivalency. For these reasons, recommendations on generic substitution differ according to medical speciality. The aim of our study was to explore if substitution ratio differ according to pharmacological classes used in primary care.
Materials and methods: We conducted a descriptive study in the French Health Insurance Database using reimbursement data on drugs prescribed and delivered in the Midi-Pyrénées county between March 2010 and March 2011. We selected different pharmacological classes largely used in primary care: proton pump inhibitors, oral hypoglycemics, antiplatelet drugs, diuretics, beta-blockers, calcium antagonists, angiotensin converting enzyme inhibitors, sartans, statins, thyroid hormones, antibiotics, antiepileptics, antiparkinsonians, benzodiazepines and serotonin reuptake inhibitors. For each class, a substitution ratio was calculated (DDD of generics delivered/DDD of brand name plus generics). A Chi-square test was used in order to detect a difference between these substitution ratios.
Results: The global substitution ratio was 68.7%. Values varied between 11.2% for antiparkinsonians and 90.7% for antibiotics, with significant statistical difference ($P < 0.001$). Substitution ratio was inferior to 50% for thyroid hormones (28.4%) and antiepileptics (46.1%). Higher substitution ratios were observed for statins (88.6%), serotonin reuptake inhibitors (86.2%) and diuretics (86.1%).
Discussion: This study underlines that major differences exist in terms of generic substitution ratio between different pharmacological classes. Recommendations could partly explain them. Further studies are needed in order to precise point of view of patients, general practitioners and pharmacists on this problematic.

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Prospective observational study of angiotensin converting enzyme inhibitors -induced hyperkaliemia in hospitalized patients with chronic renal failure

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Objective: To study the incidence and risk factors of angiotensin converting enzyme inhibitors-induced hyperkalemia in hospitalized patients with hypertension and pre-existing chronic renal failure.

Methodology: Two months prospective observational study was used including all hospitalized patients older than18 years with a history of hypertension, non dialyzed chronic renal failure and who had angiotensin converting enzyme prescription at the time of the admission. Hyperkalemia ≥5 mM was detected in these patients. The studied variables were demographic, clinical, biological and therapeutic.

Results: Eight patients, among 27 included, had a hyperkalemia (29.63%). They were 73 ± 15 years old. Factors that predispose to hyperkalemia were present in all patients. Hyperkalemia was associated in six cases with decompensation of renal function. The age was associated with hyperkalaemia in patients treated with angiotensin converting enzyme inhibitors (RC = 1.21; CI95% 1.11–1.46; $P = 0.021$). Diabetes is a possible risk factor (OR = 5.9 021et, 95% CI0.93–24.10, $P = 0.053$). Compared with patients who did not develop hyperkalemia, the occurrence of hyperkalemia in patients included was associated with a longer duration of hospitalization (OR = 1.30, 95 1.12–1.60, $P = 0$, 022)

Conclusion: The Prescription of angiotensin converting enzyme inhibitors in the elderly with chronic renal failure and diabetes requires careful monitoring of serum potassium.

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Estimation of the number of malignant and premalignant skin lesions induced by voriconazole in France by two sources capture-recapture method

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Introduction: Voriconazole is a highly efficient broad-spectrum second-generation azole antifungal agent. Unlike other azoles, it is associated with a risk of phototoxicity and photocarcinogenesis. Numerous reports describing induction of premalignant skin lesions and severe skin carcinoma lead EMEA to request to a risk management plan and to include the Voripeau French registry in. The aim of this retrospective and prospective registry, built by medical scientific French societies, is to collect as many cases as possible to further identify risk factors of this particular disease.

Method: We used the two sources capture-recapture method to estimate the number of cases of skin premalignant and malignant lesions occurred in France since the marketing authorisation in 2002. The first source was spontaneous notifications originating from the regional 31 pharmacovigilance centers or from Pfizer France. The second source was the Voripeau registry.

Results: Spontaneous reporting retrieved 16 cases, Voripeau registry retrieved 13 cases. Five common cases were identified. A first estimation of the total number of French cases is obtained by the maximum likelywood estimates method: 41.6 (IC95%: 14.9–68.3). As the number of common cases was low, we used the Chapman Seber correction: the estimate becamed 38.7 (IC95%: 22–55.4). Odds ratio calculated from the contingency table was 0.83 (IC 95%: 0.21–3.32) indicating independency of the two sources. Reporting rates of each source could be estimated: 41.3% (IC95%: 28.9–72.7) for spontaneous notification, 33.6% (IC95%: 23.5–59.1) for the Voripeau registry. But the inhomogeneity of reporting within the French territory is noteworthy.

Conclusion: As the observed population is not formally closed (new treatments or deaths), we can suspect a diminution of the probability of capture, leading to a low number of common cases and therefore an underestimation the total population. On the other hand, the severity and the atypical evolution of these cases make a memorisation bias highly probable (high reporting rates of both source are consistent with this hypothesis). Whatever the final tendency, this simple method can be considered as a survey indicator.

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Capture-recapture estimates of drug abuse related disorders

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Aim: It is necessary to quantify serious hazards of psychoactive drug abuse or misuse to better understand the harm caused. One of the major difficulties of assessing psychoactive substance use disorders is that addictive behaviours are often associated with hidden characteristics that are difficult to detect by usual approaches. This study aimed to estimate the incidence of serious drug-related complications by using the capture-recapture method in defined geographical area.

Methods: Serious drug related complications were defined as hospitalizations. We crossed three sources of data: the spontaneous reports (NotS) collected by the regional drug abuse monitoring centre (addictovigilance centre), the computerised hospital database PMSI (Programme de medicalisation des systemes d'information), and the toxicological laboratory which provided the toxicological analyses (TA) carried out for patients admitted to hospital.

Results: In 2007 and 2008, analysis of the three data sources captured 1509 distinct cases. After they were modelled, the estimated number of psychoactive drug-related hospitalizations was 4 744 (95% confidence interval (CI): 4060–5429). Products most frequently identified were opioids (NotS: 59%, PMSI: 30%, TA: 44%), cannabis (NotS: 29%, PMSI: 19%, TA: 20%) and cocaine (NotS: 5%, PMSI: 8%, TA: 24%). 'Multiple drugs' were observed in 40% of PMSI cases. In Toulouse area, the incidence of serious drug-related complications should be estimated at 5.7 (5.5–5.9) per thousand inhabitants aged 15–64. Exhaustiveness of sources were 0.4% (95%CI: 0.2–0.6) for NotS, 11.6% (95%CI: 10.7–12.5) for toxicological analyses and 22.6% (95%CI: 21.4–23.8) for PMSI. The latter was relatively higher than the other two but remained low in absolute numbers, indicating that administrative hospital databases are valuable but not sufficient pharmacoepidemiological tools.

Discussion: The 'real' number of cases far exceeds that of cases which can be identified through simple counts. In particular, it confirms the under-reporting and even quantifies its magnitude. These results confirm that drug users are hospitalised and require heavy medical management. Moreover, these results show the real, although limited, advantage of PMSI in detecting drug associated disorders in epidemiological studies.

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Drug intoxication in patients registered for liver transplantation for acute liver failure: results from 7-country SALT study

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Introduction: Intentional or non-intentional drug overdose may cause acute liver failure (ALF) leading to transplantation. Such cases identified in the SALT (Study of Acute Liver Transplant) study were evaluated.

Methods: SALT study was a multinational, multicentre, retrospective, case-population study performed in France, Greece, Ireland, Italy, Netherlands, Portugal, and UK over 2005–2007 in adults. Data of ALF cases were sought through liver transplant registries and hospital records. ALF cases were classified as (i) with an identified clinical cause (were not further considered for drug exposure), (ii) exposed to drugs (including herbal and homeopathic medicines) within 30 days of index date (ID, initial symptoms of liver disease). Drug-exposed cases were again subdivided into i) acute drug intoxication (ADI), with or without suicidal intent, ii) non-ADI. Demographic, clinical, and drug use data in the 30 days prior to ID were collected for all drug-exposed ALF cases. Drug-exposed cases of ALF were assessed individually by a case adjudication committee (CAC) using WHO causality scale.

Results: Fifty-two of the 57 eligible transplant centres contributed data (91.2%). A total of 9479 cases registered for transplantation at the contributing centres, of which 600 were ALF, 302 were exposed to a drug within 30 days prior to ID. Of these, 114 were ADI (72 intentional, 10 non-intentional, 32 intentionality not clearly defined). ADI was responsible for 19% of all cause ALF in the seven participating countries; the highest in Ireland (52%), followed by the UK (28%), France (18%), the Netherlands (8%), Italy (1%). No ADI cases of ALF were identified in Greece and Portugal. Cases of ADI were mostly females (61.4%); mean age was 33.6 (±std10.9) years. One hundred and eleven (97.4%) of the 114 ADI cases were exposed to paracetamol, all had the causality score possible, probable or highly probable concluded by the CAC. Thirty-one (27.2%) ADI cases were exposed to antidepressants, and 30 (26.3%) to psycholeptics. For three non-paracetamol cases, causal drugs were benzodiazepin derivatives+opioids, ectasy, and diclofenac+iron.

Conclusion: These results show that ALF cases leading to registration for transplantation with paracetamol overdose with or without suicidal intent represented 97.4% of all intoxication drug-exposed cases of ALF.

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Analysis of prescription in hemodialysis patients

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Introduction: Hemodialysis only partially corrects the effects of kidney failure, requiring adjuvant therapy plus medications associated with pathologies common in this population. Thus these patients are polymedicated, which increases the risks of treatment.

Objective: The objective of our work is to analyse prescriptions in hemodialysis population.

Method: Descriptive study conducted among 39 patients undergoing hemodialysis at renal unit at University Hospital Mohammed VI in Marrakech. It was noted all treatments taken by patients. Analysis of drug prescriptions was carried out in comparison to the recommendations made in the summaries of product characteristics.

Results: The average age of patients was 44 years old, female gender represented 56.41%. The seniority of hemodialysis was 10-year period from 1 month to 21 years. The cause of kidney failure was unknown in 53.85% of cases, hypertension was the cause of 28.21% of renal failure. On average 3.33 drugs were taken per patient (seven drugs in the maximum and one drug in a minimum). The oral route was preferred in 36 patients, other routes have been used for medicines that can't be administered by oral route. The frequency of administration was one time per day for 104 drugs (86.67% of prescription). The good adherence to treatment was noted in 30 patients (86.34%). Drug interactions were noted in 16 patients (44.44%), in 75% of cases it was only one drug interaction. Side effects were noted in three patients.

Discussion: This study shows that most hemodialysis patients are polymedicated with a risk of lower compliance and drug interactions. The consequences of these interactions in our study were generally mild, however, this risk must be considered before any drug prescription in hemodialysis patients.

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Antipsychotic drugs and risk of QT prolongation

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Introduction: The cardiovascular mortality of psychiatric patients is higher than the general population. Antipsychotics and their property of QT prolongation seems a possible cause of this mortality.

Objective: The objective of our work is to estimate the prevalence of QT prolongation in a population of patients treated with antipsychotics and identify key risk factors for this prolongation.

Method: We conducted a retrospective study in psychiatric center Ibn Rochd of Casablanca over a year, including 134 patients receiving antipsychotic treatment. We analyzed the epidemiological, clinical, biological, therapeutic and electrocardiographic data in these patients, to determine the prevalence of long QT syndrome and research its key risk factors.

Results: The prevalence of long QT syndrome in our series is 4.5%. The risk factors for QT prolongation (except drugs) are infrequent. The risk factor for drug is present in the majority of patients: drug combinations are numerous. Almost half of patients (43.3%) were traited by triple therapy, among those associated treatments, many molecules are presented as lengthening the QT. The risk is higher with increasing number of prescriptions. The lowest QTc interval was measured in patients receiving only one drug. Patients with six associated drugs have the longest QTc. Fifty percent of patients with a long QT are treated by high doses of neuroleptics.

Discussion: It seems important that the prescriber of antipsychotic drugs takes into account factors of prolongation of QT, especially drugs. But we must remember that despite the proven toxicity of antipsychotic drugs, QT prolongation does not necessarily a negative risk-benefit balance. This is assessed on a case-by-case basis to determine the right practice.

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ALPHO: ALlergy to PHOlcdine and NBMBA anaphylaxis

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Objective: Immediate hypersensitivity during anesthesia is mainly due to neuromuscular blocking agents (NMBAs) by an IgE dependant mechanism. No previous exposure to NMBA is found in 15–85% of patients. Therefore, other agents are suspected to induce cross sensitivity. According to Scandinavian authors, pholcodine, an antitussive agent could be one of them. Indeed, NMBA anaphylaxis dramatically decreased in Sweden after pholcodine withdrawal in 1998 [1], and later in 2007 in Norway [2]. Pholcodine is largely consumed in France, but from April 2011, a medical prescription is now necessary for its delivery. Moreover, following its benefit risk reassessment, less drugs containing pholcodine are available.

We propose to study, from 2012 to 2016, the impact of decreased pholcodine consumption on both pholcodine hypersensitivity and NMBA anaphylaxis incidence by the synergistic activities of two French networks: the GERAP (Groupe d'Etude des Réactions Anaphylactiques Peranesthésiques) network and the Regional Pharmacovigilance Centers network.

Patients and Method: Every year, about 150–170 patients who experienced anaphylaxis during anesthesia with NMBA injection are tested by the allergologists of the GERAP network. All these patients will be informed about the study and if they agree they will be tested for pholcodine sensitization by i) intradermal tests with diluted pholcodine and ii) dosage of IgE specific for pholcodine. Exposition to pholcodine will also be evaluated by standardized inquiry about antitussive and antalgic drugs intake during the six preceding months.

NMBA anaphylaxis annual incidences will be calculated by capture-recapture method from the databases of the two networks. The results will be compared to those obtained before pholcodine delivery modifications.

Simultaneously, NMBA and pholcodine national consumptions will be monitored, as well as other antitussive specialties in order to evaluate if pholcodine decreased consumption is related to another antitussive increase.

First results are expected in June 2014 and complete results are expected in October 2016.

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What will become of prescribed drugs?*

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Do patients exactly follow their prescriptions or are they rather free in prescribed drug use?

A survey has been performed by a pharmacy student in Haute Garonne in order to evaluate patients' behaviour after prescription of drugs. The questionnaire which has been used concerned drug dispensation by the pharmacist: did the patient ask the pharmacist not to give him all medications and the written dose? At the end of the treatment, the patient was asked about what he had consumed and the reasons for this attitude.

One hundred and fifty patients have accepted to participate. When they went to the pharmacy, one out of five patients did not take all the medications (or whole the dose) which have been prescribed. Nearly 3/4 of the patients did not consume all prescribed drugs Dosage was not respected for 65% of the cases and the entire treatment was not taken for 70% of the patients: 38% of the patients have decided not to take the drugs, 58% have decreased the daily dose and 4% have increased it. For 8% of the patients, it was too difficult to take the drug since, for example, the pharmaceutical form was not easy to carry. Seven percent of the patients thought that the drug was not effective enough. Four percent of them have stopped the drug because of a side effect. Eighty percent of the non-consumed remaining drugs have been kept at home for a next use.

Taking these results into account, one of the first questions that could be asked to the patient by the prescriber could be: did you take this drug?

*According to an idea from Professeur Paul Montastruc.

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Treatment costs of early breast cancer in Morocco: evaluation of cost of two chemotherapy regimens

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Introduction: The aim of this study was to evaluate the cost of the management of early breast cancer by analyzing the data of 400 patients treated at the National Institute of Oncology (NIO) in Rabat with two different protocols.

Patients and methods: The cost of treatment was assessed by using the microcosting method that considers as more as possible all determinants of cost. We focused on the total cost of treatment protocols (surgery, chemotherapy, radiotherapy…). The calculations were made with the costs already fixed by using the monetary values.

Results: The direct cost of care per patient was estimated at $1451.35 (CMF) and $1615.29 (Anthracyclin) depending on the protocol. Chemotherapy represents a small proportion of the total cost between 2% and 4% ($3518.45 for CMF and $14485.54 for anthracyclin), while surgery and radiation represent the larger proportion of the total cost ($219154.22 for CMF and $381208.54 for anthracyclin). The difference between the prices of the same drug can range from single to double ($50.31 in NIO vs. $125.02 in officine for AC 60) and whether it is a price approved by the Ministry of Health or negotiated within the framework of public procurement. According to our study, the cheaper chemotherapy protocol (2.6 times less) seems not to be the cheapest if the global cost of the treatment is considered (1.1 times price). This is mainly due to the small proportion of the chemotherapy drugs in the overall cost and because of the less efficacy of the protocol based on CMF in local control and progression free survival, hence the interest in this type of study to reach the optimal decision by care providers and policy makers.

Discussion: The regimens based on anthracycline were more expensive for the health care system that the regimen based on CMF. However, the difference in cost can be largely justified by the lower results in efficacy in the CMF group. In addition, our study showed that chemotherapy represents only a small proportion of total cost as compared with other treatments. The decision to adopt the protocol based on anthracycline as a reference, should take into account theses results.

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Analyze of internet narratives on patient websites before and after benfluorex withdrawal and media coverage

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Background: Chat group is a new way of communication which allow people to share experiences about different topics. Recently, Butt and al. (1) explored narratives of survivors of Stevens-Johnson syndrome posted on patient websites. In France, benfluorex (Mediator®) was withdrawn in November 2009, the risk communication about valvulopathy – induced by benfluorex (Mediator®) began in the end of 2010 related to publication of data about the estimation of mortality of patients exposed to benfluorex during 30 years (2).

Objectives: The aim of our study was to explore the evolution of narratives posted on patient websites according to three dates indexes: before benfluorex withdrawal, after its withdrawal, after its media coverage.

Methods: After exploration of several blogs, three were chosen: *Elle, Vive les rondes and Doctissimo*. Three periods have been identified: before November 2009 (benfluorex withdrawal), between November 2009 and media coverage in November 2010 and after November 2010.

Results: Around 140 posting from three blogs are analyzed. Different topics were posted concerning mainly: efficacy of drugs or general diet advice, dosage, adverse drug reactions (diarrhea, valvulopathy or anxiety), anger towards pharmaceutical companies and physicians, deny or safety of the benfluorex, warning, …According to the three dates indexes, a predominance of posting about efficacy of drug was identified (52/87, 60%), how to find benfluorex after withdrawal,… before and also after November 2009 until November 2010. As expected, posting about anger towards healthcare system (19/33, 58%) and anxiety about cardiovascular adverse effects (10/33, 30%) were predominated after the media coverage.

Conclusions: Withdrawal of benfluorex do not change perception of patients about risk of drugs. Significant changes were only observed after media coverage and then 1 year after its withdrawal.

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Pharmaceutical Sales Representatives and Patient Safety: a comparative cross-sectional survey of information quality in Canada, France and the U.S.

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Introduction: The information provided by sales representatives has been shown to influence physicians' prescribing decisions, an influence that is often underestimated. For accuracy and balance, and to enable safe prescribing, all medicines information must include harm as well as benefit.

Methods: This is the first comparison of safety-related information provided by sales representatives in different regulatory environments. We carried out a comparative cross-sectional study in Vancouver, Montreal, Sacramento and Toulouse. Physicians who regularly see sales representatives were recruited to report on consecutive sales visits. These sites represent three national regulatory environments: France has the strictest standards; the French and US governments directly enforce regulations; and Canada relies primarily on industry self-regulation. We asked how often 'minimally adequate information for safe and appropriate use' was provided. This was defined a priori to include: ≥1 serious adverse event, ≥1 common adverse event, ≥1 contra-indication, ≥1 indication, and no unapproved indications or unqualified safety claims.

Results: 'Minimally adequate information' was rarely provided in any of the sites, and no information on harmful effects was provided in two-thirds of promotions in Vancouver, Montreal, and Sacramento. Qualified and unqualified safety claims, health benefits, and insurance status were noted more frequently. In Toulouse, free samples, and 'drug lunches' were rarely provided, and harm was mentioned more often, mainly common adverse events.

Conclusions: Some important differences were consistent with stronger information standards in France. However, in all four sites, physicians received inadequate information on serious harmful effects of medicines, raising questions about whether information quality is compromising protection of patients' health.

Keywords: sales representatives, drug information, prescription, safety, international, comparative study

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The cost of management intracranial aneurysms by embolisation in Morocco

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Introduction: Exhibit the cost of care by embolisation of intracranial aneurysm and understand relation betwen the cost and clinical parametres of patient.

Patients and methods: This is a prospective study between January 2010 and November 2011, for patients treated with embolization of cerebral aneurysms. The cost of pharmaceutical product (drug and medical devices) and the overall cost of care were calculated using data from the information system of the hospital.

Results: In total, 40 patients were treated, mean age 51.5 ± 12.5 years. The sex ratio M/F = 0.53. Twenty patients were covered by health insurance (50%). The median overall stay was 8 days [4–11] whose ICU stay was 1 day [1–2.5]. The overall average cost of treatment was 9 277.6€, it varies from 4784.3€ to 32 172.2€. The cost of pharmaceuticals products is 57.6% on average in the overall cost. The average cost of consumables was 5 352.9€ with a range of 2331.4€ to 13 454.4€. Length of stay significantly influence the overall cost of ownership ($r = 0.839$, $P < 0.001$), especially when it comes to admission to intensive care service ($r = 0.894$, $P < 0.001$).

Discussion: The cost of pharmaceuticals in the endovascular treatment of intracranial aneurysms remains high and represents a major handicap for the development of this technique in countries with high coverage by a health plan is low (34% in Morocco).

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Circulation of information on adverse effects of medicines: true or utopic?

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Objective: to assess the opinion of the general population regarding medicines safety and the quality of information on adverse effects.

Methods: five 2nd year pharmacy students interviewed people randomly in the street, with a standardized anonymous questionnaire. There were seven closed questions on their medicine-taking habits, their awareness of adverse effects, of where to report and their prevention. For example: Do you consider that you take too much medicines? Are you well enough informed about adverse effects of medicines? Responders were classified by sex and age groups: 18–24 years, 24–50, and over 50 years old.

Results: One hundred and fifty persons participated to the survey. There were 62.6% of women and 37.3% of men. Twenty percent were in the 18–24 years age group, 33.3% in the 24–50 years group and 46.7% in the over 50 years group (seniors). Most participants (80.7%) thought they don't take too much drugs. Among participants, 77.5% of women and seniors read the patient information leaflet before using a new medicine. Fifty-two percent of the participants considered themselves not sufficiently informed on adverse effects and did not know what to do, or where to go in case of adverse effect. Only 20% of them knew the pharmacovigilance system. Even though, 44% thought that they have already had an adverse effect in their lifetime. Paradoxically, 82% have heard about adverse effects in the media, but were not able to give more details.

Discussion: According to these results, the awareness of responders of their own drug-taking habits is distorted; the majority seems to believe that their drug use is low. However, 50 medicines are still used by every inhabitant each year in France. There is also a lack of information from the system on prevention of adverse effects. This is in contrast with the expectations of the public who needs more information. A poster on the proper use of medicines was realized to help the general public to better understand the importance of pharmacovigilance in preventing adverse effects of medicines.

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Influence of GNB3 C825T polymorphism on antidepressant efficacy and metabolic effects

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Background: Recent evidence suggest that the T allele of the C825T genetic polymorphism of the ß3 subunit of G protein (GNB3) was associated with depressive disorders and arterial hypertension. The association between GNB3 C825T polymorphism and antidepressant efficacy is still unclear. Our objectives were to determine the influence of the GNB3 C825T genetic polymorphism on antidepressant efficacy and metabolic effects in a sample of depressed patients.

Methods: In a prospective, open and naturalistic study, 397 adult patients recruited from 2007 until now, suffering from a major depressive disorder and requiring an antidepressant treatment were genotyped for GNB3 C825T single nucleotide polymorphism. They also received clinical measurements (height, weight and blood pressure), psychometric assessments (the Hamilton Rating Scale for Depression 17-items, the Clinical Global Impressions and the Quick Inventory for Depressive Symptomatology) and blood samples (metabolic parameters) before and after 1, 3 and 6 months of treatment.

Results: Genotype frequencies were in Hardy-Weinberg equilibrium ($\chi^2 = 0.73$: $P = 0.12$). GNB3 C825T polymorphism was not associated with antidepressant efficacy. But TT patients developed more arterial hypertension after 6 months (TT vs. CC/CT: $P = 0.02$). Furthermore, they had more metabolic syndrome after 3 months when they were treated by venlafaxine (TT vs. CC/CT: $P = 0.02$)

Conclusions: TT depressed patients receiving antidepressant treatment would run more the risk of developing metabolic symptoms. Clinical practice should be changed if these results were confirmed.

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Beating the odds: efficacy and toxicity in cancer patients with DPD-driven pharmacogenetic adaptive dosing of 5-FU

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Background: 5-FU is the backbone of most chemotherapies used in digestive oncology. Dihydropyrimidine dehydrogenase (DPD) deficiency is a pharmacogenetic syndrome associated with increased risk of experiencing life-threatening toxicities upon 5-FU intake. Tailoring 5-FU dose according to the DPD status of patients should help to reduce this risk through a reduction in dosing in deficient patients. However, maintaining an optimal efficacy in patients whom dosages have been cut is a challenging issue, and whether cutting the dosage could reduce the response rates eventually is still widely debated.

Patients and methods: A total of 39 adult patients (23M/16W, mean age 60.5 years, range 38–83 years) with digestive cancers were included. Treatment consisted of Folfox-4 (33%), LV-5-FU2 (33%), Folfiri (21%) or other 5-FU-based combinations (13%). Primitive tumor localization was colorectal (30%), rectal (22%), and others, including pancreatic, stomach, coecum (48%). DPD status was evaluated using the standard U-to-UH_2 ratio chromatographic determination in plasma. Additionally, screening for the IVS14 + 1G > A single nucleotide polymorphism was performed in patients with Poor Metabolizer profile. When evidenced, DPD deficiency led to a 15 down to 30% reduction in 5-FU dosage. Toxicity was monitored following standard CTC guidelines after the first and the second course of administration. Efficacy was evaluated at 3-month following the RECIST criteria.

Results: Seven out of 39 patients (18%) exhibited U/UH2 ratios indicative of mild (five patients, 13%) or severely (two patients, 5%) deficiency in DPD. 5-FU dosage was reduced accordingly. Mean 5-FU dose was 2322 mg/m^2 in patients with normal DPD status, and 1871 mg/m^2 in patients displaying DPD deficiency. No difference in toxicities were observed between the groups. Interestingly, efficacy remaiend the same in DPD-deficient patients treated with reduced dosage as compared with full-dose patients (CR: 29% vs. 28%, PR + SD: 57% vs. 47%, PD: 14% vs. 16%).

Conclusion: Preliminary determination of DPD status associated with adaptive dosing can secure the administration of 5-FU while maintaining an optimal efficacy. Our study strongly suggest that DPD-based tailoring of 5-FU dosage could help to improve clinical outcome, because usually 10–20% of severe toxicities are reported when treating patients with 5-FU standard dosage.

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Allele frequencies of CYP2D6 and CYP2C19 variants in a hospital psychiatric population and their clinical relevance

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Background: CYP2D6 and 2C19 polymorphisms are currently investigated in our hospital psychiatric unit to help explaining, at least in part, drug resistance and/or side effects, and selecting right psychotropic medications.

We here report CYP2D6 and CYP2C19 allele frequencies and genotyped-predicted phenotypes in 146 patients.

Methods: Genomic DNA was extracted after informed written consent in schizophrenic or depressive patients. CYP2D6 (*2, *3 *4, *6,*9,*10 and *41) variants were detected by DNA sequencing, *5 variant and duplicated alleles *1xN, *2xN by long range PCR and CYP2C19 *2 and *17 variants by allele specific amplification.

Results: One hundred and forty-six patients, mean age 43 years (17–84), were genotyped for CYP2D6 and 116 for both CYP2D6 and CYP2C19.

The most frequent CYP2D6 alleles were *1 (0.38),*2 (0.20),*4 (0.16),*41 (0.11).

Nine patients carried functional allele duplications, one of the inactive *4 associated with decreased-activity alle *9 (previously described in Zimbabwe populations).

CYP2D6 genotyped-predicted phenotypes identified 4% ultrarapid, 4.8% poor (all of them homozygous *4/*4 except one *4/*5), 30% intermediate and 61% extensive metabolizers.

CYP2C19*2 and *17 allele frequencies were 0.13 and 0.21. Genotyping led to expect 1.7% poor (homozygous *2/*2) and 3% ultrarapid metabolizers (homozygous *17/*17).

None was found to be defective homozygous for both CYP2D6 and CYP2C19.

Discussion: CYP2D6*4 allele frequency was close to values reported in caucasian populations. Duplications were more abundant in our psychiatric subjects (6.8% vs. 1–4% in volunteers from northern Europe).

CYP2C19*2 and *17 frequencies were very similar to other observations in Europe.

Genotypes, genotype-predicted phenotypes and clinical observations were often closely related. A patient with a long history of treatment resistance with antipsychotic like risperidone and aripiprazole was found to be carrier of CYP2D6*2xN which can lead to an insufficient exposure. Another patient with depressive symptoms showed poor response after a well-conducted treatment by escitalopram was homozygous for the CYP2C19*17 which increases enzymatic activity.

Nevertheless, there is still a need for studies to explore complex relationships between genotypes, genotype-predicted phenotypes and 'real life'.

In all cases, therapeutic advice provided after metabolic enzyme genotyping, should not be restricted to the initial query but always extended to all pharmacological and clinical informations.

P277

Genotype/phenotype double expertise for predicting CYP2D6 status

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Objectives: To develop an easy functional approach to identify the CYP2D6 status in support of a genotypic approach for patients treated by tamoxifen-based homonotherapy, and to conduct a feasibility study to identify the most relevant time-point to establish the CYP2D6 phenotype.

Material/Patients and methods: We have developed a double screening strategy for patients scheduled for tamoxifen hormonotherapy: genotypic detection of the most frequent allelic variants, associated with a simple and rapid phenotypic expertise. Our method is based on the oral administration of a standard tracer (i.e., 30 mg of dextromethorphan) and the calculation of a plasmatic metabolic ratio after measurement of three metabolites concentrations by Fluorometric HPLC. In parallel, a genotypic study has been conducted, using Real-Time PCR with Taqman® technology on LightCycler® and screening for CYP2D6*3, *4 and *6 allelic variants, accounting 95% of PM in the Caucasian population. Five healthy volunteers were included in this feasibility study to determine their metabolic ratios associated with their respective genotype.

Results: All our volunteers were wild-type. Their metabolic ratios ranged from 0.103 to 0.242. The most relevant observation time to determine the ratio was 2 h.

Conclusion: Our phenotypic method only requires a single blood sample, affordable analytical technology with simple liquid/liquid extraction, thus making it cheap and suitable to large scale screening. Identifying CYP2D6 phenotype from a single plasma sample is a patient-friendly alternative to the standard determination of CYP2D6 status from 24-h urine. A phenotypic approach seems to be more efficient to detect Cyp2D6 deficiency, regardless of upstream genetic events. Further study is currently ongoing to determine the cut-off value of metabolic ratio for CYP2D6 deficiency.

Reference:

1. Afshar M et al. 2005.

P278

Sudden toxic-death in a patient upon Xeliri (capecitabine + irinotecan) plus bevacizumab intake: pharmacogenetic implications

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Introduction: The triple combination Avastin-Folfiri is the fist-line regimen for treating metastatic colorectal cancer. Both 5-FU and irinotecan can have their toxic profiles dramatically worsen by genetic polymorphisms affecting *DPYD* and *UGT1A1*, respectively. In some cases, a 5-FU can be switched to capecitabine (a.k.a. avastin-xeliri regimen) which is considered as a safer alternative. However, identifying biomarkers predictive of life-theratening toxicities is still an ongoing story.

Observation: We report here the case of a 57-year old male patient, who was treated with Folfox4, then bevacizumab (Avastin)-Folfiri regimen over a period of 2 years, with no major toxicities. However, due to local intolerance, central catheter had to be removed and 5-FU was then switched to oral capecitabine. Extremely severe toxicities (e.g., grade 4-diarrhea, grade 4-neutropenia, sepsis) showed soon after the treatment was resumed. Despite symptomatic treatment, immediate stop of the chemotherapy and transfer into intensive-care unit, fatal outcome was observed at Day-12. To understand this toxic-death, comprehensive pharmacogenetic investigations were performed. This patient had been previously screened for DPD deficiency and had not been identified as Poor Metabolizer. Complementary investigations on the *DPYD* gene (eg, c.464 T>A (exon 5), c.1679 T>G (exon 13), IVS14 + 1 G>A (exon 14), rs3918290 or c.2846 A>T (exon 22) polymorphisms) confirmed this normal DPD status. Further screening on the TYMS gene (28 bp tandem repeat (rs34743033), c.-58G>C (rs2853542) or 1494del TTAAAG, (rs34489327) polymorphisms) was performed and proved to be negative too. In addition, no *UGT1A1*28* allelic variant (28 (TA)6 > (TA)7 (rs8175347)) was found, an observation consistent with the fact that irinotecan proved to be well tolerated over the previous line. Finally, our investigations focused on cytidine deaminase (CDA). Based upon phenotypic evaluation, this patient was identified as ultra-Metabolizer (UM). Complementary genotypic investigations confirmed this status because deletion on the *CDA* gene promoter (-31delC, rs3215400) was evidenced, a condition associated with increased transcription.

Conclusions: Overall, this case illustrates how complex can be the identification of genetic causes for severe toxicities in patients undergoing combinational therapies. We demonstrate here that the rs3215400 deletion accross the CDA promoter is associated with a risk of lethal toxicities in capecitabine-containing regimen.

P279

Double: 79C>A and -del31C CDA contradictory genotypes in a patient undergoing cytarabine therapy: impact on treatment-related toxicities

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Introduction: The antimetabolite cytarabine (Ara-C) is the backbone of the consolidation phase in combination with mercaptopurine for treating lymphomas, a frequent disease in children. Toxicities are usually related to genetic polymorphisms affecting TPMT, the enzyme responsible for the liver detoxification of mercaptopurine. To date, little data are available about constitutive genetic variations associated with toxicities upon cytarabine administration.

Observations: A 7-year-old girl was treated in our institute for compressive mediastinal, metastatic T-cell lymphoblastic lymphoma. Four cytarabine blocks (75 mg/m^2/day for 4 days followed by a 4 days break), once daily purinethol (50 mg/m^2/day) and cyclophosphamide (1 g/m^2) were initiated. During the second block of aracytine, a rapid drop in neutrophils, white blood cell count, platelets resulting in grade-4 toxicities was observed. treatment was discontinued and search for TPMT genetic polymorphism perfomed. In the meantime, mercaptopurine was precluded, and low-dose cytarabine (i.e., 33% then 50% dose reduction) was resumed. Severe toxicities showed again, and treatment was stopped and switched to maintenance. Pharmacogenetic investigations on TPMT found none of the polymorphisms usually associated with severe toxicities with mercaptopurine (i.e., c.238 G>C (exon 5, rs1800462), c.460 G>A (exon 7, rs1800460) and c.719A>G (exon 10, rs1142345) mutations). Surprinsingly, further genotypic screening focusing on CDA, the liver enzyme responsible for the disposition of cytarabine, showed that this patient was bearing both the 79A>C polymorphism and the -del31C deletion accross the CDA gene promoter, two genotypes with theoritically opposite results in term of CDA expression and activity. To determine univoqually the patient's CDA status, phenotypic determination was perfomed eventually and revealed a mild deficiency (i.e., CDA = 1.6 U/mg, 50% lower than mean values usually reported in children).

Conclusions: this is the first time that severe toxicities in a patients treated with a cytarabine-containing regimen can be attributed to CDA deficiency, a pharmacogenetic syndrome mostly associated with gemcitabine toxicity thus far. Beside, the complex genotypic status displayed by this patient highlights the need for developing functionnal approaches, in support to genotypic screening, to better determine patient's status prior to administrating cytotoxics.

P280

Influence of CYP2C9 and SLCO1B1 genotype on bosentan-induced liver toxicity

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Introduction: Bosentan is a first-line endothelin receptor antagonist for the treatment of pulmonary arterial hypertension (PAH); its main adverse drug reaction is an increase in aminotransferase level. As bosentan is partly metabolized through cytochrome (CYP) 2C9 and transported into hepatocytes through organic-anion-transporting polypeptides (OATP/SLCO) 1B1, we aimed at testing whether CYP2C9 and SLCO1B1 genotype was associated with bosentan-induced liver toxicity.

Methods: In this case-control association study DNA was isolated from peripheral blood of nine PAH patients who experienced liver toxicity (aminotransferases >3 times the upper limit of normal) and 18 PAH patients who did not (controls), matched on age and gender. Genotyping of SLCO1B1*1B (rs2306283) and SLCO1B1*5 (rs4149056) SNPs was performed using predeveloped TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA). Genotyping of CYP2C9*2

and CYP2C9*3 allelic variants of CYP2C9 was performed by PCR followed by restriction enzyme analysis. Genotypic tests based on a logistic regression model were used.
Results: Among 10 patients who had heterozygous loss-of-function for CYP2C9, three experienced liver toxicity, whereas six of 17 patients with functional CYP2C9 did ($P = 0.78$). Fifteen patients had heterozygous loss-of-function for SLCO1B1, among whom five experienced liver toxicity; one patient had heterozygous loss-of-function (and no toxicity); and four of 11 patients with functional SLCO1B1 showed increased aminotransferases ($P = 0.76$).
Conclusion: We did not show any association between CYP2C9 and SCLO1B1 genotype and bosentan-induced liver toxicity. Other transporters could be targeted, such as bile salt export pump or Na+-taurocholate cotransporting polypeptide.

P283
In vitro DNA damage and cytotoxicity of hexachlorobenzene
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Purpose: Hexachlorobenzene (HCB) is one of the most persistent environmental pollutants, can cause a wide range of toxic effects. To obtain more insight into HCB-induced mechanism of toxicity, the present study was undertaken to determine the possible effects of low doses of HCB on DNA integrity, cellular viability, and differentiation in vitro in human colonic cell line Caco-2.
Methods: HCB was dissolved in absolute ethanol and tested at low doses (0.04, 0.4, 4, 40, 400 nM and 2 μM) Cytotoxicity was evaluated by MTT assay and DNA damage was assessed by comet assay. Data were compared by one way ANOVA and Tukey's post hoc test with significance level set at $P < 0.05$ level.
Results: We observed that alkaline phosphatase activity of caco-2 cells was not changed; suggesting that cell differentiation was not affected upon pesticide treatment. On the other hand our results clearly showed an impact of HCB on cell viability. This effect was observed at 4 nM and was dose dependent. In addition, cell exposed to 0.4 and 400 nM of HCB showed a significant increase of the percentage of tail DNA compared to untreated control cells.
Conclusions: These findings indicate that exposure of caco-2 cells in HCB, at low doses, induced cytotoxicity and have a genotoxic risk without affecting cell differentiation.

P284
Role of the CD47 receptor in regulating UHRF1 and NF-KB proteins expression in human astrocytoma cell lines
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CD47 protein is a membrane receptor that plays pivotal roles in many pathophysiological processes, including infection, inflammation (Sick et al; 2009), cell spreading, proliferation, and apoptosis. We have shown that activation of CD47 increases proliferation of human astrocytomas, whereas blocking it has the opposite result but has no effects on normal cells suggesting that CD47 may be constitutively activated in astrocytomas (Sick et al; 2011). Our objective was to study the intracellular pathways activated by CD47 receptor by targeting the Ubiquitin-like containing PHD and Ring Finger domains 1 (UHRF1), p16 (a negative regulator of cell cycle), NF-kB (a transcription factor) and cytokines pathways.
Human astrocytoma cell lines U87, CCF-STTG1 (WHO grade IV) and normal human astrocytes (NHA) were incubated with 50 and 100 μM of CD47 agonist peptide 4N1 or with 0.2 and 2 μg/mL of blocking monoclonal antibody against the extracellular domain of CD47 (mAb B6H12). CD47 receptor, UHRF1, p16, NF-kBp65 and cytokines (IL6, IL7, MCP-1) expressions were evaluated by Real-time polymerase chain reaction and western blot.
We have shown that UHRF1 is more expressed on human astrocytomas than on normal astrocytes whereas there is no change on CD47 expression between the different cells. Activation of CD47 receptor by 4N1 increases UHRF1, NF-kB and cytokines expression on human astrocytomas, while blocking it with anti-CD47 mAb (B6H12) decreases the expression of the same proteins. In addition, blocking CD47 increases p16 protein expression on human astrocytomas, suggesting that CD47 pathway is implicated in down-regulation of p16.
In the same condition no effects on UHRF1 expression where observed on normal human astrocytes (NHA).
Our data indicate that CD47 receptor increases the UHRF1, NF-kB and cytokines expression, thereby likely contributing to tumor aggressiveness by enhancing the proliferation.
Sick et al., Cell. Mol. Life Sci., 2009, 66:1271–1282; Sick et al., Glia, 2011, 59: 308–319

P285
Nitric oxide limits the production of reactive oxygen species induced by mitochondrial reverse electron flow
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Introduction: Mitochondrial electron transport chain (mETC) is a major source of reactive oxygen species (ROS) in cells making it a central player in ischemia/reperfusion damage and a main target for cardioprotective strategies. Succinate driven reverse electron flow from complex II to complex I of mETC yields the highest rate of ROS production. This can contribute to cell death during myocardial ischemia-reperfusion as succinate concentration increases in these conditions. Complex I inhibitors have been shown to suppress reverse electron flow and nitric oxide (NO) is known to alter the activity of complex I. Therefore, the objective of this study was to investigate the effect of NO on mitochondrial reverse electron flow.
Methods: Mitochondrial ROS generation was assessed by measuring the rate of H_2O_2 production by spectrofluorimetry (oxidation of amplex red to resorufin). ROS production was measured in cardiac mitochondria isolated from: (i) wild type (WT) mice and feeded with succinate in the presence of increasing concentrations (50–500 nM) of the NO donor DETA-NONOate and (ii) transgenic mice (TG) overexpressing H11Kinase specifically in the heart. These mice are characterized by an increased expression of cardiac inducible NO Synthase (NOS) expression and are powerfully protected against myocardial infarction. Mitochondrial NO production and mitochondrial NOS expression were measured using the fluorescent probe DAF-FM and by western blot analysis, respectively.
Results: Our data demonstrate that NONOate inhibited mitochondrial reverse electron flow in a concentration-dependent manner and this effect was reversed by the NO scavenger phenyl-tetramethylimidazoline-1-oxyl-3oxide (PTIO). Transgenic mice showed an increase in mitochondrial NOS expression and produced significantly more NO with a faster rate compared to WT mice. This was associated with a decrease in H_2O_2 production induced by the reverse electron flow. Pre-treating the animals with the NOS inhibitor L-NG-Nitroarginine Methyl Ester (L-NAME) (20 mg/kg for 3 days) abolished the increase in NO production and restored reverse electron flow.
Conclusion: Taken together, these results show that ROS production induced by mitochondrial reverse electron flow is inhibited by delivering NO in vitro or increasing NO production in vivo. This could contribute to the cardioprotective properties of NO.

P286
A new CD47-derived peptide acting as a TSP-1 ligand and exhibiting anti-angiogenic properties
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Introduction: CD47 is a ubiquitously expressed membrane receptor implicated in many pathophysiological processes including apoptosis, cell proliferation, inflammation and cardiovascular responses. CD47 and its endogenous agonist thrombospondin-1 (TSP-1) might therefore represent key targets in tumor microenvironment for development of innovative therapeutic strategies against tumorigenesis and metastasis. The purpose of the study was to evaluate the biological effects of CD47-derived peptides designed by molecular modeling, paying particular attention to their anti-angiogenic potential.
Materials and methods: Bioinformatic studies led to identify CD47-derived peptides (IESQLLKGDAS and its disulfide-bound analogue CEVSQLLKGDAC) acting as putative antagonists of TSP-1/CD47 interaction, as revealed by co-immunoprecipitation experiments. HUVECs 2D and 3D migration was analyzed by scratch assay and Transwell migration in the presence of 100 μM peptides. Anti-angiogenic effects of CD47-derived peptides were evaluated in vitro by quantification of pseudotubes formation and ex vivo by mouse aortic rings sprouting assays. In vivo studies were performed on a homograft model of murine B16F1 melanoma cells. Tumor morphology and necrosis were evaluated by MRI and H&E staining.
Results: TSP-1 co-immunoprecipitation with CD47 was reduced by 50% in several stromal and tumoral cell lines in the presence of 100 μM peptides, confirming the predictive molecular modeling studies. In vitro, a twofold diminution in 2D and 3D endothelial cells migration was observed under IESQLLKGDAS or CEVSQLLKGDAC treatment. HUVECs tube formation was also strongly disturbed in the presence of CD47-derived peptides in accordance with migration assays. CEVSQLLKGDAC induced a 25% inhibition of ex vivo Matrigel-induced angiogenesis on aortic rings explants after 6 days incubation. Finally, CEVSQLLKGDAC induces in vivo tumor necrosis, which was three times higher than in non-treated mice.
Discussion: CD47-derived peptides antagonism toward TSP-1/CD47 interaction was confirmed by co-immunoprecipitation, validating our molecular modeling original approach. These peptides exhibit strong anti-angiogenic activities in vitro, ex vivo and in vivo and could therefore represent new exciting tools for cancer treatment in association with classical anti-mitotic agents. Further studies will focus on the characterization of the molecular mechanism of action on endothelial cells, considering the intracellular signaling and the putative contribution of CD47 membrane partners (integrins, CD36, LRP-1).

P287
Effects of leptin on human myometrial cell proliferation
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Background: Maternal obesity is associated with delivery disorders, such as delayed or difficult onset of labour leading to higher rates of post-dates pregnancies. During pregnancy, myometrial cells undergo phenotypic changes, from an early proliferative phase to a contractile state in order to initiate labour. Leptin might be involved in obesity-associated delivery disorders as leptin inhibits, in vitro, myometrial contractility.
Objective: This study was aimed to assess the ability of leptin to induce human myometrial primary cell lines proliferation.
Study design: Primary cell lines were established from myometrial biopsies obtained from women with uncomplicated pregnancies at term before the onset of labor. To investigate the effects of leptin on proliferation, we performed a time course (8, 24, 48 h) and dose response (12.5, 25, 50, 100 ng/mL) stimulation.
Results: This is a currently ongoing study, results have to be considered with caution. We first showed that both long and short leptin receptor isoforms are expressed in myometrial cells of pregnant women, predominantly the short isoform. Leptin increases total cell number in a concentration-dependent manner but more experiments are necessary to perform formal statistical analysis (+2%, +16%, +7%, +1% compared with control, for 12.5, 25, 50 and 100 ng/mL of leptin respectively, $n = 5$). We further investigated the cell cycle phase involved in this effect and showed that leptin seems to increase percentage of cells in S-phase (+13%, +48%, +100%, +39% compared with control, for 12.5, 25, 50 and 100 ng/mL of leptin respectively, $n = 3$) and to up-regulate cyclin E expression after 8 h of treatment at

a concentration of 25 ng/mL (Arbitrary Density Unit: 1.00 (ref), 1.98 ± 0.40, for control and leptin respectively, $n = 5$). Finally, we suggest that leptin-induced myometrial cell proliferation is mediated through ERK1/2 signaling pathway, as an increase in ERK1/2 phosphorylation associated with its translocation to the nucleus was observed after 30 and 40 min of leptin treatment at 25 ng/mL (ADU: 1.00 (ref), 1.79 ± 0.24, 2.09 ± 0.42 for control and leptin 25 ng/mL after 30 and 40 min respectively, $n = 6$).

Conclusion: Our preliminary results suggest that leptin induces myometrial cell proliferation, which supports the contribution of leptin in the development of parturition-related disorders observed in obese women.

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Antioxidant, cytoprotective and anti-inflammatory activities of fruit extracts of date (Phoenix dactylifera L.) from Algeria

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Dates, the fruit of the date palm (*Phoenix dactylifera*), represent an important source of nutrients and energy as well as a source of dietary fibres minerals lipids and protein. Dates are also used in traditional medicine and their pharmacological properties may be attributed to the presence of phenolic compounds, anthocyanin, sterols, carotenoids, procyanidins and flavonoids (review in Baliga et al., 2011). A series of acetone extracts from 10 date varieties was assayed for their antioxidant property by ORAC method in comparison with Trolox activity. Then cytoprotective effect and anti-inflammatory activity of the date extracts have been determined in THP-1 human monocytic cell line. Results show strong variations between date varieties (from 790.82 µmol Trolox equivalent/100 g of fresh weight to 2802.20 µmol TE/100 g). Most of date varieties extracts enhanced cell viability and showed protective effects against oxidative stress induced by hydrogen peroxide (H_2O_2). Anti-inflammatory activities, measured by IL-1β production, have revealed heterogeneity of activity among date varieties. The highest activity was demonstrated with Helwa variety (82% reduction of IL-1β production). Our research confirms antioxidant and cytoprotective activities and demonstrates in vitro anti-inflammatory activities of extracts from a series of Algerian date varieties. These properties are different according to date varieties and maturity state. Experiments are on-going to identify the main components responsible for these activities.

Reference:

1. Baliga MS, Baliga BRV, Kandathil SM, Bhat HP, Vayali PK. A review of the chemistry and pharmacology of the date fruits (*Phoenix dactylifera* L.) Food Research International 2011, 44: 1812–1822.

P289

Development and validation of a reliable LC-MS/MS method for extraction and quantitative analysis of acetaminophen and its main metabolites in small plasma volume

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Background: The painkiller acetaminophen (APAP) is mainly metabolized via a non-oxidative pathway to glucuronide (APAP-GLU) and sulfate (APAP-SUL) conjugates (50% and 30% of the initial APAP dosage, respectively). Obesity might changes APAP biotransformation, in particular in mice. However, in these animals, only small volumes of blood can be withdrawn to study APAP metabolism. We describe herein a validated LC-MS/MS method for simultaneous quantification of APAP and its metabolites in small plasma volumes. The method includes a simple step of sample extraction and reversed-phase HPLC chromatographic separation using multiple reaction monitoring (MRM) detection.

Methods: Experiments were performed on a Thermo Scientific LC-MS/MS system including a TSQ Quantum Ultra tandem triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source. Separation of the analytes was carried out on a Thermo Hypersild Gold C18 column (3.0 µm, 2.1 × 100 mm). The mobile phase consisted of aqueous 1% formic acid and 95% methanol (80:20, v/v). Calibration, or mouse plasma, samples (25–100 µL) were supplemented with the internal standard (IS), APAP-deuterated-analog, and treated with methanol. Calibration curves were obtained by spiking drug free human plasma.

Results: The retention times (RT) for APAP-GLU, APAP-SUL, APAP and IS were approximately 2.3, 2.9, 3.5 and 3.5 min, respectively. MRM transitions were used for quantitative determination whereas the second transitions were employed for confirmation purpose. The developed LC-MS/MS method was validated for linearity, accuracy, precision and recovery according to the currently accepted method validation procedures. Next, eight young C57B16J wild-type and eight obese ob/ob female mice were treated with 500 mg/kg APAP intraperitoneally. After 0.5, 2, 4 or 8 h, blood was withdrawn and centrifuged for plasma collection. Our results showed that APAP-GLU plasma levels in ob/ob mice were higher than wild type mice after 2 h, in keeping with increased glucuronidation in obesity.

Summary and conclusion: In summary, we developed an original LC-MS/MS method for the simultaneous quantification of APAP and its main metabolites in small volume of plasma, after a simple extraction procedure. This method could be helpful in other murine models to get more information on APAP metabolism in the context of obesity.

P290

Proton pump inhibitors inhibit estrone-sulfate uptake by organic anion transporter 3:

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Background: The human organic anion transporter (hOAT3), is mainly expressed into the renal proximal tubular cells. It plays a critical role in renal drug elimination, by mediating the elimination of organic anions, including a number of commonly used drugs such as: antivirals (e.g., acyclovir), nonsteroidal anti-inflammatory (e.g., Ibuprofen, Indomethacin), diuretics (e.g., furosemide, bendroflumethiazide), antibiotics (e.g., Tetracycline, quinolone). Drug-drug interaction by inhibition of hOAT3 transporters may be important. So far, comprehensive data on the interaction of PPIs with OATs are missing, although PPIs are frequently used in combination with drugs eliminated by hOAT3. The purpose of this study was to evaluate in vitro the inhibitory effect of the PPIs on the uptake of the E-S by hOAT3.

Methods: An in vitro model to study drug interactions was developed. We transfected HEK Flp-In 293, with the SLC22A8 gene which codes for hOAT3 transporter, by using the Flp-In™ System recombinase (invitrogen™). The validation of the model was performed on the basis of the expression and functional activity of OAT3 compared to control cells (HEK Mock), their expression level was measured by immunoblotting and qPCR. hOAT3 activity was tested by uptake of [^{3}H]E-S. After validating the model we carried out the tests of inhibitions by the PPIs.

Results: We observed a positive uptake of [^{3}H]E-S in HEK-hOAT3 cells compared to HEK Mock and confirmed the inhibition of hOAT3 by positive control (probenecid IC50 = 1.06 µM and ibuprofen IC50 = 0.78 µM). E-S uptake into HEK-hOAT3 cells was inhibited by all IPPs in a concentration-dependent manner. We confirmed our assumption concerning the inhibition of the hOAT3 by the PPIs tested: IC50 of 8.10 µM for the pantoprazole, 3.74 µM of lansoprazole and 14.06 µM for the omeprazole.

Conclusion: We established and characterized HEK cells stably expressing hOAT3, for the detection of substrates or inhibitors of this transporter. We also confirmed PPIs could affect significantly uptake of different drugs transported in kidney by hOAT3 in the range of plasmatic concentration.

Keywords: OAT3; Estrone-Sulfate; Proton Pump Inhibitors.

P291

Leptin prevents MMP activation in an in vitro model of myometrial inflammation

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Objective: Maternal obesity is associated with delivery disorders, such as delayed or post-term delivery, that might be explained partly by the increase in plasma leptin level in obese women, as leptin inhibits in vitro myometrial contractility. Delivery implies uterine remodeling of the extracellular matrix, via matrix metalloproteinases (MMP) activation. An association between term or preterm labor and increased levels of MMP in reproductive tissues has been suggested. This study was aimed to assess the ability of leptin to interfere with LPS-induced collagen degradation and MMPs activity and expression, in a model of human myometrial inflammation.

Materials and Methods: Myometrial biopsies, obtained from women with uncomplicated pregnancy who delivered between 38 and 40 weeks by caesarean section, prior to the onset of labor, were stimulated for 48 h with LPS (10 µg/mL) without or with leptin (10^{-10} to 10^{-8} M). MMP2, MMP9 and MMP13 expression was investigated by immunohistochemistry and western blotting, and proteolytic activity of gelatinases by zymography Total collagen degradation was assessed by Red Sirius staining. The specificity of leptin effects was assessed by a pretreatment with a selective leptin receptor antagonist (LPrA).

Results: LPS challenge was associated with a marked diminution of total collagen content and an increase in MMP2 and MMP9 activity and expression, which were both prevented by leptin treatment in a concentration dependent manner (MMP9 expression (Arbitrary Density Unit): 1.00 (ref), 3.46 ± 0.53, 1.87 ± 0.25 for Control, LPS, LPS+leptin respectively, $P < 0.001$; MMP2 expression (ADU): 1.00 (ref), 4.16 ± 0.62, 1.35 ± 0.21 for Control, LPS, LPS+leptin, respectively, $P < 0.001$). Leptin effects on collagen content and MMP2 and MMP9 activity were abolished after pretreatment with LPrA at 10^{-5} M (total collagen content (ADU): 40.7 ± 0.8, 30.3 ± 0.9, 40.0 ± 1.3, 28.9 ± 1.5 for Control, LPS, LPS+leptin, LPS+leptin+LPrA respectively, $P < 0.001$; MMP2 activity (ADU): 1.00 (ref), 1.82 ± 0.18, 0.92 ± 0.11, 1.63 ± 0.28 for Control, LPS, LPS+leptin, LPS+leptin+LPrA, respectively, $P < 0.001$; MMP9 activity (ADU): 1.00 (ref), 1.42 ± 0.09, 1.09 ± 0.04, 1.52 ± 0.13 for Control, LPS, LPS+leptin, LPS+leptin+LPrA, respectively, $P < 0.001$).

Conclusion: These results show that leptin prevents LPS-induced collagen degradation and MMP2 and MMP9 activation and expression in human myometrium, and provide new insight into the pathophysiology of delivery disorders in obese women.

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Effects of *N,N*-diéthyl-m-toluamide (DEET) in the cellular processes leading to angiogenesis

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Insect repellents containing DEET are highly effective through an inhibition of acetylcholinesterase (AChE) activity and are potentially harmful to human and animal health. Indeed, these pesticides have been reported to play a role in tumor development. Different mechanisms can lead to stimulate angiogenesis, such as the inhibition of AChE activity, a critical step in tumorigenesis. Thus, the present study was designed to test the potential effect of DEET on different processes leading to angiogenesis on human endothelial cells including proliferation, adhesion and migration through an involvement of cholinergic receptors. DEET was incubated for 24 h on human umbilical vein endothelial cells (HUVECs) at concentrations similar to that found in plasma of exposed individuals (10^{-5} M) or present in the environment (10^{-8} M). VEGF (20 ng/mL) was used as a positive control. Proliferation was analyzed by using CyQUANT Cell Proliferation Assay Kit. Quantitation of AchE activity was performed by Ellman colorimetric assay. Evaluation of adhesion was performed using crystal violet staining. Cell migration was evaluated using Transwell® migration kit. Analysis of both superoxide anion (O_2^-) and nitric oxide (NO) productions were performed using electronic paramagnetic resonance technique.

At both concentrations DEET inhibited endothelial AChE activity and increased cell proliferation. Moreover, it potentiated cell migration that was associated with enhanced MMP2 activity and endothelial cell adhesion through an enhancement of FAK phosphorylation and stress fibers. Although it did not modify O_2^- production, DEET increased NO production through an increase of peNOS-Ser/peNOS-Thr ratio. All of these cellular processes were partially prevented by methoctramide and pFHHSiD, M2 and M3 antagonist, respectively.

Altogether, these data highlight that DEET modulates cellular angiogenic processes through an activation of muscarinic receptors. In addition to their toxic effects on nervous system, this study underscores that DEET may affect the generation of vascular network that could potentiate proliferative diseases.

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Quercetin potentiates insulin secretion in INS-1 pancreatic beta cells through L-type voltage-dependent calcium channels

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We previously showed that the flavonoïd quercetin (Q) potentiated insulin secretion and ERK1/2 phosphorylation induced by glucose or glibenclamide (G) in the INS-1 β cell line. It is known that G induces insulin secretion by closing ATP-dependent potassium channels (K_{ATP}), producing beta-cell membrane depolarization and entry of calcium through the L-type voltage-dependent calcium channels. We therefore analyzed the effect of Q on intracellular calcium concentration ($[Ca^{2+}]_i$) after membrane depolarization.

To this aim, we evaluated the effects of the L-type voltage-dependent calcium channels antagonist nifedipine or the inhibitor of sarcoendoplasmic reticulum (SR) calcium transport ATPase (SERCA) thapsigargin on Q-induced potentiation of insulin secretion and Q-induced intracellular calcium changes. Insulin secretion and ERK1/2 activation were evaluated after 60 min of incubation, using transfer of fluorescence (HTRF) and Western blot, respectively. $[Ca^{2+}]_i$ was determined by imagery using the fluorescent probe fura-2.

Under low glucose concentration, Q (20 μM) only moderatly stimulated insulin secretion (x1.3), activated ERK1/2 (×2.5) and caused a weak increase in $[Ca^{2+}]_i$. Q also potentiated (x2) KCl-induced insulin secretion and amplified (x1.6) depolarization (15 mM KCl)-induced $[Ca^{2+}]_i$ increase. Nifedipine (1 μM) inhibited the amplificating effect of Q on $[Ca^{2+}]_i$ and insulin secretion. Thapsigargin (1 μM) did not modify the potentiating effects of Q.

Our results suggest that Q induces intracellular $[Ca^{2+}]_i$ increase in the pancreatic beta cell by opening L-type voltage-dependent calcium channels without influencing SERCA. By increasing basal $[Ca^{2+}]_i$ and leading to the activation of ERK1/2, Q could sensitize beta cells to the effects of stimulants of insulin secretion. Further studies will determine the possible interaction between Q and L-type voltage-dependent calcium channels.

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Probiotic effects of lactic bacteria isolated from algerian camel milk on rabbits and chicken

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The beneficial effects of probiotics and their safety-related pathogenic strains isolated were shown, which is why they are now widely used in the dairy industry and are present in a wide range of commercially products.

We reviewed the main properties that must submit a probiotic bacteria in several strains isolated in our laboratory of local Algerian products (the Algerian camel milk and the machroub). Strains of lactobacilli are used they have been subjected to tests in vitro to select the most resilient with the conditions encountered in the gut (Acidity, bile salts and their behavior with a range of antibiotics tested and their potential antagonism to enterobacteria that we isolated from raw milk, and feces of babies, we also tested their ability to absorb cholesterol.

Then we tested the prebiotic effect exerted by different honey in western Algeria on these strains in comparison with the effect of fructo-oligosaccharides, which are widely used as good prebiotics.

The second step is to test the effect of the most efficient strains in different batches of chickens Algerians on zootechnical parameters, microbiological and hematological ones.

These tests are a continuation of a study that involved testing the effect of two potential probiotic strains (isolated from camel milk and chicken meat) on local rabbits, the results obtained from two milk consumption fermented by our strains showed some interesting effects on the health of animals compared to controls, these beneficial effects were shown by improvement in the rate of plasma components, the reduction of blood cholesterol and triglycerides as well as the load Enterobacteriaceae in the digestive tract.

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P298

Physicochemical incompatibility of injectable drugs (assessment of knowledge care professionals)

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Introduction: In the absence of precipitation or formation of a toxic compound, a mixture is called compatible when 90% of the active substance is still available in the mixture at the end of the observation period. The AIM of this study is to evaluate the knowledge of care professional about physicochemical incompatibility of injectable drugs.

Materials and methods: this is a randomized cross-sectional study done on a homogeneous population of 100 nurses of the University Hospital of Fez. A detailed anonymously and an original questionnaire was developed specifically for this study.

The data were based on personal data such as age, gender, diploma, seniority, service, training, and medical data for the assessment of knowledge was based on the compatibility of certain solutes and drugs used in daily practice. About 20 variables were collected and analyzed and compared between units. The results were analyzed by appropriate statistical tests.

Results: The age of nurses in this study is between (26–32 years) with an average of 28 years old ±2. Nursing experience in the majority of participants is 3 years ±1. Only a very small percentage to 0.9% received ongoing training. Theoretical knowledge nurses vary, depending on the drug and in injection solutions. The questions concerned the widely used solutions (saline) and little-used solutions (Ringer). For the saline <50% know the drug's compatibility; for the ringer solution compatibility Less than 15% know the drug's compatibility. Tests of correlation between knowledge and nursing experience show no significant difference between the participants with 2 years and participants with 5 years of nursing experience.

Conclusion: Detecting a medical error in our context is a difficult work; this study represents the best way to evaluate the quality of the act and knowledge of nurses. It allows also creating a procedure to promote safe for patients.

Keywords: physicochemical incompatibility, injectable drugs, medical pharmacology.

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Antituberculosis dosing and galenic considerations in an overweight patient

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Introduction: As recommended by the American Thoracic Society and the Centers for Disease Control , the planned treatment of active tuberculosis is a 6-month regimen consisting of 2 months with isoniazid, rifampin, and pyrazinamide followed by 4 months of isoniazid and rifampin. The use of fixed-dose-combination (FDC) chemotherapies as first-line treatment of tuberculosis has been recommended since 1998 by the WHO and the International Union Against Tuberculosis and Lung Disease because of its easier administration, minimized risk of prescription errors, improved treatment adherence, and possible limited risk of drug-resistant tuberculosis, along with increased safety and efficacy.

Observation: We report on a case of a 36-year-old overweight man with lymph node tuberculosis that presented delayed response to treatment. Following International guidelines, the use of fixed-dose-combination (FDC) chemotherapies as first-line treatment of tuberculosis and prescribing the maximum recommended doses of FDC led to suboptimal dosage of antibiotics in this patient, which may have contributed to this evolution.

Discussion: Whatever the used size descriptor, limiting the range of increase in dosage as currently recommended by guidelines may lead to low drug concentrations in overweight patients. Dosing regimen consideration of antituberculosis treatment in overweight and obese patients is discussed.

P300

Role of rt-pa on bdnf metabolism in stroke

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Aim: Recombinant tissue activator plasminogen (rt-PA) is the only curative approach in the treatment of ischemic stroke. rt-PA cleaves the plasminogen into plasmin that in turn dissolves the clot of the occluded vessels. In addition to this fibrinolytic role, recent in vitro studies have shown that plasmin can also cleave the extracellular pro-BDNF into mature BDNF (mBDNF). This process may be clinically

relevant since the mature form of this neurotrophin play a key role in the induction of post-stroke neuronal plasticity. In this context, our study was designed to investigate the in vivo effect of rt-PA on the cerebral expression of mBDNF in rats with (ischemic animals) or without ischemic stroke (intact animals).
Methods: Experiments were carried out on adult male Wistar rats (290–350 g). Stroke was induced by embolization of the brain with plasmin-resistant carbonized microspheres (50 μm). This model results in multiple infarcts disseminated in the embolized hemisphere. Using western blot analysis, mBDNF expression was measured 1 h after the infusion of rt-PA (0.9 or 10 mg/kg, i.v, 1 h or saline). The infusion was administered in control and ischemic rats in which it was started 30 min after the stroke onset.
Results: In intact rats, rt-PA (0.9 or 10 mg/kg) changed the expression of mBDNF neither in the whole hemisphere nor in the hippocampus. In ischemic rats, no difference in hemispherical mBDNF expression was observed between saline and rt-PA (0.9 mg/kg).
Discussion: Our results suggest that rt-PA-induced cleavage of extracellular pro-BDNF into mBDNF does not occur in vivo or is too low to be detectable by Western blot analysis.

P301

De-escalation of antibacterial therapy in urinary tract infections: a neglected opportunity to save broad-spectrum agents

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Objective: Fluoroquinolones and 3rd-generation cephalosporins are the cornerstone of the empiric treatment of urinary tract infections, and should be replaced by antibacterial agents with narrower antibacterial spectrum as soon as susceptibility tests are available (the so-called de-escalation). Our objectives were to assess the frequency of de-escalation when treating inpatients for urinary tract infections, and frequency of conditions justifying not to de-escalate antibacterial therapy.
Methods: Retrospective study of adult inpatients that were empirically treated for urinary tract infection due to *Escherichia coli* during 2010 in Nantes University Hospital. Strains had to be susceptible to at least one of the following antibacterial agents: amoxicillin, co-amoxiclav, cotrimoxazole. De-escalation was defined as the replacement of the empiric therapy by an antibiotic with a narrower antibacterial spectrum, according to the susceptibility test.
Results: Eighty patients were included (mean age, 70 ± 22 years, females, $n = 62$). Diagnosis was: cystitis, $n = 10$; pyelonephritis, $n = 47$; prostatitis, $n = 13$; unspecified urinary tract infection, $n = 10$. De-escalation was prescribed in 31 patients (39%). Initial treatment was switched to amoxicillin ($n = 21$), co-amoxiclav ($n = 2$) or cotrimoxazole ($n = 8$). Thirteen conditions justifying not to de-escalate antibacterial therapy were detected in 11 patients: shock, $n = 5$; renal abscess, $n = 1$; obstructive uropathy, $n = 4$; bacterial resistance or clinical contra-indication to both cotrimoxazole and beta-lactams, $n = 3$. Hence, de-escalation could have been prescribed in 38 patients for whom it has not been done (38/49, 78%). Finally, de-escalation was prescribed in 31 patients among 69 (45%) for whom it was possible both from a bacteriological and a clinical point of view.
Discussion: Because fluoroquinolones and 3rd-generation cephalosporins select multiple bacterial resistances, usage of both classes should be restricted. As susceptibility tests are available in the majority of cases of urinary tract infection, de-escalation should be systematically considered in these infections. However, de-escalation is under-prescribed in our institution, and should be actively implemented.

P302

No expression of an alternative CD20 transcript variant in B cells from patients with rheumatoid arthritis

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Introduction: Targeting B cells is an effective therapy in rheumatoid arthritis (RA). Rituximab (RTX) is a chimeric monoclonal antibody directed against the membrane CD20 protein present on B cells. Recently, a spliced mRNA transcript of CD20 (ΔCD20) has been identified in B cell lines from patients with lymphoma and leukaemia. This transcript is coding for a non anchored membrane protein and its expression is associated with resistance to RTX in patients with haematological malignancies.
Objective: To determine whether ΔCD20 is expressed by circulating B cells from patients with RA and whether it could be a factor for non response to RTX therapy.
Patients and methods: Twenty-three RA patients (17 F, age (mean ± SEM): 60.1 ± 2.7 years; disease duration: 13.3 ± 1.7 years, positive rheumatoid factors: 19/23; positive anti- CCP antibodies: 19/23) and 20 healthy controls (15 F, age: 59.6 ± 2.5 years) were evaluated. Patients were under DMARDs, low corticosteroids or anti TNFa agents but none received or had received RTX. CD20 mRNA expression study was performed using RT-PCR assay allowing first to discriminate full length CD20 (membrane CD20) from ΔCD20 transcripts. A more sensitive RT-PCR assay, using a specific primer spanning the splice fusion area was then used to detect specifically only the ΔCD20 transcript.
Results: RA patients had mild active disease (DAS28 score: 3.3 ± 0.3; CRP levels: 6.8 ± 1.9 mg/L). Number of circulating B cells per μL was not different between RA patients and controls (mean ± SEM, range: 184 ± 22, 18–437 vs. 211 ± 27, 63–408, respectively). Among all the 23 RA samples, although full length CD20 expression was always detected, we were unable to detect ΔCD20, even with the more sensitive RT-PCR assay permitting to identify the spliced transcript form.
Conclusion: The present study showed that, on the contrary of leukemic or lymphoma B cells, RA B-cells do not express ΔCD20, suggesting that this transcript may be a molecular marker of malignancies rather than of autoimmune diseases like RA. Study of RTX-non responders or -escaping RA patients may be relevant to know if ΔCD20 expression may be detected under the pressure of RTX therapy.

P303

Increased visceral adiposity during anti TNFa treatment for inflammatory rheumatic disease is associated with various changes on serum adipokines: a 2 year prospective study

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Introduction: TNFa blocking agents are effective in rheumatoid arthritis (RA) or ankylosing spondylitis (AS). Adipokines are proteins produced by the adipose tissue and may influence the inflammatory response.
Objective: To evaluate the long term effects of anti TNFa treatment on body composition and the changes on the serum levels of adipokines and ghrelin.
Patients and methods: Twenty patients under anti TNFa (6F) were evaluated (12 AS and 8 RA). Body weight, body mass index (BMI), serum levels of adipokines (leptin, adiponectin, resistin) and ghrelin were measured at baseline and then at months (M) 1, 3, 6, 12, 18 and 24. Body composition was evaluated at baseline and then at M6, 12 and 24. Total and regional body fat and lean masses were measured by total body DEXA (Lunar iDXA). Fat distribution was evaluated as the relative proportion of fat tissue in the android (central) and the gynoid (hip and thigh) regions.
Results: there was a slight but significant increase in body weight (+ 1.35 kg) and BMI (+ 0.55 kg/cm^2) ($P < 0.005$). Although fluctuating, leptin and resistin levels did not significantly change. By contrast, adiponectin increased at M1, and then decreased slowly and reached lower values at M24 compared to baseline (baseline: 12.1 ± 4.8; M24: 11.4 ± 5.4 mg/mL; $P = 0.01$). We also observed a rapid decline in ghrelin which remained low until the end of the study (baseline: 1256 ± 387; M24: 1117 ± 429 pg/mL; $P = 0.035$). Lean mass and fat mass in the gynoid region did not change while there was a significant increase at M24 in adiposity (+ 1.36%; NS), in total fat mass (+ 2.4 kg; $P = 0.003$) and in fat tissue located in the android region (+ 366 g; $P = 0.025$).
Conclusion: long term administration of TNFa blocking agents was associated with gain of body weight and BMI. Leptin and resistin levels fluctuated while ghrelin decreased. However, TNFa blockade is associated with a modest gain in total fat and especially fat in the android region. These changes, together with the decrease in adiponectin serum levels, could negatively influence the cardiovascular risk of these patients.

P304

Are there factors modifying the expression of enzymes of drug metabolism in obese subjects?

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Objective: To describe, in morbid obese subjects, the jejunal expression of efflux transporters and metabolizing enzymes, that the intestinal microbiota and the low-grade inflammation may change.
Methods: The subjects were non-diabetic obese patients (BMI > 35 kg/m^2). A fragment of jejunal epithelium located about 40 cm after the gastroduodenal junction and considered as a surgical waste was preserved during gastric bypass surgery and frozen at −80°C. The expressions of genes encoding the transporters: *ABCB1/MDR1*, *ABCC2/MRP2*, and *ABCC3/MRP3*; in addition to the enzymes: *CYP3A4* and *UGT2B7* were analyzed by qRT-PCR. Patients were genotyped for the ABCB1 C3435T polymorphism.
Results: The intestinal epithelium of 28 obese subjects (23 women and five men) were analyzed. This population, with a mean age of 40 ± 9.9 years, had a mean BMI of 44.6 ± 5.9 kg/m^2. Of those, 10.7% were smokers, 32.1% were hypertensive and 28.6% had a treated sleep apnea syndrome. No chronic treatment of the patients was known to induce transporters and enzymes expression.
qRT-PCR showed that *ABCB1/MDR1* was the mainly expressed (84.1 ± 48.8) gene, followed by *CYP3A4* (43.2 ± 19.9), *ABCC2/MRP2* (22.7 ± 23.3), *UGT2B7* (14.5 ± 7.4) and *ABCC3/MRP3* (2.0 ± 1.2). A difference between sex in the expression of ABCB1 was at the limit of significativity (136.6 ± 75.3 in men vs. 72.7 ± 33.7 in women, $P = 0.055$). Patients homozygous for the T allele of the gene *ABCB1/MDR1* expressed significantly more *ABCB1/MDR1* than subjects heterozygous CT and homozygous CC (123.2 ± 59.5 vs. 63.5 ± 23.7 and 60.7 ± 22.8 respectively). A correlation between the expression of *ABCB1/MDR1* and weight, fat mass, truncal fat mass, BMI, liver enzymes (AST, ALT, GGT) disappeared after adjusting for sex. Similarly, there was no correlation between the markers of inflammation and the gene expression. There was a strong correlation between the expression of each gene.
Conclusion: In obese subjects, inflammation and body composition do not influence jejunal expression of the genes studied. Carriers of the C allele of the gene *ABCB1/MDR1* encoding P-gp express significantly less *ABCB1/MDR1* gene transcripts than the others.

P305

Sodium lactate in the treatment of severe hyponatremia in cirrhotic patients. Cases report

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Background and goal of the study: Hyponatremia, which remains difficult to treat, is one of the main predictive factors for a poor prognosis in cirrhotic patients. Serum lactate infusion has been demonstrated to be efficient on free water

elimination after coronary artery bypass grafting. We report two cases of cirrhotic patients with ascitis and severe spontaneous hyponatremia corrected by sodium lactate infusion.
Patients: Two women (aged 49 and 47 years) suffering from alcoholic cirrhosis were admitted for hyponatremia lower than 130 mM resistant to fluid restriction. In the first case, hyponatremia was associated with renal failure due to hepatorenal syndrome resistant to terlipressine. Molar serum lactate infusion (5 mL/kg/day for 4–5 days) was responsible for an increase of natremia above 135 mM. The natremia mean daily increase (4.9 ± 3.4 mM) remained below the cut-off of 12 mM recommended to prevent the risk of central pontine myelolysis. Renal function normalized in case 1. Bicarbonates increased due to cellular lactate metabolism, explaining at least in large part, a hypokalemia requiring daily potassium infusion. Hormone dosages completed in case 2, showed a decrease of renin from 2177 to 1226 UI and of aldosterone from 13420 to 283 UI after sodium lactate treatment. After the end of infusion, natremia decreased progressively to reach its initial value in about 4 weeks. The first patient was successfully transplanted in August 2010 after two lactate treatments; the second patient, currently waiting for transplantation, has received two infusion courses.
Discussion: Lactate infusion corrected hypervolemic hyponatremia thanks to mechanisms discussed in this presentation. These mechanisms involve for a large part a water transfer from cells to the vascular sector, restoring volemia and permitting the free water excess to be excreted. Sodium lactate probably also works as an energy supplier improving the potential defect in cellular energy status which could contribute to cellular dysfunction and hemodilution.
Conclusion: Despite its transient effects, sodium lactate may open new routes for the therapeutic approach of hypervolemic hyponatremic cirrhotic patients.

P309

Biotherapies' therapeutic maintenance rate in rheumatoid arthritis in current rheumatology practice

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Objective: To determine the treatment maintenance rates of biotherapy (anti-TNF, abatacept, rituximab, tocilizumab) prescribed from 2004 to 2009 in patients with rheumatoid arthritis (RA) followed in hospital rheumatology department and in rheumatology secondary care. Secondary Objective:
to study the reasons for discontinuation of biotherapy and to identify serious adverse events.
Patients and methods: In this retrospective study of 201 patients, mean age 57.8 ± 8.5 years, we studied the overall therapeutic maintenance rate, the biotherapy treatment maintenance of first, second and third lines and therapeutic maintenance rates compared in first-line treatment with infliximab, etanercept and adalimumab. We identified the number of treatment discontinuation and the reason of them, especially deaths and serious adverse events.
Results: The mean follow-up was 33.5 ± 22.6 months. The treatment sequence is specified for 194 patients who underwent 281 different biotherapies. The overall therapeutic maintenance rates varied from 93% at 6 months to 78% at 5 years. It diminished from the first line to the second and third line therapies. It was comparable in the first line, for infliximab, etanercept and adalimumab. There were a total of 125 discontinuations of biotherapy (44% of prescribed treatments), temporary (91 rotations) or permanent (34). There were 91 rotations of treatment (32% of prescribed treatments), mostly (67%) for lack of efficacy. There were 34 definitive discontinuations of treatment (12% of prescribed treatments), mostly (82%) for intolerance or serious adverse events and mostly in the first 26 months of treatment. The reason for discontinuation was not significantly different between infliximab, etanercept and adalimumab. We have identified one death from heart failure, 10 serious infections (5% of patients) and seven cancers (3.5% of patients).
Conclusion: The overall therapeutic maintenance rates are high, but with some decrease during the rotation of biotherapy. Serious adverse events are occurring relatively early in the biotherapy with quite high rate of serious infections and cancers.

P310

Can General practitioners 'deprescribe' drugs? Results of a preliminary prospective study

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Objective: The aim of drug 'deprescribing' is to take out drugs which are not necessary or potentially dangerous a patient. The aim of this prospective study was to determine if deprescribing drugs was really possible by general practitioners (GPs), what were the main deprescribed drugs, and if there was behavioural brakes to deprescription.
Method: A total of 100 GPs were contacted by phone and by mail. They were informed about drug deprescribing and they were requested to participate to an investigation about drug deprescribing. The investigation file had two parts: a first part about the activity of the GPs (age, conditions of activity) and a second part about each drug deprescribed during the 3 days of the study: pharmacologic class, reasons and brakes for deprescribing, and final result (real deprescribing or not).
Results: 47/100 GPs gave their agreement and sent one or more name of deprescribed drug. During the 3 days of the study, GPs wished to deprescribe 314 drugs from 249 prescriptions (6.7 drugs per GP) and deprescribing was really successful for 65.5% of these drugs. The main reasons for deprescribing drugs were the disappearance of the drug's indication (16.2%), drugs excess (15.7%) and the occurrence of an adverse drug event (13.4%). Psychotropic drugs were the main group of drugs that the GP wished to deprescribe (27%), but the rate of succes for deprescribing was the lowest (32.9%). The main behaviour brakes for deprescribing were the rejection from the patients to take out drugs (43.5%). There was a link between the age of the GPs and the ratio of real deprescribing/wish for deprescribing ($P < 0.001$). Amonf the GPs who treated the most important number of patients by day, the wish for deprescribing were the lowest ($P < 0.01$), but the ratio of real deprescribing/wish for deprescribing was higher.
Discussion: The aim of drug deprescribing is to ameliorate the medical care of patients. This preliminary prospective study shows that GPs can really deprescribe drugs. The success of this action would be higher after an educational approach of the patients because the patients remain an important brake for deprescribing drugs.

P311

Pregabalin beneficial effects on sleep quality or health-related quality of life are poorly correlated with reduction on pain intensity after an 8-week treatment course

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Background: Pregabalin (PGB) has been shown to improve sleep quality and Health-related Quality of Life (HRQoL) as well as pain intensity in neuropathic patients.
Objective: to explore the magnitude of the correlations between changes in pain intensity, sleep quality and HRQoL after PGB treatment.
Methods: One hundred and thirty-eight patients suffering from neuropathic pain of any origin and without an adequate response to analgesics received an 8-week treatment course of PGB in an open-labeled fashion. Pain intensity, sleep quality and HRQoL outcomes were evaluated at baseline and at week 8 by means of an 11 points (0–10) numerical rating scale (NRS), the Pittsburgh Sleep Quality Index (PSQI) and the EuroQuol health-state visuoanalogic (EQ-5D VAS) score, respectively.
Results: At week 8, mean PGB dose was 166.7 ± 7.8 mg/day. Pain intensity NRS score, PSQI total score and EQ-5D VAS score were improved by 66.5 ± 1.9%, 40.0 ± 3.6% and 26.4 ± 4.7% (all $P < 0.01$) respectively. Correlations between percent change from baseline in pain NRS score and PSQI total score or EQ-5D VAS scores were $r = 0.36$ ($P < 0.01$, $R^2 = 0.11$) and $r = -0.20$ ($P < 0.02$, $R^2 = 0.05$) respectively. A multivariate logistic regression analysis disclosed that PSQI score change below the median (i.e. a better outcome) was related to higher EQ-5D VAS score change (OR = 2.15 [95%CI = 1.09–4.25]) whereas pain intensity NRS score change below the median was not (1.58 [0.78–3.23]).
Conclusion: In our study PGB-related improvements in sleep quality and HRQoL were marginally related to reductions in pain intensity in neuropathic patients. Improvement in sleep quality was a significant predictor of better HRQoL whereas pain intensity reduction was not.

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Placental transfer of maraviroc in the ex vivo human cotyledon perfusion model and influence of placental ABC transporters expression

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Objective: Our objective was to investigate the placental transfer of Maraviroc, an antiretroviral drug, and determine the potential influence of ABC transporters' expression levels on this transfer.
Material and methods: Placentas were obtained from uncomplicated vaginal or caesarean deliveries. Then, they were perfused for 90 min in an open double circuit with maraviroc at 600 ng/mL. Ten and six placentas were perfused in the maternal-to-fetal or fetal-to-maternal directions, respectively. Maternal and fetal plasma concentrations were determined by High-Performance-Liquid-Chromatography. The fetal transfer rate (FTR) was measured as the ratio of fetal to maternal concentrations and the clearance index (CLI) as the ratio of the FTR of maraviroc to that of antipyrine. ABC transporters expression levels were quantified by quantitative RT-PCR. Immunodetection of placental ABCB1 and ABCG2 was conducted by Western Blotting.
Results: For the 10 placentas perfused in the maternal-to-fetal direction, the mean FTR and mean CLI were 7.0% ± 3.0% and 0.25 ± 0.08, respectively. For the six placentas perfused in the fetal-to-maternal direction, the mean CLI was 0.52 ± 0.24. We showed a significant inverse correlation between maraviroc clearance index and *ABCC2, ABCC10 and ABCC11* placental expression level ($P < 0.05$).
Discussion: In the present work, we report original data on maraviroc placental transfer using an ex vivo human perfusion model. The low clearance index and fetal transfer rate of maraviroc may be explained by its relatively high molecular weight (513 Da) and protein binding capacity (76%). This corresponds to a low maraviroc placental transfer in accordance with Winters *et al.* who found that maraviroc plasma concentrations in newborn macaques were <1% of mothers'. We also showed a significant inverse correlation between maraviroc clearance index and several ABCC transporter placental expression levels. These data would yet need to be completed by clinical studies with paired maternal and cord blood samples, even if placental dual perfusion is recognized as the gold standard to study drug placental transfer.

P313

Prevalence and factors related to somnolence in Parkinson's disease Results from the French DoPaMiP cross-sectional study

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Objective: To assess the prevalence and the factors related to somnolence.

Methods: Four hundred and seventeen idiopathic Parkinson disease (PD) patients and 93 age- and sex-matched patients with chronic disorders other than PD underwent structured standardized clinical examination and completed self-reported questionnaires in this cross-sectional study. PD characteristics were assessed by the Unified PD Rating Scale (UPDRS), parts I (mentation), II (ADL), III (motor) and IV (motor fluctuations). Data about medication was obtained from the clinical records. Dementia was assessed by the Mini mental-state examination (MMSE). Anxious and depressive symptoms were explored by Hospital Anxiety and Depression Scale (HADS). Sleep quality was evaluated by the Pittsburg Sleep Quality Scale. Somnolence was defined based on question #8 as 'troubles staying awake while eating, driving or having social interactions once or more times/week'. Data was analyzed by chi-square test followed by logistic regression.

Results: Somnolence was reported 20% of PD patients vs. 3% of controls ($P < 0.01$). PD Patients with somnolence were less frequently retired/inactive (83% vs. 93%, OR[95%CI]= 0.3[0.2–0.7]. $P < 0.01$), reported hallucinations more frequently (36% vs. 20%, OR = 1.9[1.1–3.4], $P < 0.05$), were more frequently exposed to dopamine agonists (62% vs. 49%, OR = 1.6[1.1–2.8], $P < 0.01$) and were more frequently depressed as shown by HADS depression score > 7 (51% vs. 31%, OR = 1.8[1.1–3.0], $P < 0.01$).

Conclusion: PD patients suffered from somnolence more frequently than a matched sample of patients with other chronic conditions. Being active, presence of hallucinations, exposure to dopamine agonists and depression were related to somnolence in PD.

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Relationship between polypharmacy and frequency of adverse events to antiparkinsonian drugs: a preliminary study

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Objective: To evaluate the relationship between polypharmacy and most frequent AEs to antiparkinsonian drugs (APDs) reported by Parkinson disease patients.

Methods: Two hundred and three non-demented, non-operated Parkinson's disease out-patients were recruited at the Toulouse and Bordeaux Movement Disorder Clinic (mean age 67 years, 62% males, and mean disease duration 9 years, mean UPDRS II+III in ON-state 37). They were systematically questioned about the presence of a predefined list of the most common AEs to APDs. Polypharmacy was arbitrarily defined as consumption of >5 medicaments (i.e. the median in the sample). Medications were coded by ATC.

Results: Most frequent explored AEs were: weight loss, fatigue, leg oedemas, dry mouth, nausea/vomiting (NV), memory complaints, somnolence, impulse-control disorders (ICDs), orthostatic hypotension (OH), and psychotic events (PE). Polypharmacy was present in 68 patients (34%) and was related by multivariated logistic regression to weight loss (OR [95% CI]: 2.44 [1.07–5.56]), dry mouth (2.33 [1.25–4.37]), NV (2.72 [1.32–5.62]) and somnolence (1.87 [1.03–3.42]). On the other hand fatigue, oedemas, memory complaints, ICDs, OH and PE were not related to polypharmacy. Some medications were related to each of these AEs. Weight loss was independently related to H+-pump inhibitors (2.71 [1.00–7.45]) or domperidone (2.68 [1.12–6.41]); oedemas to dopamine agonists (2.42 [1.08–5.41]); dry mouth to amantadine (4.03[1.74–11.12]), opioids (OR could not be calculated) or antidepressants (3.16 [1.47–6.79]); memory complaints to statins (2.85 [1.09–7.45]); ICDs to dopamine agonists (OR could not be calculated) and MAO-B inhibitors (3.76 [1.12–12.67]); OH to lack of MAO-B inhibitors (0.08 [0.01–0.68]) and non-benzodiacepinic hypnotics (3.64 [1.46–9.04]); and PE to amantadine (3.03 [1.18–7.77]). Fatigue, NV and somnolence were not related to any particular drug.

Discussion: Polypharmacy was related to the occurrence of some AEs to APDs in this preliminary study including: weight loss, dry mouth, NV and somnolence. Moreover, the presence of several AEs was also related with particular drugs (leg oedemas, memory complaints, ICDs, OH and PE).

P315

Mid-regional pro-adrenomedullin: a link between nocturnal abnormalities in blood pressure and neurogenic orthostatic hypotension in Parkinson's disease

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Rationale/Objectives: Neurogenic orthostatic hypotension (NOH) is frequently associated with nocturnal abnormalities in blood pressure (NABP) in autonomic failure but the link between both is not understood. The aim of the work was to investigate if adrenomedullin, which is elevated in hypertension and is a potent vasodilator, could be involved in both NABP and NOH.

Methods: Twenty-three patients suffering from Parkinson's disease (61 ± 2 years, 56.5% of males, H&Y stage: 2.1 ± 0.2) and autonomic failure (at least two abnormal responses to classical autonomic testing) were included. Patients with arterial hypertension, obesity, renal failure and diabetes mellitus were excluded. According to the results of a 24 h blood pressure recording and of 60° head-up tilt they were classified as normal or suffering from NABP with or without NOH. Plasma MR-pro-AM, a surrogate for adrenomedullin, was measured by fluoroimmunoassays using TRACE (Time Resolved Amplified Cryptate Emission) technology on the automated system KRYPTOR® analyzer in supine position and at the end of a 10 min head-up tilt.

Results: NOH was significantly more frequent in patients with NABP (78% vs. 20% in patients without NABP). MR-pro-AM plasma levels were positively correlated with night systolic (r^2: 0.269; P: 0.03) and diastolic (r^2: 0.307; P: 0.02) blood pressure and were significantly higher in patients with NABP than in controls (0.54 ± 0.03 vs. 0.39 ± 0.04 nM, $P < 0.05$) as well as in patients with NOH (0.55 ± 0.03 vs. 0.38 ± 0.04 nM in the absence of NOH, $P < 0.001$).

Conclusion: Our data suggest that adrenomedullin plasma levels are elevated in NABP. This could explain the occurrence of NOH is these patients.

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PhenoMAP study evaluation of the prognostic value of monocytes and lymphocytes phenotyping: new predictive markers for threat of preterm labour (PTL)

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Background: Pregnancy and delivery are both complex immune situations, as immune cells need to switch from a tolerant to an inflammatory status to allow delivery. Mechanisms leading to preterm labour PTL are still poorly understood, but are thought to be the same as for term labour. In this context immune cells phenotyping seems to be a good approach to select accurate biological markers of PTL.

Objective: This study was aimed to seek for an immunological signature and to find clinical useful tools to confirm or deny the true onset of PTL.

Study design: We present preliminary results of an ongoing observational prospective study conducted in two centres (Dijon, France; Galway, Ireland) is planned to include 200 patients with a singleton pregnancy hospitalized for PTL between 24 and 34 weeks of amenorrhea. Peripheral blood samples are drawn upon admission (D0), at D1, D2, D4, D6 and D3 after delivery (if delivery occurs before D7). Study was approved by ethics committee and written informed consent obtained from all patients. Leucocytes (monocytes, granulocytes and lymphocytes NK, B, T4, T8, Treg, Th1, Th2, Th17) are characterized by flow cytometry.

Results: Fifteen women have already been included and patients who delivered within 7 days post admission (DP, $n = 4$) were compared to non delivering patients (NDP, $n = 11$). DP shows an increase in proportion of MCP-1-expressing-monocytes (73 ± 2.8% vs. 48.9 ± 19.7% respectively in DP and NDP, $P = 0.0028$). Additional results are as follow (all are for DP an NDP respectively): CCR2 expression in granulocytes (expressed as mean fluorescence intensity, MFI) 299 ± 180 vs. 223.89 ± 156.37; proportion of activated T cells 25 ± 11.34% vs. 16.22 ± 9.16%; and Th17 and Th1 responses 6.6 ± 5.5% vs. 3.2 ± 1.7% and 7.8 ± 5.4% vs. 6.1 ± 2.1% in for Th17 and respectively. So far, effectives are too small to observe statistically significant differences.

Conclusion: PTL seems to be associated with T cells and Th1/Th17 activation. These data needs to be confirmed but might bring new insights on the immunological mechanisms associated with labour onset and help identifying new markers.

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Systemic increase in the proportion of maternal circulating MCP-1 activated CD14+CD16− monocytes is associated with the onset of labor

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Background: Pregnancy and delivery are both complex immune situations, as immune tolerance toward fetus is necessary for successful pregnancy and delivery is an inflammatory process. Monocytes seem to play a pivotal role since MCP-1 that activates and recruits monocytes/macrophages is increased in utero-placental sphere during labor.

Study design: We conducted a prospective observational study between April 2009 and October 2010. Peripheral blood samples were obtained from healthy non-pregnant female volunteers (NP, $n = 6$); third-trimester healthy pregnant patients (HP, $n = 18$); and patients with Preterm premature rupture of membranes (PROM, $n = 46$). Monocyte subpopulations were characterized by flow cytometry with monoclonal antibodies against CD14, CD16, CCR2 and MCP1 in order to investigate the proportion and the level of activation of each monocyte subpopulations.

Results: Whereas pregnancy, either healthy or complicated, didn't influence the relative proportion of each monocyte subset, it was associated with a strong diminution in MCP-1 expressing monocytes (79.5 ± 19.75% vs. 9.3 ± 6.8% and 11.9 ± 8.3, respectively for NP, HP and PROM, $P < 0.05$). Compared to the women delivered by caesarean section prior to the onset of labor, in women with vaginal delivery he proportion of MCP-1 expressing monocyte had return to non-pregnant value, at the time of delivery both in normal (74.4%±16.9) and PPROM pregnancy (68.4%± 35.6,), despite a significant difference in terms at delivery (39.3 ± 1.4 vs. 31.8 ± 3.5 weeks respectively). Finally, CCR-2 (MCP-1 receptor) expression was not modified in monocytes, during labor, but was significantly increased in granulocytes (3646 ± 1080 vs. 7338 ± 2718 in MFI, respectively for non-laboring PROM and laboring PROM, $P < 0.05$).

Conclusion: These results suggest that the down regulation of the level of activation of monocytes, *via* MCP-1 expression might be pivotal for the fetal tolerance within the maternal environment and that the activation of specific monocytes subtypes might be critical for the onset of labor.

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Voriconazole and fluconazole in kidney transplant recipient receiving tacrolimus: a case of drug interaction

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Infection occurs frequently in the organ transplant recipients during the post-transplant period because of immunosuppression. Therefore, prophylactic antimicrobial agents are often used. The azole antifungals, widely prescribed prophylactically, are known to have many drug-drug interactions.

This report presents a case of drug-drug interaction between voriconazole/fluconazole and tacrolimus in a kidney transplant recipient.
A 31-year-old man had received a renal transplantation 4 years earlier. Immunosuppressive agents including mycophenolate 1500 mg/day, tacrolimus 2 mg/day, and prednisolone 10 mg/day were continued. Meanwhile, the tacrolimus level (8–10 ng/mL) was within the therapeutic range (5–15 ng/mL).
Voriconazole 400 mg/day was administered for pulmonary aspergillus. Few days after, the patient present tremor and the tacrolimus level markedly increased (41.8 ng/mL). Tacrolimus was discontinued for 5 days and then the daily dose was reduced to 1 mg. then tacrolimus level were stabilised between 9 and 11 ng/mL. After voriconazole withdraw, the tacrolimus dose was increased to 2 mg/day.
Three months later, the patient was treated by fluconazole 200 mg/mL for skin infection of the lower limbs due to microsporum canis. Tacrolimus levels were increased, 26 ng/mL after 3 days and 33.6 ng/mL after 8 days. Tacrolimus was discontinued for 2 days and then the daily dose was reduced to 1 mg/day. Fluconazole was continued for 4 months and the tacrolimus levels were between 9 and 11 ng/mL.
The present drug-drug interaction can be attributed to a strong inhibitory effect on cytochrome P450-3A4 activity by voriconazole and fluconazole. Our observations suggest that hepatic metabolism inhibition of tacrolimus is more important of 21% with voriconazole that with fluconazole.
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P321

Convulsion and Posterior Reversible Encephalopathy Syndrome (PRES) during a nephrotic syndrome treated with tacrolimus and a transient association with a macrolide

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Aim: the posterior reversible encephalopathy syndrome (PRES) aetiology is widely associated with drug overdose. We reported a single case of convulsion and PRES in a 11 years old boy who suffered of a corticosteroid resistant nephrotic syndrome (NS) that needed an immunosuppressive therapy with Tacrolimus [TL] which level was transiently increased by a macrolide antibiotherapy.
Observation: The boy was macrosomic and obese (1.54 m; 72 kg; BMI = 30.4). Few months prior he began an acute NS, which was cortico-resistant. In such cases an immunosuppressive drug is recommended. The anti-calcineurin Tacrolimus [TL] was prescribed, at low dose (8 mg/day, i.e. 0.11 mg/kg/D). The creatinine clearance was initially normal (> 80 mL/min/1.73 m^2) but became degraded when the tacrolimus overdose occurred. On day 7 he received josamycine for an acute otitis. On day 10, he displayed a status epilepticus needing intensive cares. A transient PRES was confirmed by RMI. Associated treatments were: Propranolol [160 mg/D]; Prednisone [60 mg/D]; Esomeprazole [10 mg/D]; calcium 500 mg/D + vitamine D3 400 IU/D; spironolactone [50 mg/D] and a restricted hydration.
Results: Since the TL administration was interrupted and the blood concentration decreased (usual therapeutic range: 8–10 μg/L) from 40 μg/L (D9) to 9.7 (D12). During this period creatinine increased from 76 to 123 μM/L ($N < 110/1.73$ m^2). Tacrolimus was rechallenged on D13 (3 mg, 2 times/D); TL concentration increased dramatically (29.2 at 8 a.m. and 56.6 at 3 p.m.) while an acute renal failure occurred (creatinine increased from 150 (D13) to 178 (D16). Consequently the drug was definitively stopped and the child health rapidly improved (creat. 87 μM/L at D20).
Discussion: Both Tacrolimus and Ciclosporin are extensively metabolized by Cytochromes, especially of the 3A4 subtype (CYP 3A4). The macrolide family, including Josamycine, are powerful inhibitors of CYP3A4 leading to toxic effects. The rechallenge induced a dramatic increase of TL and creatinine. The recovery occurred in 2 weeks, except the renal function due to NS. We emphasize the early toxic effect occurred 2 days after macrolide addition. The TL overdose triggered convulsions, PRES and an acute pulmonary oedema. The drug re-introduction, even at low dose, induced dramatic toxic effects.

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Base excess is an accurate predictor of elevated lactate in ED septic patients

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Objective: Prior studies showed that lactate is a useful marker in sepsis. However, lactate is often not routinely drawn or rapidly available in the emergency department (ED). The study aimed to determine if base excess (BE), widely and rapidly available in the ED, could be used as a surrogate marker for elevated lactate in ED septic patients.
Methods: This was a prospective and observational cohort study. From March 2009 to March 2010, consecutive patients 18 years or older who presented to the ED with a suspected severe sepsis were enrolled in the study. Lactate and BE measurements were performed. We defined, a priori, a clinically significant lactate to be >3 mM and BE less than −4 mM.
Results: A total of 224 patients were enrolled in the study. The average BE was −4.5 mM (SD, 4.9) and the average lactate was 3.5 mM (SD, 2.9). The sensitivity of a BE less than −4 mM in predicting elevated lactate >3 mM was 91.1% (95% confidence interval, 85.5–96.6%) and the specificity was 88.6% (95% confidence interval, 83.0–94.2%). The area under the curve was 0.95.
Discussion: Base excess is an accurate marker for the prediction of elevated lactate in the ED. The measurement of BE, obtained in a few minutes in the ED, provides a secure and quick method, similar to the electrocardiogram at triage for patients with chest pain, to determine the patients with sepsis who need an early aggressive resuscitation.

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Evolution under antithrombotic therapy of patients with chronic obstructive pulmonary disease and venous thromboembolism, according to their initial clinical presentation

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Background: We recently found that patients with chronic obstructive pulmonary disease (COPD) present more frequently with pulmonary embolism (PE) than deep venous thrombosis (DVT)[1]. They also experiment more frequently mortality, bleeding or first venous thromboembolism (VTE) recurrences as PE than non-COPD patients. The aim of this study was to compare the evolution under antithrombotic therapy of patients with COPD and acute VTE, according to their initial clinical presentation (PE or DVT), in the RIETE registry.
Methods: In a prospective cohort study of COPD and VTE patients, the RIETE registry, demographic data, PE and DVT characteristics, and known risk factors were recorded in addition to events (death, bleeding, VTE recurrence) occurring within a 3-month follow-up. Cox regression models were performed to calculate adjusted hazard ratios (HR) and 95% confidence intervals (CI) for initial VTE presentation as a potential predictor for overall death, fatal PE, first recurrent VTE as PE and major bleeding in COPD patients whom require antithrombotic therapy for VTE.
Results: By June 2011, 4036 (11%) of the 37 090 patients included in the RIETE registry had COPD (67% men, median age: 75 years). The initial VTE presentation was PE in 2452 (60%). Under treatment, 3-month cumulative incidence of overall death (including fatal PE), first recurrent VTE as PE and major bleeding were: 11% (Fatal PE: 2%), 2% and 2.5%, respectively. During the 3 month follow-up period, risk of fatal PE (HR = 8.21, 95%CI: 3.57–18.87), recurrent VTE as PE (3.87, 2.31–6.49) and death (1.46, 1.2–1.8) were significantly higher in COPD patients presenting with PE than in COPD patients presenting with DVT. There was a trend towards an increased risk of major bleeding (1.47, 0.96–2.24).
Conclusions: During the first 3 months of VTE treatment, the risk of death, fatal PE and reccurent VTE as PE were significantly increased for COPD patients presenting with PE compared to COPD patients presenting with DVT.
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P324

May specific allergen tolerance induction (immunotherapy) replace allergen avoidance as treatment of food allergy?

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Introduction: Food allergy and severe anaphylactic reactions prevalence in children and adolescents increase in western countries. The treatment of food allergy by immunotherapy was reported as an alternative to dietary allergen avoidance in children.
Objective: We systematically reviewed and assessed whether specific oral tolerance induction (SOTI) or intravenous rush are associated with an increased threshold dose for allergic symptoms and with a reduced risk of severe reactions after inadvertent ingestion, in children with food allergies.
Methods: We performed a PubMed literature search from 1992 to November 2011. Two reviewers separately verified the inclusion criteria of observational studies and randomized clinical trials relevant for the objective of the review. The data extracted in the patient and control groups were samples size, age, gender, measurement of the dose of allergen challenge by methods of double-blind, placebo-controlled food challenge (DBPCFC) before and after tolerance allergen induction and statistical analysis results.
Results: From 540 citations, 29 articles concerned SOTI or intravenous rush, 20 observational studies with case-control design and nine randomised control trials met the inclusion criteria for the different types of allergy: 11 in cow's milk (CM), six in hen's egg (HE), eight in peanut (P), two in nut tree (NT), two in fruits. According to the allergen type, five articles showed a significantly increased dose of CM at DBPCFC compared with the control group, four in HE allergy, two in P allergy, one in NT allergy. No severe allergic effects were reported during allergen tolerance immunotherapy in all but one study. Half studies reported a significant reduced risk of severe allergic reaction and a better quality of life in children and their family.
Discussion: Despite the heterogeneity of protocols and the kind of allergen administration, specific allergen immunotherapy in food allergy could be considered as a way of food acquired tolerance induction. Data concerning the long term efficacy of acquired tolerance were not yet reported. The allergen maintenance doses and their duration to avoid the risk of severe allergic reactions during immunotherapy should be further defined.

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Evaluation of the management of asthma: Multicenter study

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Objective: Asthma remains a public health problem in Morocco. The implementation of a standardized management of asthma can improve the quality of life of patients. The objective of this study is to determine how to care about patients affected with asthma.

Patients and methods: This is a prospective observational multicenter study involving asthma diagnosed for more than 3 months, seen in consultation in three respiratory centers of Rabat University and started in September 2010.

Results: Among 3150 consultants, 220 patients with asthma were compiled including 129 patients (58.7%) in the dry season (June-October). All aged between 17 and 64 years with a female predominance (77.6%). Asthma was well controlled in 43% and not well controlled in 41.4%. Spirometry was performed in 31 cases of which 17.6% was reversible. The obstructive syndrome was noted in nine patients. From the therapeutic side, the association beta-2- adrenergic agonists/glycocorticosteroids was used in 37.6% of cases. Inhaled glycocorticosteroids alone were used in the treatment of substance in 44% of cases of mild to moderate persistent asthma. The use of short-acting inhaled beta-2- adrenergic agonists way was advised in all patients for the prevention of exacerbations, regardless of the level of control and long-term treatment.

Adjuvant therapy was prescribed in cases of associated pathology, as the main risk factors at the same time triggering and aggravating the disease, including H1 antihistamines in case of allergic rhinitis in 42 cases (19%), and antibiotics in case of bronchial infection in 33 cases (15%).

Discussion: Despite the existence of effective therapies against asthma, many patients with asthma (74.8%) remain symptomatic.

The Beta-2-adrenergics occupy a major part of the consumption of antiasthma. Inhaled glucocorticoids alone is the treatment of asthma symptoms that meet the various recommendations advocating a glucocorticoidtherapy as soon as possible. The use of association provides great ease of use but they are indicated in second intention.

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Optimization of drug prescription in the elderly: an exploratory study

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Introduction: Polypharmacy and inappropriate medication use is frequent in elderly patients. The role of university hospital is to promote a safe and rational use of drugs. The aim of our study was to analyse the drug prescription changes during a hospitalization in a post emergency Unit and to focus on the ambulatory management after discharge.

Methods: This prospective study included all hospitalized patients over 65 years old and receiving at least one of the following drug classes: antihypertensive, oral hypoglycemic, oral anticoagulant and hypolipidemic drugs. The inclusion was performed during a 1-month period (August 2011). For each patient, total number of drugs taken, before and at the end of hospitalization, was assessed. Any modification in drug regimen (drug added or stopped, dosage modification) was recorded. Reasons for drug regimen modifications, such as underuse, misuse, inappropriate or adverse drug reactions (ADRs), were evaluated. One month after discharge, general practitioners were interviewed regarding post-discharge drug modifications. An interview was also planned 3 months after discharge.

Results: Sixty-one patients (mean age: 84.7 years, sex ratio (M/F): 2/3) were included. When admitted to hospital, 90% of patients were treated by antihypertensive, 34% by hypolipidemic, 26% by oral anticoagulant and 16.4% by oral hypoglycemic drugs. Number of drugs slightly decreased after hospital discharge (from 6.6 to 5.9 daily). During hospitalization, ACE inhibitors were the main added drugs whereas calcium-channel blockers and thiazide diuretics, the most frequently stopped. Statins were stopped in 83% of patients and oral hypoglycemic in 67%. Reasons for drug regimen modification were mainly inappropriate treatment (49%) or ADRs (34%). Up to now, follow-up at 1 month was obtained for 38 patients. Modifications in drug regimen were observed in 47% of these patients. A re-hospitalisation was reported in six patients.

Conclusion: These results suggest that hospitalization can improve drug prescription in elderly. However, almost half of patient experienced a drug regimen modification 1 month after discharge. Finally, the study also underlines the important role of multidisciplinary intervention on rational and safe use of drugs.

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5-FU-induced neurotoxicity in two cancer patients with profound DPD deficiency syndrome

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Introduction: 5-Fluorouracil (5-FU) is a mainstay for treating various solid tumors in adults. The intensity of 5-FU-induced toxicities can dramatically increase to become life-threatening in patients with dihydropyrimidine dehydrogenase (DPD) deficiency. This pharmacogenetic syndrome results in a partial or complete loss of ability to detoxify fluoropyrimidine derivatives in the liver. We report two cases of sudden drug-induced neurotoxicities that occurred in patients undergoing 5-FU-based chemotherapy.

Case reports: Patient 1 was a 65-year-old woman treated for sigmoid carcinoma. Eleven days after 5-FU administration, she presented mucosities, several skin toxicities, and biological signs of haematologic toxicity. Neurological disorders gradually happened, such as drowsiness, confusion and dysarthria. Electroencephalogram revealed diffuse cerebral suffering without any epileptic discharge. Brain CT scan, MRI and cerebrospinal fluid analysis were normal. Conservative treatment, granulocyte growth factors and parenteral nutrition were undertaken. Neurological disorders decreased after several weeks, except for a persistent frontal lobe syndrome.

Patient 2 was a 55-year-old man treated for head and neck cancer. Five days after 5-FU infusion, he presented convulsive seizures and persisting loss of consciousness. Biological analysis, brain CT scan and cerebrospinal fluid analysis were normal. Electroencephalogram showed slow and unresponsive brain activity, suggesting metabolic encephalopathy. In intensive care unit, he developed neutropenia and sepsis. With probabilistic antibiotherapy, growth factor administration and supportive treatment, he fully recovered from both his neurological and hematological toxicities after 7 days.

Discussion: In these two cases, central nervous system disorders occurred after the first course of standard 5-FU-containing regimen. None of these patients had any previous neurological disorder history. It was assumed that overexposure to 5-FU could explain the severe toxicities encountered.

We retrospectively evaluated the DPD activity of these patients with a phenotypic investigation. Evaluation of the uracil-to-di-hydrouracil (U/UH2) ratio in plasma revealed a profound DPD deficiency syndrome in both patients. In these patients, 5-FU standard dosage administration led to strong overexposure, responsible for the severe toxicities observed, including the neurological features. Prospective screening of DPD deficiency and development of DPD-based dose tailoring strategies could prevent such side effects in the future.

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Heterogeneity of pholcodine dosing recommendations in paediatric population according to the French drug compendium specifications

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Background: Pholcodine is a very old drug used for its anti-tussive propriety since nearly 50 years. Actually 17 liquid preparations containing Pholcodine are available in France: two containing pholcodine alone, five associated with chlorpheniramine (anti-histaminic), 10 associated with natural extract (erysinum, biclotymol). Among them, seven are indicated for children from 15 to 50 kg.

We have compared the maximal dosing purposed for each product avaible on the market.

Methods and results: In a strange way, reading of the French drug compendium specifications, each pharmaceutical industry seems to suggest its own reference for maximal dose and propose besides its own weight and age bracket. Unfortunately, for same weight child's, recommended maximal dosage of pholcodine can double or even more. As an exemple, for a child from 15 to 18 kg, maximal dosage do not exceed of 1 mg six per day with Polery®, while for the same child the laboratory marketing Hexapneumine® recommends a maximal posology of 2.5 mg 6 per day, which means more than twice as much.

Conclusion: Revealing incoherence in French drug compendium specifications is not new (see Laroche ML et coll. Fundamental Clin Pharmacol 2007; 21 (suppl 1): 59 (abstract 291)). However, such of discrepancy between recommendations remain staggering, for the same active substance with the same indication for the same population.

What is the 'good' dose of this opiate for child population? Even if it is well tolerated, high doses of pholcodine can induce severe lethargy. Actually, none study is available for this population, while pholcodine is usually prescribed for child cough. For clarification, French drug agency should intercede to achieve both more readily understandable and harmonized recommendations.

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Activation of muscarinic receptors inhibits neurogenic nitric oxide in the corpus cavernosum

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The functional role of cholinergic transmission in erection is still far from being fully elucidated. This work aims to further elucidate the modulatory role of neostigmine on NO in the corpus cavernosum and to highlight whether cholinergic transmission in the penis modulates sildenafil action. The isolated rabbit corpus cavernosum and measurement of intracavernosal pressure in the anesthetized rat model were used. Neostigmine (0.02 mg/kg) inhibited intracavernosal pressure/mean arterial pressure (ICP/MAP). Higher doses (0.06 and 0.4 mg/kg) potentiated ICP/MAP and atropine (1.5 and 10 mg/kg) did the opposite. In vitro, neostigmine (10^{-5} and 10^{-4} M) potentiated cavernosal relaxations and this effect was significantly inhibited by hexamethonium (10^{-4} M) or N^{ω}-propyl-L-arginine (3×10^{-5} M) and partially but significantly reduced in the presence of atropine. Lower dose neostigmine (10^{-7} M), on the other hand, inhibited electrically-induced relaxation over the range of 1–4 Hz, and atropine (10^{-6} M) almost abolished this inhibitory effect as well as N^{G}-nitro-L-arginine (10^{-5} M). It was also significantly reduced by selective nNOS inhibitor N^{ω}-propyl-L-arginine (3×10^{-5} M). Nicotine (10^{-4} M) significantly potentiated electrically-induced relaxations amounting to $84.625 \pm 8.06\%$ at 1 Hz and potentiated the effect of sildenafil synergistically. Hexamthonium did the opposite. The potentiatory effect of sildenafil on erection was significantly reduced in the presence of low dose neostigmine both in-vivo and in-vivo. This study provides evidence that muscarinic receptors may modulate NO synthesis in nitrergic nerves by inhibiting nNOS and high level of cholinergic stimulation may activate nicotinic receptors to promote erection probably by potentiating NO synthesis in nitrergic nerves.

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Inhibitory potential of omega-3 fatty and fenugreek terpenenes on key knzymes of carbohydrate-digestion and hypertension in diabetes rats

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Background: Diabetes is a serious health problem and a source of risk for numerous severe complications such as obesity and hypertension. Treatment of diabetes and its related diseases can be achieved by inhibiting key digestives enzymes-related to starch digestion secreted by pancreas.

Methods: The formulation omega-3 with fenugreek terpenenes was administrated to surviving diabetic rats. The inhibitory effects of this oil on rat pancreas α-amylase and maltase and plasma angiotensin-converting enzyme (ACE) were determined.

Results: The findings revealed that administration of formulation omega-3 with fenugreek terpenenes (Om3/terp) considerably inhibited key enzymes-related to diabetes such as α-amylase activity by 46% and 52% and maltase activity by 37% and 35% respectively in pancreas and plasma. Moreover, the findings revealed that this supplement helped protect the β-Cells of the rats from death and damage. Interestingly, the formulation Om3/terp modulated key enzyme related to hypertension such as ACE by 37% in plasma and kidney. Moreover administration of fenugreek essential oil to surviving diabetic rats improved starch and glucose oral tolerance additively. Furthermore, the Om3/terp also decreased significantly the glucose, triglyceride (TG) and total-cholesterol (TC) and LDL-cholesterol (LDL-C) rates in the plasma and liver of diabetic rats and increased the HDL-Cholesterol (HDL-Ch) level, which helped maintain the homeostasis of blood lipid.

Conclusion: Overall, the findings of the current study indicate that this formulation Om3/terp exhibit attractive properties and can, therefore, be considered for future application in the development of anti-diabetic, anti-hypertensive and hypolipidemic foods.

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ABC drug transporter and nuclear receptor expression in human cytotrophoblasts: influence of cell differentiation and induction by glucocorticoids

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ABC transporters on the human placenta play a major role in protecting the fetus against potential toxic drugs. Dexamethasone (DEXA), a glucocorticoid (GC), has been shown to induce ABCB1 expression in enterocytes and hepatocytes. However, no data exists in placental cells neither for DEXA nor for betamethasone (BETA) and prednisone (PRED), while these GCs are used during pregnancy. We investigated the modulation of placental ABC transporter and nuclear receptor expression by these drugs.

Placentas from normal full-term pregnancies were obtained and cytotrophoblasts isolated. We first assessed the influence of cell differentiation on transporter and nuclear receptor gene expression by taking samples of cytotrophoblasts after 24, 48 and 72 h of cell culture ($n = 7$ placentas). Then, incubations were conducted with DEXA, BETA and PRED vs. no drug for 24 h ($n = 6$). mRNA expression was quantified by qRT-PCR.

No influence of syncytialisation was observed, except for *ABCB1*, *ABCC2* and *ABCC5* expression between t = 24 and 48 h ($P < 0.05$). DEXA (50, 200 nM, 1 μM) and BETA (20, 100, 400 nM) significantly induced *ABCB1* gene expression by around 4-fold (3.54 ± 1.79, 3.96 ± 2.49 and 3.82 ± 1.82, $P < 10^{-2}$ for DEXA and 3.42 ± 1.59, 3.97 ± 2.83, 4.16 ± 2.64, $P < 10^{-3}$ for BETA, respectively). However, BETA decreased *GR* mRNA amount by 4–22% (0.96 ± 0.20, 0.78 ± 0.13 and 0.84 ± 0.06, respectively; $P < 10^{-2}$). Conversely, PRED showed no effect on transporter or receptor expression.

As a conclusion, we showed that DEXA and BETA, which are in particular administered to women at risk of preterm birth to prevent hyaline membrane disease, can induce ABCB1 expression in the human placental barrier. Given ABCB1 apical localization, its induction may potentiate the efflux of its substrates when co-administered with GCs, entailing variable drug exposure for the fetus. This altered transfer may be protective for the fetus, notably when the mother needs to receive anticancer drugs. However, it may conversely be deleterious in particular cases in which fetal exposure is wanted, like in the prevention of HIV mother-to-child transmission.

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Cystic fibrosis related bone disease: F508del CFTR reduces the rate of bone formation and osteoprotegerin production

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Cystic fibrosis (CF) is an autosomal recessive disorder caused by mutations of the cystic fibrosis transmembrane conductance regulator (CFTR), a cAMP-dependent anion channel expressed mostly in epithelia. Low bone mass affects children and young adults with CF and is associated with significant morbidity due to fractures and decreased lung function. To date, the effect of mutations in CFTR (specifically the most frequent F508del allele) in bone cell metabolism remains unknown. We recently reported the expression of CFTR mRNA and protein in primary human osteoblasts and showed that reduction in CFTR-dependent chloride activity affects the production of osteoprotegerin (OPG) and prostaglandin E2, two key regulators of bone formation/resorption process (Le Heron L et al. 2010). Here, we report both a defective CFTR-mediated Cl^-channel activity and a severe deficit of OPG production by cultured osteoblasts isolated from a 25-yr-old CF patient with the F508del/G542X mutation in CFTR. A total absence of CFTR-dependent (Cl_{CFTR}) chloride response in F508del-CFTR osteoblasts was observed compared to normal osteoblasts. Interestingly, we found that both the basal and stimulated (TNF-α, 20 ng/mL, 4 h) OPG protein released by F508del-CFTR osteoblasts was considerably reduced (8–10%) compared to normal osteoblasts.

To evaluate whether the severe osteopenia in CF is directly linked to the F508del mutation, we used quantitative computed tomography (microCT) and bone histomorphometry in adult F508del-CFTR homozygous mice ($cftr^{tm1Eur}$ in a FVB genetic background) compared to wtCFTR littermate controls. Bone mineral density (BMD) was measured by DEXA in groups of F508del and WtCFTR 14-week-old gender-matched mice. Smaller bones with decreased BMD, cortical bone thinning and altered trabecular bone architecture was found in F508del CFTR mice compared to wtCFTR mice. Dual tetracycline and calcein in vivo labeling showed lower bone formation rate in both F508del male and female mice compared to wtCFTR mice. Our results showing a defective CFTR-mediated chloride activity, a marked deficit of OPG production by human F508del-CFTR osteoblasts, and a severe osteopenia associated with reduced bone formation in F508del CFTR mice indicate a possible role of the *Cftr* gene in bone development and CF pathophysiology. (supported by Vaincre la Mucoviscidose and the Region Champagne Ardenne, France).

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Implication of Poly(ADP-ribose) polymerase in the effects of the recombinant tissue plasminogen activator (rt-PA) on the blood-brain barrier after cerebral ischemia in mouse

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Introduction: Thrombolysis with recombinant tissue plasminogen activator (rt-PA) is presently the only pharmacological treatment approved for patients suffering from ischemic stroke. However, rt-PA exerts vascular toxicity which contributes to an increased risk of hemorrhagic transformations. The aim of this study was to determine if the poly(ADP-ribose) polymerase (PARP) is implicated in the vascular toxicity of rt-PA, especially on interendothelial jonctions of the blood-brain barrier (BBB).

Methods: Permanent focal cerebral ischemia was performed in male Swiss mice (anesthetized with ketamine 50 mg/kg and xylazine 6 mg/kg) by endovascular occlusion of the left middle cerebral artery. Six hours after the onset of ischemia, mice were given an intravenous perfusion of rt-PA (10 mg/kg). PJ34 (*N*-(6-oxo-5,6-dihydrophenanthridin-2-yl)-2-(*N*,*N*-diméthylamino)acétamide), a powerful inhibitor of PARP, was administrated twice, immediately and 4 h after ischemia (1 or 3 mg/kg *via* intraperitoneal route).

Twenty-four hours after ischemia, expression of the tight junction proteins claudin-5, occludin and zonula occludens-1 (ZO-1), and of the adherent jonction protein VE-cadherin were studied by western blot.

Results: Twenty-four hours after cerebral ischemia, there was a marked decrease in claudin-5, ZO-1 and VE-cadherin expression (33%, 32% and 57% respectively). The degradation of these proteins was aggravated by rt-PA (25%, 32% and 29% respectively).

At 1 mg/kg, PJ34 increased the expression of claudin-5, ZO-1 and VE-cadherin compared with rt-PA alone. At 3 mg/kg, only ZO-1 and VE-cadherin were enhanced by PJ34.

Expression of occludin was changed neither by ischemia nor by rt-PA.

Conclusion: Our results showed that PARP is implicated in post-ischemic damages of the BBB induced by rt-PA. Thus PARP inhibition would be a promising strategy to associate with rt-PA for the treatment of ischemic stroke.

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Atorvastatin protective effects against deleterious cardiovascular consequences induced by chronic intermittent hypoxia

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Introduction: Obstructive sleep apnea (OSA) characterized by chronic intermittent hypoxia (IH) is an important risk factor for cardiovascular morbidity and mortality. We have previously demonstrated that oxidative stress is involved in the hypertension and the hypersensibility to myocardial infarction induced by IH. Knowing the antioxidant activity of atorvastatin, the aim of this study was to evaluate the beneficial effects of this treatment against deleterious cardiovascular consequences of IH.

Methods: Male Wistar rats were exposed to 14 days of IH (cyclic 21–5% FiO_2, 60 s cycle, 8 h/day) or normoxie(N) and received 10 mg/kg/day atorvastatin or its vehicle by intraperitoneal injection. After 14 days of exposure and treatment, mean arterial blood pressure (MABP) was measured before isolated hearts were submitted to an ischemia reperfusion (I/R) protocol. Oxidative stress was quantified in myocardial tissue by measuring the dihydroethidium (DHE) level, p47-phox expression (the cytosolic protein required for the activation of the NADPH oxidase) and superoxide dismutase (SOD) activity.

Results: MABP was significantly increased by IH exposure (132.7 ± 3.7 mmHg in IH group vs. 119.8 ± 4.8 mmHg in N group). Likewise, chronic IH aggravated myocardial susceptibility to infarction (infarct sizes in percent of ventricles, 53.0 ± 12.7% in IH group vs. 41.0 ± 7.8% in N group) and increased cardiac oxidative stress. These deleterious effects of IH were significantly prevented by atorvastatin treatment, which was able to abolish the increased MABP and infarct size induced by HI. Atorvastatin also prevented the IH-induced increase in DHE level and p47-phox expression.

Conclusion: These results suggest that atorvastatin prevented deleterious cardiovascular consequences of IH. This atorvastatin protective effect might be due to its antioxidative property.

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Activities extract from Trigonella foenum-graecum L. (fenugreek) seed on experimental pulmonary fibrosis

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Objectives: The present work aimed to enlighten the relationship between oxidant-antioxidant balance and inflammatory mechanisms in experimental pulmonary fibrosis.

Methods: Three days after the induction of fibrosis in 20 healthy male albino Wistar rats, weighing 233 ± 35 g, by intratracheally injection of Bleomycin (4 mg/kg), the animals were divided randomly in two groups (10 in each group). The first group treated with fenugreek seed polyphenol extract (FSPE) which was administered orally at a dose of 200 mg/kg/day, approximately equivalent to 6.5 mL/kg/day, by using intragastric intubation. The second group (control) received an equal volume of sterilized distilled water (6.5 mL/kg/day). Two weeks later, blood samples were obtained from rats for Malondialdehyde (MDA) and Total Antioxidant Status (TAS) determination. The severity of inflammation was estimated using the semi quantitative grading system from 0 to 5 in lung sections stained with haematoxylin-eosin (H&E). Statistical analysis values were reported as mean ± standard deviation using One-Way ANOVA test (software SPSS 11.5).

Results: MDA levels in treated group (0.281 ± 0.0537 nmol/mg protein) decrease significantly in comparison with control group (0.434 ± 0.043 nmol/mg protein). In contrast, TAS increased in the serum of treated rats: 0.888 ± 0.0868 mM against 0.345 ± 0.043 mM in control rats. Concerning inflammation, the histopathological changes correlated with biochemical results showed that fenugreek seed have anti-inflammatory effect: In fact, the entire pulmonary parenchyma of untreated group appeared affected by the inflammatory process (Inflammatory Index: 4.70 ± 0.48): lungs from rats showed marked peribronchiolar and interstitial infiltration which inflammatory cells (predominantly mononuclear cells including macrophages and lymphocytes), extensive thickening of interalveolar septa, interstitial oedema, and increase in interstitial cells. However treated group showed a reduction of inflammation (Inflammatory Index: 3.29 ± 0.49): alternating zones of normal and inflammatory/fibrosing lung parenchyma. The process of inflammation became patchy and only little zones are affected by inflammation.

Conclusions: The highly levels of inflammation in control group confirm the idea that an acute inflammation characterizes the initial reaction of Bleomycin in the lung and the decrease of this levels in treat group is a proof that fenugreek seed extract have anti-inflammatory effect.

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Effects of ocimum basilicum L. extracts on lipid metabolism and oxidant status in hyperlipidemic rats

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Background: Ocimum Basilicum L (OBL) has effets on blood pressure and platelet function and is traditionally used in metabolic diseases. To study a possible effect on lipid metabolism, we tested OBL in rats fed a high-fat emulsion (HFE).

Methods: Male Wistar rats fed HFE by gavage were treated with aqueous (A-) and ethanol (E-) OBL extracts 100, 200 or 400 mg/kg, lovastatin 2 mg/kg or Xuezhikang 125 mg/kg for 28 days, then tested for serum and hepatic total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL), liver malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), hepatic index (liver weight/body weight), and whole blood viscosity.

Results: HFE increased TC (1.61 ± 0.09–3.29 ± 0.09 mM), TG (1.37 ± 0.18–2.07 ± 0.38 mM), LDL (0.74 ± 0.03–1.04 ± 0.04 mM) and MDA (11.2 ± 0.92–22.2 ± 11.6 nM), decreased HDL (0.70 ± 0.04–0.54 ± 0.04 mM), SOD (497.6 ± 17.3–440.0 ± 36.0 μ/mL) and GSH-Px (150.3 ± 8.69–117.3 ± 29.5 μ/mL) in the plasma or serum with similar alterations in hepatic concentrations. These alteractions were opposed by OBL extracts in a dose dependent manner. E-OBL was more effective than A-OBL, and at 400 mg/kg/day was at least as active as lovastatin (TC 2.79 ± 0.15 vs. 2.70 ± 0.10; LDL 0.65 ± 0.05 vs. 0.66 ± 0.09; HDL 0.73 ± 0.04 vs. 0.72 ± 0.10; MDA 11.0 ± 1.04 vs. 12.9 ± 2.12; SOD 523.2 ± 24.9 vs. 506.3 ± 13.7) or Xuezhikang. OBL, lovastatin and Xuezhikang also opposed HFE-induced increases in blood or plasma viscosity.

Conclusion: OBL extracts improve lipid profile and antioxidant status of rats fed with high-fat emulsion. Further studies of their effects in atherosclerosis or cardiovascular disease appear warranted.

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Studies on the anti-thrombotic activities and mechanism of action of ocimum basilicum L. in mice and rats

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Objective: Ocimum Basilicum L. (OBL), sweet basil, has been shown to have antiplatelet activities. THe objective of the present study was to complete the investigation of the antithrombotic effects of OBL on coagulation and fibrinolysis.

Methods: Three experimental models in vivo: pulmonary thromboembolism in mice induced by collagen-epinephrine, FeCl3-induced arterial thrombosis and inferior vena cava ligation thrombosis in rats were used to examine the antithrombotic activity of aqueous and alcohol extract of OBL. The concentration or activity of anti-thrombin III (AT-?), protein C (PC), plasminogen (PLG), tissue plasminogen activator (t-PA) and plasminogen activator inhibitor-1 (PAI-1) in plasma were determined by ELISA.

Results: Oral administration of OBL extract significantly increased the survival of mice with experimental pulmonary thrombosis, and significantly decreased the weight of the thrombus induced by ligation of inferior vena cava. AT-3 concentration was decreased, PC concentration was increased; the activity of PLG and t-PA were increased, the activity of PAI-1 was decreased slightly, the t-PA/PAI-1 ratio was increased.

Conclusion: OBL extracts have strong anti-thrombotic activity in experimental models in vivo, by activating the anticoagulant and fibrinolytic systems.

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Effect of cydonia oblonga on experimental thrombosis in rats

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Background: Cydonia Oblonga Miller. (COM) leaf is used in traditional Uyghur medicine for prevention of cardiovascular disease.

Objective: To investigate the effects of COM as an antithrombotic agent.

Methods: Two models of experimental thrombosis: common carotid artery FeCl3-induced thrombus formation and inferior vena cava thrombosis occlusion time were used to evaluate the effects of COM on arterial and venous thrombosis in rats. Low 20 mg/kg, medium 40 mg/kg, high 80 mg/kg doses of COM aqueous extracts were compared with the model group, aspirin 5 mg/kg and Ginkgo 20 mg/kg. The plasma concentrations of thromboxane B2 (TXB2) and 6-keto-prostaglandine F1 alpha (6-keto-PGF1a) were measured.

Results and conclusions: The venous occlusion time (OT) was prolonged and arterial and venous thrombus weights were dose-dependently reduced in COM treated groups compared with the model group ($P < 0.01$). Aspirin and gingko had effects similar to the effects of the medium dose of COM. TXB2 concentrations were dose-dependently decreased and the concentrations of 6-keto-PGF 1a increased in all COM-treated groups and aspirin-treated group compared with the model group ($P < 0.05$). There was a clear association between 6-keto-PGF 1a/TXB2 concentration ratios and arterial or venous thrombus weight for all products, and for occlusion time cith COM but not aspirin or gingko.

COM confirmed in vivo antithrombotic effects that may be related to its traditional use in uyghur medicine to prevent cardiovascular disease.

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A study of the effects of the leaf extract of cydonia oblonga miller. on blood hemostasis, coagulation and fibrinolysis in mice

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Objective: To study the anti-thrombotic effect of Cydonia Oblonga Miller (COM) leaf extracts, which are used for cardiovascular prevention in traditional Uyghur medicine.

Methods: The effects of three doses of COM (20, 40, 80 mg/kg/day), aspirin (5 mg/kg/day) and gingko biloba extracts (20 mg/kg/day) in mice were compared to untreated controls. Products were given orally for 14 days before the experiments. Bleeding time and clotting time were measured in mice using the tail cutting method and glass slide method, respectively. The death rates in pulmonary thrombosis induced by collagen-epinephrine, thrombolysis in vitro and euglobulin lysis time (ELT) were also measured,

Results: Low, medium and high doses of COM leaf extracts dose-dependently prolonged the bleeding time (by 2.17, 2.78 and 3.63 times, respectively, vs. 2.58 for aspirin) and the clotting time (by 1.44, 2.47 and 2.48 times compared to controls, vs. aspirin 1.91 times), and reduced the mortality rates in the pulmonary embolus model (by 27%, 40% and 53%, respectively, compared to 47% for aspirin). All three doses significantly and dose-dependently increased spontaneous thrombolysis (by 45%, 55% and 63%, respectively, compared to 56% for aspirin) and shortened ELT to 71%, 61% and 43% of controls ($P < 0.01$), vs. 43% for aspirin.

Conclusion: The effects of COM on hemostasis, coagulation and fibrinolysis warrant further exploration.

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Pharmacological evaluation of anti-inflammatory and anti-proliferative potential of some marine organisms' extracts and fractions from Mediterranean Tunisian coasts

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Objective: Extracts and fractions from three brown algae of the genus *Cystoseira, Zonaria,* and *Dictyopteris* and from the defensive secretion of the sponge of the genus *Spongia* collected from Mediterranean Tunisian coasts were evaluated for their anti-inflammatory and anti-proliferative activities.

Materials and methods: The anti-inflammatory activity was determined in vivo, using carageenan induced rat paw oedema model (Winter et al, 1962). Then, in vitro, anti-proliferative effect against three human tumour cell lines (A549, MCF7 and HCT15) was investigated using the MTT assay (Marine et al, 2008).

Results: We established that the extracts from three brown algae and from the defensive secretion of the sponge, tested at different doses (50, 100, 200 mg/kg), in comparison to reference drug (Acetylsalicylic-lysine, 300 mg/kg), exhibited, in a dose dependent manner, a significant inhibitory effect on the rat paw edema. The inhibition's percentage of oedema, 3 h after carrageenan injection, ranged from 64% to 86%, whereas the reference drug produced 67% of inhibition. We also established that the fraction F3 from the sponge's defensive secretion showed a

significant anti-proliferative activity against the three tumour cell lines. At concentrations from 50 to 500 µg/mL, this fraction F3 suppressed, dose dependently, the proliferation of the three cell lines. The IC_{50} values ranged from 100 to 250 µg/mL.
Discussion: This study revealed that aqueous extracts and a fraction from marine organisms (brown algae and defensive secretion of the sponge) provide an interesting anti-inflammatory activity associated with a significant anti-proliferative activity. These pharmacological efficacies of brown algae extracts were positively correlated with their total phenol and polysaccharides content. The purification and the determination of chemical structures of compounds of these active extracts and fraction are under investigation.
Reference:
1. Dellai et al, 2010; Ben Aoun et al, 2010; Gupta & Abu-Ghannam, 2011; Martine et al, 2008; Meyers et al, 2010; Winter et al, 1962.

P341
Pharmacological investigation of Captopril® in the rat Bleomycin model
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Aim of study: Recent studies showed that Angiotensin Converting Enzyme Inhibitors family can attenuate lung fibrosis. This study examined the dose-response effect of Captopril in Bleomycin model of experimental pulmonary fibrosis.
Methods: To create an experimental model, Bleomycin was injected intratracheally in single dose (4 mg/kg). After 3 days, male wistar rats (weighing 270 ± 3 g) were divided randomly into three groups. The first experimental group (n = 10) was treated with a Captopril at dose of 25 mg/kg per day, the second experimental group (n = 10) was treated with a Captopril at dose of 50 mg/kg per day and the control group (n = 10) did not received any treatment. After 15 days of orally treatment, the rats were sacrificed and simples of the lungs underwent an immunohistochimical analysis to determine density of TGFβ in several areas of the lung (Bronchiolar Epithelia, Macrophages, Alveolar walls, Lung parenchyma and Peri-bronchial area) by using the semiquantitative grading scale (0 = absence density, 1 = low density, 2 = moderate density and 3 = important density). Data comparison among the studied groups were analysed by one-way ANOVA and Tukey post-hoc comparison tests.
Results: Statistical results showed that TGFβ density decreased in most areas of both treatment groups compared to control and this decrease is most notable in the group receiving the high dose of treatment (50 mg/kg per day), with only exception in Macrophages (results are not significant between the three groups). TGFβ density in Bronchiolar epithelia (ANOVA, F = 13 030, $P \leq 0.0001$), in Alveolar wall (ANOVA, F = 10 886, $P \leq 0.0001$), in Peri-bronchial area (ANOVA, F = 6862, $P \leq 0.004$), in Lung parenchyma (ANOVA, F = 8680, $P \leq 0.0001$), in Macrophages (ANOVA, F = 1427, $P > 0.05$).
Conclusions: This data suggest that Captoril® diminishes the density of TGFβ in the lung of rats injected with Bleomycin and therefore reduces the degree of pulmonary inflammation mainly at dose of 50 mg/kg per day. The results of this work, encourages to evaluate the effect of this drug on the pulmonary fibrosis in combination with the conventionnal treatement.

P344
Evaluation of residual attentional effects of chronic treatment by zolpidem in attentional set shifting test in the rat
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Object: Recent studies have revealed that zolpidem, a benzodiazepine analog and the most prescribed hypnotic drug, increase the risk of driving accidents[1]. The impairment in driving performance may be linked, at least in part, to alteration of the attentional processes. With the aim to compare the influence of acute and chronic administration of zolpidem on attentional processes, we developed a complet behvioral experience in the rat with the attentional set shifting test.
Method: Male Sprague Dawlays rats were used. In a preliminary study (n = 32), we determined the dose of zolpidem (1, 3, 10 mg/kg) and the delay after administration which were compatible with residual effect observation. With this aim, at 1, 3, 5 and 8 h after administration, hypolocomotor effects were recorded in an open-field test coupled with videotracking system and seric concentration of zolpidem were mesured with fluorescence liquid chromatography. Residual effects were then observed with the 3 mg/kg dose and 3 h after administration in two groups of rats (saline or treated, n = 8) trained in the attentional set shifiting test. Residual effects were assessed at J1 after one acute injection and at J7 after seven daily injections of zolpidem. To dissociate residual effect on memory, working memory performances were assessed in the same groups in an Y-maze test.
Results The acute administration of zolpidem induced 3 h after administration a decrease of attentional performances like the flexibility in rats trained in the attentional set shifting test compared to control group ($P < 0.001$). A 'cognitive strain' was also observed because some treated rats stop the test before they have ended all conditions. No impairment was found after the chronic administration. In the Y-maze, no impairment of spontaneous alternation was observed 3 h after administration.
Discussion These data suggested that the initiation of a treatment by zolpidem represent a criticize period where the residual attentional disturbances seem the highest. These data gave some preliminary explanations to the residual impairments observed in human studies on driving performance.
Reference:
1. Bocca et al. Zolpidem and zopiclone impair similarly monotonous driving performance after a single nighttime intake in aged subjects. Psychopharmacology 2010; 214: 699–706.

P345
Inhibition of nociceptive behaviors by chronic administration of a selective serotonin reuptake inhibitor in a mouse model of anxiety/depression
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Context and objectives: There is high comorbidity between depression and chronic pain. Pain is a complaint reported by 60–90% of depressed patients, and chronic pain states are often associated with depression. However, animal models of pain/depression are requested to screen centrally active drugs, but they are notoriously lacking. The first aim of this study was thus to describe pain sensitivity in mouse treated with corticosterone in drinking water. The 'CORT' mice display phenotypic alterations indicative of anxiety/depression (David et al., 2009 Neuron 62:479–493). Then, we determined the effect of chronic administration of the antidepressant fluoxetine, known to reverse anxiety/depressive-like state in the CORT model, on pain relief.
Material and methods: Modification of nociception was assessed by different behavioural pain tests, e.g., the Von Frey hair test for mechanical allodynia and hyperalgesia, the hot/cold plate tests for thermal hyperalgesia and the thermal preference plate test for thermal allodynia. In this last test, animals were free to explore an arena containing identical adjacent platforms, one set to 30°C and the other to 20°C. Effects of fluoxetine treatment were assessed after 4 week of administration in drinking water (18 mg/kg/day).
Results:
We reported modifications of pain sensitivity in CORT mice: Hot hyperalgesia as indicated by a decrease in the latency to lick hind paw (11.6 ± 0.6 vs. 17 ± 1.2 s) in the hot plate test;
Cold allodynia as indicated by a decrease in time spent on the plate set at 20°C in the thermal preference plate test (14.0 ± 5.4% vs. 31.1 ± 5.4% of time).
Chronic fluoxetine reduced both heat hyperalgesia (23.8 ± 5.1 vs. 11.6 ± 0.6 s) and cold allodynia (+ 49.3 ± 22.3 s on 20°C).
Discussion: Results showed some important modifications in nociceptive states for the first time in the CORT model. They suggested that fluoxetine should be active on pain, unveiling a link between mood and nociception in the CORT model. This hypothesis is consistent with previous clinical studies reporting the analgesic efficacy of fluoxetine in pain disorders in depressed patients, making the CORT model a good candidate for translational research.

P346
Study of the antiradical and antioxidant activities of essential oil extracted from seeds of Foeniculum vulgare Mill
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Objective: The objective of this study is to evaluate in vitro antiradical and antioxidant of the dry seeds' essential oil of *Foeniculum vulgare* Mill
Material: The material or the vegetable body selected in the present study is represented by dry seeds' bulbous fennel (*Foeniculum vulgare* Mill.). These latter are bought, in dried form, at an arborist. They are originating in Ain Ouelman, Wilaya of Setif, Algeria.
Methods: The extraction is carried out by water distillation. The antiradical activity of seeds' essential oil of fennel is measured on the basis of the scavenging activities of the stable 1, 1-diphenyl-2-picrylhydrazyl ($DPPH^{\cdot}$) free radical described by Dung *et al.* (2008). Antioxidant activity of fennel seeds' essential oil is determined using the β-cartene bleaching method described by Kulisic *et al.* (2004).
Results: Essential oil obtained is pale yellow color with aromatic odor. We could not recover a big oily quantity; the yield obtained is close to 0.79 ± 0.02%. It is very feeble compared to the yield quoted in the literature. In general, the yield of seeds' essential oil of fennel varies from 2.5 to 6% with an average of 3.5%. The EC_{50} obtained is of 752.65 ± 32.5 µg/mL. The seedss' essential oil of fennel also has show very intersting antioxidant activity (81.74 ± 3.92%) by the β-cartene bleaching method. The antiradical effect of the seeds' essential oil of fennel could be due to the presence of great proportion of the phenolic compounds (Veliioglu et al., 1998).
References:
1. Dung NT, Kim JM, Kang SC. Food and chemical Toxicology 2008;46:3632–3639.
2. Kulsic T *et al.* Food chemistry 2004;85: 633–640.
3. Velioglu YS *et al.* J Agric Food Chem 1998;46: 4113–4117.

P347
Sea urchin embryos as a model organism for studying anti-mitotic agents and multidrug efflux transporters modulators
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Objective: The development of multidrug resistance in cancer cells is a major problem in the chemotherapy. As part of our search for a model organism for studying anti-mitotic agents and efflux transporters modulators, the anti-mitotic activity of three compounds were investigated in sea urchin embryos, in absence and in presence of a specific inhibitor of multidrug resistance associated protein1 (MRP1).
Materials and methods: Adult purple sea urchins (*Strongylocentrotus purpuratus*) kept at the laboratory of marine pharmacology (UCSB), in flow-through 13 ± 2°C seawater and were fed kelp weekly. Gametes were collected and fertilized as described previously (Jacobs and Wilson, 1986). Fertilization success was checked under the microscope. All experiments were conducted at 15°C for approximately 120 min after fertilization. Thyrsiferol, dehydrothyrsiferol and colchicine were used as anti-mitotic agents and MK571 as specific inhibitor of MRP1.
Results: The present study has established that Thyrsiferol, dehydrothyrsiferol and Colchicine, inhibited the first cleavage of *S. purpuratus* embryos in a concentration dependent manner with 50% inhibitory concentration (IC_{50}) occurring at approx-

imately at 85, 280 ng/mL and 70 μg/mL, respectively. These three compounds produced 100% inhibition of the first mitosis without lysis or morphological abnormalities. However, when the efflux transporter MRP1 activity of sea urchin embryos is inhibited with 20 μM of MK571, the effective concentration of the three anti-mitotic agents was decreased. The IC_{50} of Thyrsiferol, dehydrothyrsiferol and Colchicine were reduced from 85 to 55 ng/mL, from 280 to 220 ng/mL and from 70 to 7 μg/mL, respectively.
Discussion: This study revealed the functional activity of efflux transporter, MRP1 in sea urchin embryos, and the pharmacological inhibition of mrp-mediated efflux activity with MK571 sensitizes embryos to the anti-mitotic agents. So, sea urchin embryos constitutes a fantastic model organism for in vitro evaluation and identification of potential anticancer agents and ABC transporters reversal agents.
Reference:
1. De Souza et al, 2010; Epel et al, 2006; Semenova et al, 2006; Hamdoun et al, 2004; Nishioka et al, 2003; Hansen et al, 2003; Jacobs and Wilson, 1986;

P348
The cardioprotective translocator protein ligand 4'-chlorodiazepam decreases cholesterol concentration in mitochondria during ischemia-reperfusion in rats

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Introduction: The translocator protein (TSPO) is located on the external mitochondrial membrane and is associated with the permeability transition pore. In steroidogenic tissues, TSPO regulates the transport of cholesterol across mitochondrial membranes. TSPO is also present in the heart but its function is unknown. We previously demonstrated that 4'-chlorodiazepam (CDZ), a ligand of TSPO, protects the myocardium against ischemia-reperfusion (I/R). Here, we hypothesize that mitochondrial cholesterol variations could play a role in the cardioprotective effect of CDZ during I/R.
Methods: Anesthetized rats underwent 30 min of coronary artery occlusion followed by 15 min, 2 or 24 h of reperfusion. CDZ (10 mg/kg) or its vehicle were administered 10 min before ischemia. At the end of I/R, hearts were removed, mitochondria were isolated and mitochondrial matrix and membranes were separated by centrifugations. Enzymatic activity of markers of mitochondrial matrix and membranes, mitochondrial cholesterol concentrations, lipid peroxidation of the mitochondrial membranes and mitochondrial membrane fluidity were evaluated.
Results: I/R induced a significant increase in mitochondrial cholesterol concentration after 15 min and 24 h reperfusion. This increase mainly occurred in the mitochondrial matrix. I/R also reduced mitochondrial membrane fluidity in the lipid regions and induced a reorganization of the mitochondrial membrane proteins as measured by the fluorescent probes 1,6-diphenyl-1,3,5-hexatriene and hematoporphyrin, respectively. These modifications were associated with the formation of conjugated dienes and of malondialdehyde, two markers of lipid peroxidation. Interestingly, CDZ inhibited the increase in mitochondrial cholesterol concentration after I/R both in the mitochondrial matrix and membranes. CDZ also restored the mitochondrial membrane fluidity of lipid regions without change in the organisation of proteins and decreased lipid peroxidation.
Conclusion: Taken together, these data suggest that the mechanism of action by which CDZ elicits a cardioprotective effect involves a limitation of the mitochondrial accumulation of cholesterol and an inhibition of lipid peroxidation during I/R.
Keywords: Ischemia-reperfusion, myocardium, cholesterol, mitochondria, translocator protein, 4'-chlorodiazepam

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Acute minocycline treatment improves memory function following traumatic brain injury in mice: a 12-week follow-up study

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Objective: Comorbidity of cognitive and stress disorders is a common clinical sequel of traumatic brain injury (TBI). It is essentially determined by the site and severity of the insult, but also by the extent of the ensuing neuroinflammatory response. The present study sought to examine the effects of closed-head TBI on memory function and anxiety in the mouse, in order to further examine the efficacy of an acute anti-inflammatory treatment with minocycline on these post-TBI behavioral parameters.
Materials and methods: The mouse model of closed-head injury by mechanical percussion was applied on anesthetized Swiss mice. This model is known to induce moderate to severe TBI with a pronounced neuroinflammatory response. The treatment protocol included three injections of minocycline (i.p.) at 5 min (90 mg/kg), 3 h and 9 h (45 mg/kg) post-TBI [1]. A novel object recognition test was run from 4 to 12 weeks post-TBI, to evaluate memory function. The Elevated Plus Maze test (EPM) and the Elevated Zero Maze test (EZM) were run at 3 and 7 weeks post-TBI respectively, to assess the levels of post-TBI anxiety.
Results: Our results revealed a significant post-TBI memory deficit at 4 weeks ($P < 0.001$), 9 weeks ($P < 0.001$) up to at least 12 weeks post-TBI ($P < 0.05$). Minocycline was able to efficiently attenuate the memory deficit at all three time points of the test ($P < 0.01$; $P = 0.06$; $P < 0.05$ vs. vehicle). However, neither EPM nor EZM revealed any alteration in post-TBI anxiety levels.
Discussion: Severe closed-head TBI in the mouse induces memory impairment without any alteration in stress behavior. Minocycline is able to attenuate the memory deficit in an effective and lasting manner, highlighting its therapeutic potential in TBI-induced memory dysfunctions.
Reference:
1. Homsi et al., (2010) J Neurotrauma 27:911–921.

P350
Neuroprotective effects of atorvastatine in a model of spontaneous intracerebral ischemia

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Introduction: Spontaneous intracerebral hemorrhage (ICH) are frequent (15% of strokes), and severe (40% survival at 1 year). While the neuroprotective effect of statins in cerebral ischemia is now well recognised, the relationship between statins and HIC are poorly understood.
Objective: The functional effect of preventive therapy by atorvastatine was studied on a model of experimental ICH. Comparison between the differential effect of continuation or discontinuation of atorvastatin therapy at the time of hemorrhage induction was then analysed.
Methods: ICH were induced by an intra-striatal injection of collagenase (0.2 U) in rats randomly assigned to three groups: a group of rats treated with atorvastatin (2 mg/kg/day) for 28 days, a group of control rats treated with vehicle, a group of rats treated with atorvastatin (2 mg/kg/day) for 14 days and then treated with vehicle for 14 days. The comparison of the three groups was based on behavioural tests, brain imaging data and immunohistochemistry data.
Results: Rats treated with atorvastatine significantly had better functional performance 7 days and 14 days after induction of ICH than rats treated with vehicle. Moreover, rats treated with atorvastatine during 28 days have better performance than rats only treated by atorvastatine 14 days before induction of ICH. The volumes of ICH at H24, J7 and J14 tended to be smaller after atorvastatine treatment but these results were not significant. In magnetic resonance spectroscopy, the ratio lactate/creatinine, choline/creatinine, glutamate/creatinine were significantly lower in the atorvastatine groups vs. the vehicle group and in the group treated with atorvastatine during 28 days than in the group treated during 14 days. Fourteen days after ICH, PNN infiltration was not significantly different between the three groups.
Conclusion: Our study demonstrated that administration of atorvastatine as a preventive treatment (similar to chronic treatment) before the induction of cerebral hemorrhage is associated with improved functional recovery and limited inflammatory reactions at the site of ICH, especially at H24. Our study also demonstrated the interest of maintaining treatment with atorvastatin in the first days and weeks of the hemorrhagic stroke.

P351
Metabolic disorders in a rat model of metabolic syndrome and hypertension can be reversed by centrally-acting antihypertensive drugs

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Objective: Cardiovascular and metabolic risk factors in the context of the metabolic syndrome including high blood pressure, obesity and glucose intolerance are are accompanied by alterations in the autonomic nervous system. Moreover, sympathetic hyperactivity might play an important role in the pathophysiology of these alterations.
To investigate the effects of a chronic oral antihypertensive treatment using centrally-acting sympatho-inhibitory drugs on the metabolic and hemodynamic parameters in rats under long-term high-fat/high-salt diet.
Methods: Fifty male adult Wistar rats were maintained under normal (CON, $n = 10$) or high-fat/high-salt diet (HFD, $n = 40$) during 20 weeks. Thereafter, the HFD group were treated with sympathetic nervous system inhibitors that presents differents affinity for the α_2 and I_1 receptors, clonidine (HFD-CLO, 0.1 mg/kg), rilmenidine (HFD-RIL, 1 mg/kg), LNP 599 (HFD-LNP, 10 mg/kg) or vehicle (HFD-CON) during 4 weeks by gavage. Systolic blood pressure was assessed weekly by photoplethysmography. Plasma glucose, triglycerides and cholesterol were determined by enzymatic assays. Visceral and epididymal fat were evaluated.
Results: The groups of animals submitted to HFD and treated with clonidine, rilmenidine and LNP 599 presented a similar reduction in food intake and weight gain. Nevertheless, there was a reduction in the visceral and epididymal fat deposition only in the rilmenidine treated animals. The systolic blood pressure and heart rate was increased in the HFD-CON compared to the CON group, and was reduced by all the treatments. The HFD-CON group also presented an increase in fasting glucose, compared to CON group, that was normalized only in the group treated by rilmenidine and LNP 599. In addition, the HFD-RIL group was able to reduce LDL and total cholesterol.
Discussion: These results show that chronic antihypertensive treatment with centrally-acting drugs induces a reduction of arterial pressure accompanied by an improvement in glucose and lipid parameters in an experimental model of metabolic syndrome in rats.

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Memory improvement by combined pharmacological modulation of 5-HT4 and 5-HT6 receptors

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Efficiency of current treatments for neurodegenerative disease, is under huge debate mainly because it often consists in a single targeted drug, having only symptomatic effects. Time is now to find curative treatments. Alzheimer disease, for instance, is multifactorial and new treatments have to consider other targets than the classical acetylcholine and glutamate systems. Serotonin system and particularly 5-HT4 and 5-HT6 serotonergic receptors (5-HT4R and 5-HT6R) are among potent targets of interest. Both are located in brain structures involved in memory processes. Neurochemical and behavioural studies have showed that activation of 5-HT4R and blockade of 5-HT6R improve memory processes. Accordingly, a therapeutic approach combining a simultaneous modulation of these two receptors could be an interesting and innovative strategy in the treatment of memory disorders associated

with different physiopathological situations. Herein, we investigated in mice the potent interest of associated 5-HT4R activation and 5-HT6R blockade on episodic-like memory and on c-Fos expression, a marker of neuronal activation.
Effects of an acute administration of RS 67333 (1 mg/kg), a 5-HT4R agonist, and/or of SB-271046 (20 mg/kg), a 5-HT6R antagonist on NMRI mice were evaluated in the novel object recognition test in mice. Thereafter, effects of these two ligands on c-Fos expression in the hippocampus were assessed.
5-HT4R activation combined to 5-HT6R blockade improved episodic-like memory performances in mice, more than if each ligand is administered alone. Concerning immunohistochemical studies, c-Fos expression tended to be increased in the CA1 field of hippocampus by the two ligands, and particularly by their co-administration.
Although further experiments are needed (analysis of pharmacologic-induced modulation c-Fos expression in other structures are in current investigation), those findings showed that the co-modulation of 5-HT4R and 5-HT6R improves recognition memory and increases neuronal activation. This combination could represent a novel therapeutic approach in the treatment of Alzheimer's disease.
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A glycogen phosphorylase inhibitor developed as a potential antidiabetic compound

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Introduction: Type 2 diabetes is a metabolic disorder resulting from abnormal insulin secretion from pancreatic b-cells associated with insulin resistance. Hyperglycemia is partly due to excessive hepatic glucose production (gluconeogenesis, glycogenolysis). Glycogenolysis is controlled by glycogen phosphorylase (GP); inhibiting GP could therefore reduce hepatic glucose output. Regulation of GP mainly depends on enzyme phosphorylation, D-glucose concentration and allosteric effectors. Recently, we have synthesized and tested glucose-based molecules that bind to the catalytic site of GP. Some of them, such as the glucose-based spiro-isoxazoline DCG-136, appeared to be potent GP inhibitors in biochemical assays. Thus, the present study was designed to evaluate the effects of DCG-136 on GP activity and glycogenolysis, by in vitro and in vivo experiments.
Methods: DCG-136 was synthesized from peracetylated exo-glucal upon 1,3-dipolar cycloaddition with 2-naphthyl nitrile oxide. This regio-, and stereoselective reaction afforded a cycloadduct (94% yield) which was deacetylated to DCG-136 (78% yield). Activity and site-specific binding of the compound were determined by enzymatic studies. Pharmacological experiments were performed on primary rat and human isolated hepatocytes. The efficacy of DCG-136 (10^{-8} to 10^{-3} M) to induce a reduction in glucagon (100 nM)-stimulated glycogenolysis was determined. In vivo experiments were performed in Zucker *fa/fa* rats by the glucagon challenge test. Animals received DCG-136 by oesophagic intubation 20 min before an acute subcutaneous administration of glucagon (200 mg/kg).
Results: Kinetic measurements showed that DCG-136 was a potent inhibitor of glycogen phosphorylase (K_i = 0.63 mM) and the X-ray structure of the enzyme-ligand complex showed that it binds preferentially at the catalytic site, stabilizing the less active T-state conformation. On isolated rat hepatocytes, DCG-136 induced a significant reduction in glucagon-stimulated glucose output (IC_{50} = 30 mM); on primary human hepatocytes, it had a similar effect (IC_{50} = 20 mM). In Zucker rats, DCG-136 significantly reduced glucagon-induced hyperglycemia at 30 mg/kg ($p < 0.01$).
Conclusion: DCG-136 significantly reduced the hepatic glucose output stimulated by glucagon in vitro and in vivo. So, the glucose-based glycogen phosphorylase inhibitor DCG-136 may be of potential interest as an antihyperglycemic compound.

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Antioxidative/oxidative status of muscular tissue surrounding strontium-substituted bioactive glass implanted in bone of ovariectomised rats

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Background: Bioglass (BG) have been used to repair bone defects. The addition of strontium (Sr) has been found to decrease bone resorption and increase bone formation [1]. During bone defect healing, the muscle surrounding biomaterial may be affected by the reactive oxygen species (ROS). The advantages of BG such as controlled ion release may make strontium-substituted Bioglass (BG-Sr) an effective biomaterial choice for antioxidative activity.
Objectives: Oxidative/antioxidative status of muscular tissue surrounding BG-Sr.
Methods: Wistar rats were divided into five Groups: (I) used as negative control (CNT), after ovariectomy, Groups II, III, IV and V used respectively as positive control (OVX), implanted bone with BG (OVX -BG), BG-Sr (OVX -BG-Sr) and not implanted (OVX- NI). The muscular tissue were used for catalase (CAT) superoxide dismutase (SOD), glutathione peroxidase (GPx), lipid peroxidation (LPO) estimation.
Results: In OVX rats, the activities of CAT, SOD and GPx in skeletal muscle decreased significantly as compared to controls ($P < 0.001$). Further, an increase in MDA levels ($P < 0.001$) was marked. The results showed that after 4 and 7 days, the activities of CAT, SOD and GPx in OVX -BG-Sr, OVX -BG groups decreased significantly ($P < 0.001$) as compared to OVX rats. Moreover, we observed, an enhancement increase of MDA level ($P < 0.001$). The results clarified that implantation of both BG and BG-Sr showed a pronounced release in the free radical content. These activities were more pronounced than the OVX-NI. After 60 days, the CAT, SOD, GPX activities in OVX –BG were increased by 102%, 97%, and 103% respectively when compared to OVX rats. In addition we noted a decrease of MDA levels by 82%. In the same way, a significant increase by 110%, 113% and 104%, in OVX –BG-Sr rats as compared to OVX groups and decrease of MDA by 86%.
Conclusion: After 2 months of implantation, protective action against ROS was clearly observed in the muscular tissue surrounding BG-Sr.
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Effects of PARP inhibitors on platelet aggregation

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Aim of the study: Using a potent poly(ADP-ribose) polymerase (PARP) inhibitor, PJ34, our laboratory has confirmed that PARP hyperactivation contributes to neurotoxicity and induces hemorrhagic transformations after cerebral ischemia[1,2]. Some studies also suggest that PARP inhibitors could exert antiplatelet effect, which could be very pertinent to reduce the reocclusion risk after a stroke event[3,4]. Particulary, it has been shown in vitro that certain PARP inhibitors reduced ADP-induced platelet aggregation[4]. In this context, we evaluated the antiaggregant effect of various PARP inhibitors including PJ34.
Materials and methods: Platelet-rich plasma, obtained by centrifugation of citrated blood collected from healthy donors, was pre-incubated with PJ34 (1–100 μM). ADP-, collagen- or TRAP-induced platelet aggregation was evaluated by optical aggregometry. For each donor, we used an ADP concentration producing a biphasic aggregation curve (1.5–5 μM), due to the activation of the platelet ADP receptors $P2Y_1$ and $P2Y_{12}$ respectively. Then we compared the effects of PJ34 to three PARP inhibitors, 3-aminobenzamide, minocycline[5] and INO-1001, at 50 μM.
Results: PJ34 inhibited the second phase of ADP-induced platelet aggregation ($P2Y_{12}$-dependent) by 60% at 10 μM ($P < 0.01$), 80% at 50 μM ($P < 0.001$) and 100% at 100 μM ($P < 0.001$), without modifying the first phase ($P2Y_1$-dependent). The more ADP concentrations increased (up to 10 μM) the less PJ34 had an inhibitory effect. Collagen- and TRAP-induced platelet aggregation were not modified.
Minocycline (50 μM) reduced platelet aggregation by 60% ($P < 0.01$) whatever the agonist used (ADP, collagen, TRAP), whereas 3-aminobenzamide and INO-1001 had no effect.
Conclusion: Our results show that not all PARP inhibitors are antiaggregant, since 3-aminobenzamide and INO-1001 have no effect. Furthermore, the antiaggregant PARP inhibitors seem to exert this effect independently of PARP inhibition: PJ34 alters only ADP-induced aggregation, possibly through a competitive binding to the $P2Y_{12}$ ADP receptor; minocycline seems to act via more general pathways, since it inhibits all agonists-induced platelet aggregation.
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Organic cation transporter 2 (OCT2) is implicated in the response to stress

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Organic cation transporters (OCTs) are atypical reuptake transporters, able to transport aminergic neurotransmitters in vitro with a low affinity and high capacity. Recently, we showed that OCT2 plays a key role in serotonin and norepinephrine clearance in the limbic regions of the brain, in complement to the high-affinity transporters. We also showed that OCT2 is an important determinant of antidepressant efficacy and mood-related behaviors. In particular, OCT2-deficient mice show a pronounced despair-like phenotype in two acute stress paradigms, the TST and FST. Contrasting with the high-affinity uptake transporters, OCTs can be inhibited by the stress hormone corticosterone in vitro, raising the possibility that these transporters might be direct targets for this hormone in vivo. This present study aimed to determine whether OCT2 was implicated in the response to stress. We investigated the distribution of OCT2 in the mouse brain, by peroxydase and fluorescent immunohistochemistry, focusing on regions known to be implicated in the response to stress. We investigated after swim stress exposure plasma corticosterone levels and the activation of brain regions involved in the stress response, monitored by c-Fos induction, in wild-type and OCT2-deficient mice. We tested whether this transporter could interact directly with corticosterone in ex vivo monoamine uptake in brain cell suspensions.
OCT2 was detected throughout the brain and present in a number of stress-related regions, including paraventricular thalamic, dorsomedial hypothalamic nuclei and prefrontal cortex. These regions control the activation of the HPA axis, leading to corticosterone secretion. Compared to wild-type, OCT2-deficient mice considerable increases (+150%) of peak levels of corticosterone after a 15 min swim-stress. In the same stress paradigm, neural activation in certain brain regions, evaluated by c-Fos induction, was impaired in OCT2-deficient mice. On the other hand, OCT-mediated ex vivo uptake was not inhibited by physiological concentrations of corticosterone.
These experiments altogether demonstrate that genetic deletion of OCT2 in mice impairs the hormonal and neural response to swim stress, suggesting disrupted HPA axis or sympatho-medullary function. These results reveal a previously

unsuspected role of OCT2 in the response to acute stress, which may participate in the behavioral despair phenotype of OCT2-deficient mice.

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Nav1.9 channel contributes to mechanical and heat pain hypersensitivity induced by subacute and chronic inflammation

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Inflammation is known to be responsible for the sensitization of peripheral sensory neurons, leading to spontaneous pain and invalidating pain hypersensitivity. Given its role in regulating neuronal excitability, the voltage-gated Nav1.9 channel is a potential target for the treatment of pathological pain, but its implication in inflammatory pain is yet not fully described. In the present study, we examined the role of the Nav1.9 channel in acute, subacute and chronic inflammatory pain using Nav1.9-*null* mice and Nav1.9 knock-down rats. In mice we found that, although the Nav1.9 channel does not contribute to basal pain thresholds, it plays an important role in heat pain hypersensitivity induced by subacute paw inflammation (intraplantar carrageenan) and chronic ankle inflammation (complete Freund's adjuvant-induced monoarthritis). We showed for the first time that Nav1.9 also contributes to mechanical hypersensitivity in both models, as assessed using von Frey and dynamic weight bearing tests. Consistently, antisense-based Nav1.9 gene silencing in rats reduced carrageenan-induced heat and mechanical pain hypersensitivity. While no changes in Nav1.9 mRNA levels were detected in dorsal root ganglia (DRGs) during subacute and chronic inflammation, a significant increase in Nav1.9 immunoreactivity was observed in ipsilateral DRGs 24 h following carrageenan injection. This was correlated with an increase in Nav1.9 immunolabeling in nerve fibers surrounding the inflamed area. No change in Nav1.9 current density could be detected in the soma of retrolabeled DRG neurons innervating inflamed tissues, suggesting that de novo expression of Nav1.9 channels may serve a peripheral function. Our results provide evidence that Nav1.9 plays a crucial role in the generation of heat and mechanical pain hypersensitivity, both in subacute and chronic inflammatory pain models. Increased expression of the channel, at some stages of the inflammation, may be involved in regulating nerve ending excitability via the delivery of Nav1.9 channels to the periphery.

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Chronic intermittent alcohol exposure leads to persistent mnesic episodic-like deficits in alcohol-preferring C57BL/6J adolescent mice, and increased neuronal hippocampal activity without any morphological changes

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The extent of new alcohol modes consumption like 'binge-drinking' in teenagers is quite alarming considering that adolescence is a critical time period with high plasticity and therefore more susceptibility to addictive drugs. Thus, such pattern exposure is associated with spatial learning and memory deficits in adolescent rats (Sircar et al, 2009) or teenagers (Tapert et al, 2004), as well as apoptosis and altered neurogenesis in rodents (Crews et al, 2006). However, neurobiological long-term consequences are still unclear. This study aimed to investigate long-term behavioral, molecular and anatomical consequences of a chronic intermittent alcohol exposure (CIAE) (2 g/kg, i.p., 14 day, every other day) in alcohol-preferring adolescent male mice C57BL/6J (P30). CIAE led to 'episodic-like' mnesic deficits at short-term (1 min-inter-trial interval) in a novel object recognition task (NOR) (discrimination index d2:approximately −90%, $P < 0.001$), without any alterations in anxiety-like behavior assessed in the elevated-plus-maze and the black and white box, or in locomotor activity. In addition, *c-fos*, an immediate early gene, protein expression was increased in dorsal hippocampus during the mnesic task (dentate gyrus approximately +50%, $P < 0.05$ and CA3 approximately +200%). Discrimination deficits persisted in adulthood (P70), (discrimination index d2: approximately−141%, $P < 0.001$), contrary to *c-fos* protein expression changes. By contrast, when conducted in adulthood (P70), CIAE was not associated with such deficits. In addition, longitudinal imaging study revealed no morphological changes of the hippocampal or ventricular volumes. These results suggest that adolescents are vulnerable to CIAE, which leads to an enhanced sensitivity of dorsal hippocampus to repeated withdrawals, without visible morphological changes, but persistent episodic-like mnesic deficits. Alterations of long-term episodic-like memory and morphological changes of other structures implicated in such memory (i.e. perirhinal cortex) are under current investigation.

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P359

Urinary biomarkers for specific and sensitive detection of colistin nephrotoxicity

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Introduction: The present study was conducted to determine whether the urinary levels of Neutrophil gelatinase-associated lipocalin (NGAL), lactate dehydrogenase (LDH), aspartate (AST) and alanine aminotransferases (ALT), can efficiently indicate within 7 days an acute nephrotoxicity due to administration of colistin.

Methods: Male wistar rats were divided into four groups ($n = 7$); group 1 (G1), group 2 (G2), group 3 (G3) and group 4 (G4) treated intramuscularly (i.m.) with colistin at doses of 150 000, 300 000, 400 000 or 450 000 IU/kg/day body weight) every 12 h for 7 days, respectively. A baseline urine (U_0) was collected from rats of each group before treatment as control samples. Thereafter, animals were returned to the metabolic cages and then urine 5 h were collected at 3rd (U_3) and 7th (U_7) days of treatment for urinary parameters. After the last urine collection, blood samples were collected for measurement of creatinine and blood urea nitrogen (BUN).

Results: A statistically significant increase in urinary ASAT, ALT and LDH on 7th day was found compared with before colistin administration ($P < 0.01$, $P < 0.05$, $P < 0.05$, respectively). Urinary NGAL concentrations showed a significant (0.01) increase than cratinine and BUN compared with before colistin administration.

Conclusion: The urinary NGAL and ASAT may represent sensitive and specific biomarkers of renal impairment after colistin treatment compared with other routine clinical indicators.

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Effects of dietary ginger (Zingiber Officinale) on reproductive functions in alloxan diabetic rats

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Introduction: The role of oxidative stress has been reported in various diabetic complications. This study aims to examine the antioxidative and androgenic activities of Zingiber officinale (ZO) rhizome on fertility of male diabetic rats.

Methods: The fertility experiment was done on three groups of male Wistar rats one of them was kept as control group, while the two others were rendered diabetic by intraperitoneal injection of alloxan (120 mg/kg). One group was left as diabetic control (diab) and the second group was treated with dietary ginger rhizome (diab+Z) for 1 month.

Results: The (Diab+Z) rats showed a significant increase of sexual organs weights, serum testosterone level, sperm motility and sperm count compared to diabetic rats. In addition, antioxidant enzyme activities such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were significantly increased in the reproductive organs compared to (Diab) rats. However, malonaldialdehyde levels (MDA) and aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) activities were significantly decreased in the reproductive organs, as compared to (Diab) rats.

Conclusion: Dietary ginger rhizome supplementation for 30 days possesses profertility properties in male diabetics rats which might be a product of both its potent antioxidant properties and androgenic activities.

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Study of antibacterial activities of essential oil extracted from dry flowers' Lavandula officinalis

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Objective: The aromatic plants represent an inexhaustible source of substances and natural bioactive compounds Their essential oil contents confer great prospects. In this context, we tried to evaluate the essential antibacterial activity in vitro.

Material: The flowers of *Lavandula officinalis* are collected from INATAA, University Mentouri Constantine. The harvest was undertaken manually in june 2011 during wich the plant was in full flowering.

Methods: the extraction of dries flowers' essential oil of *Lavandula officinalis* is made by a hydrodistillation. The essential oil obtained is kept at 4°C. The composition of essential oil is analysed by GC type VARIAN CHROMPACK-CP 3800.

The bacteria used in this stydy are: gram positive bacterium (*Staphylococcus aureus ATCC 29213*) and the other gram negative (*Pseudomonas aeruginosa* ATCC 27853). We provide them from Constantines' University Hospital. in vitro evaluation of the antibacterial activity is produced by the disk diffusion method in a culture medium Mueller Hinton used by RAZA and al, 2009.

Results and discussion: Forty-nine terpenic compounds representing the sum of the percentages of the components obtained are identified of wich 67% are oxygenated monoterpenic derivatives and 15% are oxygenated monoterpenic derivatives and 15.3% are hdrocarbons monoterpenic. The major components of this oil are: acetate linalyl (15.26%), linalool (10, 68%), 1, 8-cineole (10.25%), -terpinene (11.2%) and camphor (11.25%). The dry flowers' esential oil of Lavandula officinalis show an antibacterial activity agains *S. aureus* stronger than of *P. aeroginosa*, an inhibition zone about 13.33 ± 1.53 mm, in comparaison with the *P. aeroginosa* which has an inhibition zone about 11.33 ± 2.03 mm. Several work in particular those of Moreira and al, 2005 and Raza and al, 2009 have highlighted the great sesitivity of the gram (+) bacteria compared to gram (−).

Conclusion: We can conclude that esential oil of the dry flowers of *Lavandula officinalis* have an antibacterial which is not negligible.

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P362

Bell-shaped relationship between extracellular concentrations of NAADP and its cardioprotective effects

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Aim Extracellular UTP, ATP and pyridoxal-5′-phosphate may play a beneficial role in cardiac preconditioning and like, NAADP, they are able to stimulate the P2Y11 receptor. A polymorphism in P2Y11 receptors has been shown to increase the risk

of myocardial infarction. We investigated the effects of extracellular NAADP in order to confirm the favourable function of the P2Y11 receptor during an ischemia-reperfusion period.
Methods The study was designed to test whether pre-ischemic treatment with NAADP could enhance post-ischemic functional recovery, myocardial-necrosis and suppression of arrhythmogenesis. Animal were catecholamine depleted after 6-hydroxydopamine treatment. After a 20-min stabilization period, isolated rat hearts (randomized, $n = 6$ per group) were perfused for 20-min then subjected to a 40-min global ischemia followed by 40-min of reperfusion (I/R); before ischemia, the hearts were exposed for 10-min to NAADP alone (vehicle, 0.1, 1 or 10 μM), or to 1 μM NAADP bracketed for 20 min with 1 μM NF157, a full P2Y11 antagonist. Contracture and Rate pressure product (RPP) were measured in the left ventricle. Infarct size was measured by TTC staining. Arrhythmogenesis were characterized by the duration of ventricular tachycardia/ventricular fibrillation (VT/VF), as described*, and by the severity of VT (weighted-frequency).
Results Baseline outcome were equivalent for rhythm, developed pressure, and coronary flow. At post-ischemia, the curve of concentration-efficiency (RPP, contracture and infarct size) is in favour of 1 μM NAADP ($P < 0.001$ to $P < 0.01$ vs. all groups), followed by 0.1 μM NAADP ($P < 0.001$ to $P < 0.05$ vs. all groups), while the 10 μM NAADP concentration shows no beneficial effect. Also, 1 μM NAADP protect against post-ischemic arrhythmogenesis, and severe VT ($P < 0.01$ vs. vehicle). The effectiveness of 1 μM NAADP is completely suppressed by 1 μM NF157 (*ns*, vs. vehicle), suggesting that cardioprotection is mediated by the P2Y11.
Conclusion As previously suggested, the P2Y11 receptor appears a good therapeutic target in cardioprotection which can be triggered by extracellular NAADP. Clear evidence for agonist-specific signalling at the P2Y11 receptor has been presented. Like pyridoxal-5'-phosphate, a bell-shaped response was observed with NAADP and this might be attributable to the molecular nature of the pyridine moiety.
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Pharmacological responses following P2Y11 receptor stimulation are similar to those resulting from sympathetic stimulation via the beta1-adrenergic receptor
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Aim: P2Y11 stimulation might lead to a β1-adrenergic-like effect on myocardial contractility. Both receptors have been shown to be efficient in triggering myocardial preconditioning[2,3]. Relaxation and heart rate are improved under the influence of β1-adrenergic agonists. Because the intracellular uptake of the calcium ions after the contraction is energy-dependent, disorders of the myocardial oxidative metabolism will become apparent very early by a decrease in the rate of relaxation. The objectives of the study were (i) to validate a new composite index reflecting post-ischemia contractile recovery and cellular injury (ii) to apply it for the evaluation of P2Y11 and β1-adrenergic receptors response to their agonist.
Methods: Catecholamine-depleted rat hearts ($n = 6$ per group) were submitted to a pre-ischemic treatment with either NAADP (vehicle, 0.1, 1 or 10 μM), a P2Y11 agonist, or 10 nM Xamoterol a β1-adrenergic agonist. After 40 min of global ischemia, an index, for the reperfusion period (40 min), was constructed as the ratio of the maximum isovolumetric rate of pressure decrease $(-dP/dt)_{max}$ to the maximum isovolumetric rate of pressure increase $(+dP/dt)_{max}$ as a function of heart rate. Thereafter, the trend of the index was compared to the post-ischemia enhanced contractile recovery (time course of reperfusion) and decreased cellular injury (at the end of reperfusion).
Results: The mean lower value of the index was well correlated with contractile recovery and cellular injury. With regard to the reperfusion period, the curve of concentration-efficiency for functional recovery and index was in favour of 1 μM NAADP and to 10 nM xamoterol ($P < 0.01$ vs. all groups), followed by 0.1 μM NAADP ($P < 0.05$ vs. all groups). The trend for higher functional recovery and lower index, for 10 nM Xamoterol treated group and 1 μM NAADP treated group were comparable.
Conclusion: Using a new index, our results confirm similarities between P2Y11 and β1-adrenergic responses: before ischemia, both induce a positive inotropic response[1,3]; after global ischemia, they mediate the same effects on the functional recovery.
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P364
Candesartan markedly reduced pressure ulcer formation without beneficial effect on ulcer healing in diabetic and non-diabetic mice
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Objective: Angiotensin II type 1 receptor (AT1R) blockers had showed beneficial effects in organ's protection against ischemic conditions. Ischemic pressure can induce skin ulcer, leading to chronic wound during diabetes. We examined whether the AT1R blocker, candesartan, could have a beneficial effect on skin ulcer formation and on healing in streptozotocin (STZ)-treated mice and control mice.
Material and methods: Skin pressure ulcers were created by applying two magnetic plates to the dorsal skin for a short duration in diabetic mice and for a long duration in control mice. Control and STZ mice with 8 weeks of diabetes received either no treatment or candesartan (1 mg/kg per day orally administrated) 1- Pre-treatment study (during the last 2 weeks of diabetes before applying pressure). After pressure exposition, we assessed the size and histological depth of the skin ulcers. 2- Post-treatment studies (during 3 or 14 days after pressure). For the duration of treatment, we assessed ulcer development and healing. An additional group of control mice was treated with an angiotensin-converting enzyme inhibitor; Ramipril (0.25 mg/kg per day orally administrated).
Results: Both post and pre-treatment with candesartan markedly reduced ulcer development in diabetic and control mice, but did not to improve healing. Ramipril post-treatment had no beneficial effect on ulcer formation, and delayed healing.
Conclusion: AT1 blockade, but not ACE inhibition, is protective against pressure induced skin ulceration in both diabetic and non-diabetic mice.

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Disease modifying strategy based upon iron chelation in MPTP-treated mice
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Parkinson's disease (PD) is characterized by mitochondrial oxidative stress leading to the loss of the dopaminergic neurons in the nigrostriatal pathway. Most of the known environmental causal factors and PD animal models trigger the mitochondrial dysfunction through interplay between complex I deficit and excess of oxidative stress. Iron is an essential element for cellular metabolism but an excess of iron leads to ROS production and oxidative stress, particularly in dopaminergic neurons which are more vulnerable to oxidative stress. The aim of the present study was to assess the effects of deferiprone, a potent iron chelator, on dopaminergic neurodegenerescence, iron content in substantia nigra (SN) and oxidative stress through the study of a well-known model of PD, the MPTP treated mice.
Two doses of deferiprone or vehicle were delivered by oral gavage during 7 days after acute saline or MPTP intoxication. Iron content of SN was assessed by T2*MRI and by spectrometry. Neuroprotection was evaluated via the quantification of tyrosine hydroxylase (TH) immunoreactive neurons in SN and striatum and via the assay of dopamine and its metabolites by HPLC. Oxidative stress was evaluated through the study of different markers: glutathione, carbonylated proteins, malondialdehyde, 8 hydroxy-desoxyguanosine (8OHdG) and nitrotyrosine.
MPTP mice presented an increased iron content in SN as revealed by MRI T2* and iron spectrometry which was significantly reduced by deferiprone treatment. After 7 days, the 54% TH-ir neurons loss observed in MPTP mice in SN as compared to control mice was significantly attenuated by deferiprone until 29%, depending on the dose. Dopamine and its metabolites were highly reduced in the striatum of MPTP mice and deferiprone was able to limit this deficit. Oxidative stress was increased in MPTP mice as shown by a decrease in gluthatione ratio and an increase in 8OHdG and nitrotyrosine. Deferiprone was able to attenuate those markers and consequently the oxidative stress in SN.
Deferiprone was able to counteract the main characteristics and consequences of oxidative stress in MPTP treated mice. This iron related antioxidant strategy offers promising perspective for the treatment of PD patients.

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Pharmacovigilance in Fez;A first report of activity
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Introduction: Defined as the whole of the techniques of identification, evaluation and prevention of the risk of adverse effect of drugs; the Pharmacovigilance represents the adequate method that can increase the knowledge drugs and the risks related to its use. In order to limit this risk, the CHU Hassan II of Fez has created a Pharmacovigilance unit. In this work, we report the activity of this unit.
Material and methods: We listed all the adverse effects of the drugs prescribed for the patients hospitalized in the CHU Hassan II between June 2008 and June 2011. The French method of imputability was used.
Result: During the period between June 2008 and June 2011, 520 unexpected serious adverse reaction cases were noted in the Pharmacovigilance unit. The average of the patient's age was 40 years ± 22 (1–80) with a predominance of males (53% cases). The period of appearance was 6.5 days (few min – few month). The internal medecine unit was the best notificater which accounted for 27% of the declarations. The drugs anti-neoplasic were the most accused drugs in the development of the adverse effects.
Conclusion: The mission of Pharmacovigilance is to prevent the iatrogenic risk of drugs in terms of public health. It initiates and takes part in epidemiological studies intended for better evaluating the iatrogenic risk; the system effectiveness of Pharmacovigilance depends directly on the participation of the health professionals, without whom this system cannot function.

P367
Hypertrichosis following vaccine immunization
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Introduction: Infant vaccination is a pivotal aspect of preventive medical care. After routine vaccination, the most common adverse side effects are symptoms at the injection site and fever, considered to be the result of local inflammation. Also, other atypical reactions can occur. We report two cases of localised hypertrichosis observed after vaccination.
Observations: A 1-year-old girl received the third (12 months) injection of heptavalent pneumococcal vaccine and a first (12 months) injection of measles-mumps-rubella vaccine on the 13th of August, 2008. A few days later, she developed intense itching. Three weeks later, hypertrichosis at the injection site of heptavalent pneumococcal vaccine associated with a inflammatory granuloma was observed. The second case concerns a 2-year-old girl who presented pruritus after the third (12 months) administration of diphtheria-tetanus-pertussis-polio-haemophilus type b vaccine and pneumococcal conjugate vaccine in a few months. In December 2009, hypertrichosis and a subcutaneous nodule at the injection sites was observed after the boosted dose of diphtheria-tetanus-pertussis-polio-haemophilus type b vaccine administered in June 2009.

Discussion: Measles-mumps-rubella vaccine boosted dose (16 months) was administered without new side effect. Nodules and hypertrichosis persist. In the first case, aluminium patch tests confirmed the delayed hypersensibility reaction. Aluminium phosphate and aluminium hydroxide are component of pneumococcal conjugate and of diphtheria-tetanus-pertussis-polio-haemophilus type b vaccines respectively. Aluminium adjuvants are active components of vaccines, because they stimulate the immune system to respond more effectively to proteins or polysaccharide antigens that have been adsorbed at the surface of insoluble aluminium particles. Following single injections, occasional irritation (dermal) at the site of injection is the only effect that has been reported in the published literature. In a recent study, severe reactions, like hyperpigmentation or hypertrichosis are described as post-inflammatory reactions in up to 50% of the children after boosted doses.
Conclusion: This side effect is not reported in the French National Pharmacovigilance database and it is not listed in the Summary Caracteristics of Product of aluminium adjuvants vaccines. Because of possible misdiagnosis and its rare incidence, no estimate of the prevalence of this effect can been made. Therefore it is essential to communicate with health professionals about this phenomenon.

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Acute renal failure induced by allopurinol

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Introduction: The essential indication of allopurinol is hyperuricemia. Its fast effect and best efficiency allowed it to be the best treatment of hyperuricaemia which is often associated with kidney failure, but its poor tolerance represents the renal major limit of its utilization.
The aim of this work is to report some cases of adverse effects alloprinol notified to our unit of pharmacovigilance.
Patients: We report retrospectively 10 cases of intolerance to allopurinol collected over the last 2 years in the unit of pharmacology CHU Hassan II of Fez.
Results: These six men and four women, mean age 51 ± 7 years (30–73 years). The delay of consultation is 2 weeks (1–4 weeks). Among the 10 patients, eight are known with of chronic renal failure (CRF) pre-existing, with a creatinine clearance below 30 mL/min/1.73 m^2 in six of them. The clinical types are identified: an acute interstitial nephritis immunoallergic in three cases, acute renal failure superimposed on a single pre-existing IRC in three others, and drug hypersensitivity syndrome in four cases. Corticosteroids were prescribed in three patients. The evolution after stopping the drug is marked by a complete recovery of renal function in two cases and partial in three patients, three others were on dialysis and two died. Treatment with febuxostat was initiated in two patients with symptomatic hyperuricaemia, with a satisfactory efficacy after 1 year.
Discussion: Four types of renal secondary reaction to Allopurinol are reported in the literature: the hypersensitivity syndrome (DRESS: Drug Rash with Eosinophilia and Systemic Symptoms), acute interstitial nephritis, acute renal failure in patients with isolated a pre-existing renal disease and rare cases of urolithiasis.
Conclusion The reactions of intolerance to allopurinol are rare. They can sometimes damaged prognostic renal functional and also vital. The use of high dose, when are combined with diuretic therapy and the presence of renal failure promotes the occurrence of DRESS.

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Severe meprobamate overdose: a case serie

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Introduction: Meprobamate is an anxiolytic and sedative molecule, used in France mainly under EQUANIL® or MEPRONIZINE® (meprobamate-acepromatazin) forms. Meprobamate intoxications (5% of psychotropic drugs intoxications in France) lead to a deep sedation and to haemodynamic deficiencies, sometimes with a severe outcome. The aim of our study was to describe meprobamate overdose occurring with meprobamate blood level higher than 120 mg/L.
Methods: From December 2009 to the end of October 2010, in the Loire department, all patients with meprobamate blood level higher than 120 mg/L were selected. A data collection sheet was used on each medical file to extract parameters of interest. All cases have been then registered in Pharmacovigilance database.
Results: Eighteen meprobamate overdose have been retained, corresponding to 16 included patients. 62.5% of patients were women, average age was 45 years. Meprobamate-acepromatazine association was concerned in 66% of cases. Twenty-seven percent of all intoxications were associated to alcohol intake. Only 17% of cases were intoxications to meprobamate alone. Average meprobamate blood level was 160 mg/L (121–328 mg/L) and average Supposed Ingested Dose (SID) was 8.4 g (0.8–16 g). One patient presented a delayed sustained release of meprobamate. Sixty-seven percent of patients presented coma (GCS<8) and 39% vasoplegic shock (blood pressure<80 mmHg). Intubation was needed for all patients with coma and lasted from 7 to 92 h. Coma was more frequent for meprobamate-acepromatazine association than for meprobamate alone (75% vs. 50%). Hypotension length ranged from 1 to 11 h; all patients received filling solutions and 33% of hypotensive patients received vasoactive amines. There was no significant difference in the occurrence of haemodynamic deficiencies with or without acepromatazine intake (42% vs. 33%). Other clinical manifestations as mydriasis, inhalation pneumonia or hypothermia were noted. The average hospitalization length was 4.4 days. No patient died.
Conclusion: Association with acepromatazine increased sedation but not significantly haemodynamic deficiencies. Meprobamate overdose management was important but sufficient to limit mortality. Association to other toxic molecules, included acepromatazine, remained a poor prognosis factor. Afssaps has engaged measures to prevent these events: marketing end for EQUANIL® and MEPRONIZINE® is scheduled for 2012.

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Fixed drug eruptions induced by carbocysteine and acetylcysteine: two cases with negative skin tests

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Introduction: *N*-acetylcysteine and carbocysteine are bronchial mucolytics contained in more than 20 medicines, sometimes over-the-counter. *N*-acetylcysteine is also used to treat acetaminophen overdose. Side effects are mainly gastrointestinal. Immediate hypersensitivity reactions have been described. We report two cases of fixed drug eruption (FDE) induced by these drugs.
Case reports: ***Case 1:*** A 78-year-old-woman with chronic bronchitis had four typical FDE's episodes on the face and upper limbs after taking Bronchocod® (carbocysteine) Humex expectorant® (carbocysteine) and acetaminophen. Patch tests in healthy skin and areas of FDE with the suspected drugs (30% water) were negative. A new crisis of FDE was observed when taking Bronchocod® alone. A rechallenge test with acetaminophen was conducted later without cutaneous event.
Case 2: A 99-year-old-woman had an eruption in pigmented patches, with bullous face, forearms and hands 2 days after taking Exomuc® (acetylcysteine 5%) for a bronchial infection. The diagnosis of FDE was made clinically confirmed by histology. Patch tests in healthy skin and in pigmented sequelae of FDE with Exomuc® (acetycysteine), Mucomyst® (acetylcysteine) and Bronchocod ® (carbocysteine) were negative as the patch tests performed with both thioglycolates of the hairstyle battery.
Discussion: The FDE induced by carbocysteine and acetylcysteine are rare (two cases for carbocysteine and 10 cases for acetylcysteine). In most reported cases, patch tests were negative as in ours. Therefore, the diagnosis is almost always made by a rechallenge test. This test has not been proposed in our two patients because of the undeniable chronological accountability of the cysteine's derivates. Some authors have discussed the possible role of a *N*-acetylcysteine's metabolite, the thiodiglycolic acid, in the occurrence of the drug eruption.
Conclusion: The causative role of *N*-acetylcysteine and carbocysteine must be raised in the occurrence of FDE during a bronchial infection as well as acetaminophen and/or antibiotics.

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Vulvar edema after instillation of hydrogen peroxide for bartolinitis

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Introduction: Hydrogen peroxide is widely used in washing and cleansing of traumatic and infected wounds. The release of oxygen bubbles is bactericidal on anaerobes especially clostridium. We report the case of a large vulvar edema occurred abruptly during the instillation of the antiseptic in bartolinitis surgery
Comments: Ms. D. born in 1983, pregnant (15 weeks of gestation) was hospitalized for a left fistulising bartolinitis. A surgical incision of the abscess from the orifice of the fistula was performed. The content was evacuated and the cavity irrigated with about 20 cc of Eau Oxygénée Cooper ® (10 vol/100 mL) injected under pressure with a syringe. By the end of irrigation a cold swelling of the vulva and pubis appeared suddenly without erythema nor itching. The hemodynamic and ventilatory parameters remained stable. The Doppler ultrasonography showed absence of venous thrombosis or lymphatic compression. It was an outpouring subcutaneous air-fluid without ischemia. There was neither inflammation nor increase in serum tryptase. Corticosteroids (prednisone 1 mg/kg/j) were ineffective. The quantitative and functional C1 inhibitor was normal. Regression of oedema was obtained after a paracentesis at the 10th day.
Discussion: Hydrogen peroxide is widely used in the flattening of vulvae abscesses, dental, superficial wounds or abdominal or pelvic cellulites. However, several serious accidents were reported with the use of this antiseptic. Cases of subcutaneous emphysema or embolism sometimes lethal caused by injection capillary direct or passive diffusion of oxygen bubbles were reported. These emboli occur when using large volumes of hydrogen peroxide, when injected under pressure of disrepair with large cavities or crevices but this risk exists even when the injection of small volumes (20–25 cc). In our case, the local release of oxygen results in an important subcutaneous edema.
Conclusion: The use of hydrogen peroxide in the incision of wounds or abscesses might be a cause of serious accidents and its superiority over other antiseptic is not demonstrated. That antiseptic should be abandoned in these indications.

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Telaprevir induced transient vascular purpura

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Introduction: Telaprevir is a new antiprotease inhibitor indicated in the treatment of viral C hepatitis (VHC). Pruritus and macular exanthema have been described in about 50% of treated patients generally improving with corticosteroid ointment. We reported and report here the first case vascular purpura probably induced by telaprevir
Case report: Mr D, 58 years old, was relapsing into VHC cirrhosis treated in 2004 by Pegylated Interferon alpha2a and ribavirine. Telaprevir was introduced (6 × 325 mg daily) with Pegylated Interferon alpha2a and ribavirine. Four days later the patient presented with macular pruritic eruption on the two legs without severity symptoms. The eruption improved with local corticosteroid treatment. The antiviral treatment was continued but the patient came back 1 week later with an infiltrated non pruritic purpura on the back of the feet without fever, neither other cutaneous nor general symptoms. Infectious etiology, proteinuria and cryoglobulinaemia were negative. The skin biopsy showed a lymphocyte perivascular

infiltration, and red cells extravasations. The three antiviral drugs were considered as very useful and so, continued under survey. The eruption finally spontaneously improved. After 4 weeks of treatment the viral charge was undetectable.
Discussion: The responsibility of telaprevir is strongly suspected and consistent with the delay of occurrence, and absence of other etiology. Pegylated Interferon alpha2a and ribavirine were well tolerated during the former treatment. The spontaneous regression is difficult to explain but may be due to a transient modification of immunity or cytotoxic action of antiserine protease activity.
Conclusion: We describe the first case of vascular purpura probably linked to telaprevir. Stopping telaprevir is recommended only in grade 3 or 4 severity. We chose to continue the treatment because of the benefit for the patient, who moreover, did not exhibit any systemic symptom. Telaprevir induced cutaneous symptoms may spontaneously regress allowing continuing a very useful antiviral drug.

P373
'I gave him the sachet'
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Introduction: Face to an unexpected adverse drug reaction, confusion among the patient's drugs should be systematically searched. Our report illustrates that meticulous questioning of the patient and its relatives may be crucial to explain a mysterious diagnosis.
Case: A 16 months year-old boy was admitted at hospital for hypotonic coma. He had no antecedent. He was treated for gastroenteritis with oral rehydration solutions (ADIARIL®) for 3 days. While his condition was improving, the boy experienced somnolence and epileptic seizures the morning of his admission. Physical examination revealed general hypotonia, apyrexia, normal haemodynamics, and a urine retention. Biology showed no inflammatory syndrome. There was hypoglycemia (0.47 g/L). Arterial blood gaz analysis revealed metabolic acidosis (pH, 7.31; pCO_2, 30.7 mmHg; HCO_3^-, 15.1 mM. Serum anion gap was 22 mM ($N < 16$ mM). Serum creatinine and lactic acid dosages were normal. Ethanol assay was negative. There was no ketonuria. Salycylate dosage was eventually positive in serum and in urine. Questionning of the parents revealed that the mother was suffering from migraine. Aspirin + metoclopramide sachets (MIGPRIV®) were kept in the familial medicine cabinet. The boy's grandfather confounded these sachets with oral rehydration solution sachets and administered the boy the wrong drug. Imputability according to the French causality assessment score was scored I4 (very likely).
Discussion: Confusion among drug names is well-known. Drug names and labeling are assessed by authorities. Regarding the physician, prescription habits, use of drug generic names, writing in capitalized letters minimize the confusion risk from the person who delivers the drug. Confusion among similar galenicals has been described mainly for injectable drugs. As in our observation, stocking the similar drugs in the same place favors confusion. Patients should be educated to systematically check the drug names before administration.

P374
Adverse events occurring during hospitalization in a post-emergency unit
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Purpose: Hospital adverse events (AEs) prolong hospital length, increase costs and mortality. Thanks to a systematic recording of AEs performed in a post-emergency unit, this survey was aimed at describing AEs' characteristics and their preventability for drug-related ones; and at assessing factors associated with AEs occurring during hospitalization.
Materials and method: All AEs occurring in a post-emergency unit in a French University Hospital were prospectively recorded from September 2009 to February 2011. Adverse drug reactions' preventability was evaluated by two reviewers using a previously validated preventability scale (Olivier P., 2005). To identify factors associated with AEs, patients with AEs were compared to up to 3 age and date of admission matched control patients, hospitalized in the same unit.
Results: We identified 64 AEs (incidence: 3.4/100 patients admitted/year); 51 were drug-related. Patients' median age was 82.5 [Q1Q3: 72–86.5] with a men/women ratio of 1/1.4. These patients presented a median Charlson score of 1 [Q1Q3: 0–3] and a median number of medications of 6 [Q1Q3: 3–8]. Five percent of drug-related AEs were 'definitely preventable', 16% 'potentially preventable', 49% 'definitely non preventable' and 30% 'unclassable'. Drugs the most frequently involved were nervous system drugs (47%) and anti-infectives (22%). In multivariate analysis, a Charlson score ≥2 was the only factor associated with AEs (OR 0.4; 95%CI [0.21–0.80]).
Discussion: Our systematic recording showed that AEs observed in a post-emergency unit are not rare. Inpatients with comorbidity were less likely to present an adverse event. This unexpected result might probably be explained by more cautions taken by medical team.

P375
Imiquimod and pulmonary embolism: a case report
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Introduction: Imiquimod, a topical immunomodulator, has been approved for the treatment of superficial basal cell carcinoma (BCC), actinic keratosis and external genital warts. As cutaneous absorption is very limited (<0.9% of a topically applied single dose), the most frequent adverse effects are application site reactions (50–60%) and systemic adverse effects are expected to be very infrequent.
Observation: A 47-year-old male patient without significant medical history started a 6-week cycle of topical 5% imiquimod cream 5 days/week for BCC on the back. Sixteen days after the end of treatment, he experienced acute thoracic pain with blockpnea. The clinical and biological examinations (D-dimer = 1400 ng/mL) were strongly suggestive of pulmonary embolism that was confirmed on a thoracic CT scan evidencing bilateral segmental multifocal pulmonary embolism with left pleural effusion. Blood cell and platelet counts, the prothrombin time and activated partial thromboplastin time were normal. There was no evidence of thrombophilia as shown by the absence of lupus anticoagulant, anti-cardiolipin antibodies, anti-β_2-glycoprotein 1 antibodies and prothrombin gene mutation, the presence of normal antithrombin III, protein C and protein S levels, and normal activated protein C resistance. Pelvic and abdominal ultrasound, doppler echocardiography and lower limb venous echo-doppler evidenced no anomaly. The patient had no personal or familial history of venous thromboembolism, but made a 400-km car ride with regular breaks 4 days before. He was prescribed a 6-month fluindione treatment and no recurrent venous thromboembolism episode was noted after 1 year of follow-up.
Discussion: Based on the patient's history, it is tempting to speculate a relationship between imiquimod pharmacological target and the occurrence of this thromboembolic event. Indeed, imiquimod activates the innate immune response by binding to toll-like receptors 7 and 8 (TLR). The stimulation of TLR 7 and 8 in endothelial cells upregulates the expression of tissue factor (TF) on monocytes and endothelial cells. TF, the main initiator of the coagulation cascade, is considered to be the major trigger of thrombosis in the pathophysiology of the antiphospholipid syndrome. Therefore, indirect TF upregulation by imiquimod could be involved in the increased thromboembolic risk.

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Comparison of serious adverse events during clinical trial: recorded in the clinical trial database and reported to the sponsor
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Background: During clinical trial (CT), adverse events (AEs) are coded in the CT database by the clinical research assistant of the investigator. When they become serious (SAEs), they must be reported to the sponsor, wich recorded them in the safety database. In 2009, preparation of safety report of CTs showed differences between the databases. Record linkage seems necessary to improve the quality of safety data collection.
Objective: To analyse the concordance of SAEs, between CT database and the safety database.
Methods: Observational retrospective study on safety data of four academic CT (A, B, C, D) by merging AE from CT database and SAE from safety database. The merging was performed with SAS® software, using the patient identification number and the date of occurrence of SAE as the reference variables. Concordance was measured and validated after an overview of the recorded data of the patient.
Results: Among the 245 SAEs of the safety database (119 for CTA, 12 for CTB, 110 for CTC and four for CTD), 106 were found in the CT databases (51 for CTA, 12 for CTB, 43 for CTC, none for CTD). The concordance coefficients were respectively 39%, 50% and 70%. Half of the differences corresponded to difference in AE coding of the same symptoms. The other part was represented by a lack of data between the databases. The global analysis of the AE codes highlighted potential SAEs not reported to the sponsor (12 for CTA, three for CTB and C).
Discussion: SAEs were coded in different ways in the two databases. The safety unit (physicians, pharmacists), codes the diagnosis of the event and not just the symptoms. In the CT database, potential errors of coding for AEs and not recorded SAEs have been identified. On the opposite, potential SAEs have not been reported to the sponsor. This resulted in loss of information and potential bias for statistical analysis of the safety data starting from CT database. A validation procedure and a common variable for merging databases must be performed to insure the completeness of the safety data in the annual safety report.

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Priapism and antineoplastic chemotherapy: think about ondansetron
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Introduction: Ondansetron is a serotonin 5-HT3 receptor antagonist with antiemetic activity. We report two cases of priapism that occurred after chemotherapy, probably induced by ondansetron.
Case 1: A 70-year-old man, past smoker, with no history of priapism was diagnosed for rectal adenocarcinoma in 2006. After rectum surgery, he received radiotherapy and fluorouracile. Then, from June 2010 to September 2010, he was prescribed 12 courses of chemotherapy [FOLAVA: irinotecan, calcium levofolinate, fluorouracile, bevacizumab, atropine, alizapride and ondansetron (IV, 8 mg)]. At the eighth course, he reported priapism that occurred about 8 h after the end of each previous course. The event lasted about 7 h, with spontaneous recovery. Priapism did not recur after chemotherapy discontinuation.
Case 2: A-71-year-old man, past smoker, was diagnosed for prostate cancer in 2000. He received hormonotherapy (goserelin, bicalutamide) during several years, with progressive inefficacy. From April 2011 to June 2011, he received five docetaxel chemotherapy courses (161 mg) with ondansetron (IV, 8 mg). He reported priapism during the night following each course. He also complained for neuropathy and increasing chest pain that started at the second course. Priapism did not recur after chemotherapy discontinuation.
Discussion: These cases have strong chronological criteria for drug-induced priapism, but none of the chemotherapy drugs are known to induce priapism. The fact that ondansetron is the only common drug emphasizes its highly probable role. Priapism is a rare event (#300 hospitalisations/year in France), mostly secondary to pro-erectile medications (cavernous injections) and illegal drugs. Antidepres-

sants, neuroleptics, and heparin are also sometimes involved. To our knowledge, no case of priapism has been reported with serotonin 5-HT3 receptor antagonists. However, one case of fluoxetine-induced female sexual dysfunction was reversed by granisetron [1] and 5-HT3 receptors were involved in erectile process via relaxant activity in rabbits [2].
We suggest that 5-HT3 receptor antagonists should be considered in drug-induced priapism.
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P378
Adverse effects of cholinesterase inhibitors in France. Evaluation of data recorded from 1998 to 2010
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Introduction Since they have been put on the market (donepezil and rivastigmine in 1998, galantamine in 2001), cholinesterases inhibitors (ChI) have been more and more often prescribed to patients suffering from Alzheimer's disease. In our PEIMA study on the prevalence of adverse drug effects (ADE) in patients with Alzheimer's disease or other dementia, we have shown that ChI were the main providers of ADE regarding the gastro-intestinal or the neuropsychological systems; these ADE were often non serious.
The purpose of the present study was the description of the ChI induced ADE reported to the French pharmacovigilance network database from the various dates of marketing to 2010.
Methods All cases of ADE linked to ChI reported to the regional centres of pharmacovigilance from 1/1/1998 to 31/12/2010 were extracted from the database provided that the link between the drug and the effect was considered as likely, or there was an interaction. An analysis both global and according to the drugs used was performed so as to describe the various ADE, depending on the system-organ class (SOC) and the severity, and to evaluate the trend in the number of effects between 1998 and 2010.
Results From 1/1/1998 to 31/12/2010, 2577 ADE were registered in the pharmacovigilance database among which 1503 (58.3%) were judged serious. The number of registered ADE progressively increased whatever the molecule administered. During this period, donepezil ADE amounted to 52%, rivastigmine ADE to 27% and galantamine ADE to 21%. The proportion of serious ADE induced by each molecule was similar. The most frequently reported ADE were as follows:
- neuropsychiatry 25% (628/2577), 58% serious (366/628),
- skin and administration site 18% (475/2577), 46% serious (387/475),
- gastro-intestinal tract 15% (400/2577), 48% serious (191/400),
- heart and vessels, 12% (380/2577), 81% serious (297/380).

Conclusion The ADE reported to the pharmacovigilance network were more frequently considered as severe than those reported within the frame of a prevalence study conducted in the field of clinical practice (PEIMA study). The severity of cardiovascular ADE was especially of concern; this requires further studies on the adverse clinical impact of ChI.

P379
Transfixing ulceration of the foreskin under nicorandil
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Introduction Nicorandil is a potassium channel activator with a nitrate component. Although mouth ulceration has long been recognised as a side-effect of nicorandil treatment (1), its use has more recently been associated with ulceration of any region of the gastrointestinal tract and genital area. We report an unusual ulcer by its topography and its importance
Observation A 85 year old man with angina was treated for 27 months by Adancor (nicorandil), 40 mg per day. He was seen in a dermatologic consultation for a painful ulceration of the foreskin and failure of local treatment. The foreskin, on its dorsal face, was the seat of a transfixing ulceration (15 × 20 mm) with necrotic and sluggish edge extending to the balano-preputial sulcus. No other lesions were associated. Venereal assessment was negative. Nicorandil was stopped soon after the consultation after agreement phone by the cardiologist.
Discussion.
Since 2008, genital area ulcerations (vulva, vagina, penis, scrotum) has been described under nicorandil (2,3). Nicorandil-induced ulcerations can be very distressing for patients. These ulcers, if advanced, may develop into perforation, fistula, or abscess formation; Ulcerations are refractory to treatment, including surgery. They respond only to withdrawal of nicorandil. Nicorandil is frequently overlooked as a potential cause of such ulcers and patients may therefore undergo unnecessary and unsuccessful procedures before cause is recognized.
Conclusion General practitioners and healthcare professional's should consider nicorandil treatment as a possible cause of ulcerations. A widely information is necessary to inform them, for reducing diagnostic delay which can led to complications.
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Comparative Analysis of French Human and Veterinary PharmacoVigilance Systems
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Introduction: Since the recent Mediator affair, the French human PharmacoVigilance (PV) system is partially contested. Is the veterinary PV system currently settled in France better organized? Could the human PV system learn from the veterinary PV system organization?
Objective: The objective of this study was to compare human and veterinary PV systems in order to discuss possible improvements for each organization.
Methods: We first analyzed the French law texts related to the two PV systems. Then, we conducted telephone interviews with several professionals working at different levels of the two PV systems in order to better understand their practical organization.
Results: Our study suggests that the veterinary PV system could benefit from improvements by taking the human PV system as model. We will first retain as the main weakness of the veterinary PV system the lack of proximity (localization, missions…). Indeed, there is only 1 veterinary PV center vs. 31 regional PV centers in the human system. A consequence of this deficiency is that the veterinary PV system is associated with a lower incidence of Adverse Drug Reactions (ADRs) reports compared to the human PV one's (5700 ADRs vs. 30 000 ADRs per year). Concerning law, we found that pet owners do not have the legal right to report ADRs to the veterinary PV system, whereas patients can do it in human PV. Lastly, we underline the fact that ADRs occurring in humans exposed to veterinary drugs (which represent almost 600 cases since the implementation of the veterinary PV system), are reviewed by the veterinary PV system whereas an analysis by the human PV system could be more appropriate.
Conclusion: Both human and veterinary PV systems need improvements in order to better achieve their missions. For that purpose, more financial and human resources are needed. Although each organization presents some strengths and weaknesses, it seems that the veterinary PV system requires more extensive enhancements. In addition, gaps were highlighted in the cooperation between the two systems, and development of closer links seems therefore essential.

P383
Medicine is not candy!
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Objective: During the national week on patients' safety, on the auspices of the Ministry of Health, a Proper use of medicines Day was organized by the regional health agency. Our pharmacovigilance centre was solicited to run a stand: quizzes on the knowledge on pharmacovigilance latest news were proposed to the visitors, with candies offered for each correct answer. Flyers on proper use of benzodiazepines and posters on adverse effects were also presented. In addition, a survey was conducted to have the point of view on medicines and adverse effects of the stand's visitors.
Methods: Each person visiting the pharmacovigilance stand was asked to fill in an anonymous questionnaire with closed and open questions on age, sex, health professional or not, number of medicines currently taken, knowledge on medicines and adverse effects. Respondents were also asked to suggest a slogan to increase public awareness on the adverse effects of medicines.
Results: Thirty-four persons answered the questionnaire: 28 health professionals and six non-health professionals. There were 25 women and five men (data lacking: four cases) including two under 25 years, 15 between 25 and 44, 15 between 45 and 64 years (data lacking: one case). Nineteen were taking medicines at the time of the study. Nineteen responders said that they always read the patient information leaflet (PIL), 10 sometimes and five never; 22 persons thought that they were well informed on adverse effects of medicines; only five persons considered some medicines are risk free; 19 had never experienced any adverse effect. Fifteen persons suggested a slogan, of which five proposed 'medicine is not candy'.
Discussion: Candies, quizzes and a dose of humour were used for attracting visitors on the stand. Results are fairly encouraging in that less than half of the people questioned said that they were not taking medicines, more than 50% said that they always read the PIL and considered themselves as well informed on adverse effects. It must be remembered, however, that more than 80% of the people questioned were health professionals

P384
Safety data in clinical trials and personal data protection
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The aim of this work is to provide some points to consider (PTC) regarding the management of safety data in accordance with the data protection regulation in clinical trials, all safety information collected during the trial falling within the scope of the EU data protection directive (95/46/EC).
The sponsor had to ensure the benefits and risks of the clinical trial including the continuous safety evaluation of investigational medicinal products. And some conflicts between safety reporting and data protection requirements can exist as for the purposes of safety analysis, comprehensive clinical information is important to collect in order to perform efficient medical assessments. Moreover, some relevant personal identifiers are essential to collect to prevent duplicates in safety reports. So a right balance between the privacy protection rules and safety requirements in the processing of personal data has to be found.
A French reference methodology, published by the *Commission Nationale Informatique et Libertés*, covers all personal data processing in clinical trials. We propose to

establish PTC based on this reference methodology, to clearly define what kind of safety data could be collected by the sponsor.
The PTC should be divided in two parts: a first part being devoted to personal data of patient and the second one to investigators and other professionals involved in the clinical trial. Each part should clearly define what kind of personal data should be collected for safety purposes in compliance with the data protection directive. In these PTC, the categories of persons authorise to access these data should be precisely listed.
There is no current guideline stating how EU data privacy rules should be accommodated in adverse reaction data management and reporting. The reference methodology established by the CNIL and which complies with the EU directive 95/46/EC does not contain special statement for safety data. However we can assume that personal data in clinical trials on medicinal products for human use include all the data collected for pharmacovigilance purpose. Thus, our PTC proposal emphasises the need for a European guideline regarding safety data management in clinical trials.

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ARITMO Project. Torsade de pointe and QT prolongation: competition bias associated with AZERT drugs list. An analysis of the French spontaneous reporting database

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Background: The ARITMO project (http://www.aritmo-project.org/) aims to analyse the Torsade de pointe (TdP) and QT prolongation (QTP) potential of antipsychotics, antimicrobials and H1-antihistamines. As part of this project, French pharmacovigilance data were analysed to search for signals using disproportionality measures. Nevertheless, signal detection could be influenced by a competition bias related to the presence of reports of TdP related to well-established drug-event associations. The Arizona CERT (AZCERT, Center for Education and Research on Therapeutics, http://www.azcert.org) list maintains a comprehensive list of drugs that could cause TdP or QTP and that could reduce the sensitivity of the detection of new signals related to these events.
Aim: To explore the effects of competition bias between Arizona CERT drugs and other ARITMO drugs on signal generation for TdP or QTP.
Methods: The drugs with TdP or QTP potential in patients without congenital QTP were identified using the information reported on the AZCERT website. The French spontaneous reporting database (reporting data: January 2000–August 2010) was investigated in order to search signals of TdP and QTP applying the reporting odds ratio (ROR) for all drugs belonging to the following classes: antipsychotics (ATC Code: N05A), antibacterials (J01 and J04), antimycotics (J02), antiprotozoals (P01), antivirals (J05) and H1-antihistamines (R06). The analysis was performed before and after removing from the database all reports concerning drugs mentioned in the Arizona CERT lists.
Results: From the French Pharmacovigilance database, 517 cases of TdP or QTP were found, 208 of which belonged to ARITMO drugs. A total of 26 statistical signals were detected, 15 of which concerned ARITMO drugs not included in the Arizona CERT lists (antipsychotics: acepromazine, amisulpride, aripiprazole, cyamemazine, levomepromazine, loxapine, olanzapine, tiapride and zuclopentixol; H1-antihistamines alimemazine, cetirizine and loratadine; antimycotics: amphotericin B and itraconazole; antiprotozoals: quinine). After the removal of AZCERT drugs reports, no new signals were generated, while those related to amphotericin B, itraconazole, levomepromazine, and loratadine disappeared.
Conclusion: This study found that among the 15 signals of TdP or QTP, five could be affected by competition bias with Arizona CERT drugs.

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Benfluorex and pregnancy: a case-control study in EFEMERIS database

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Following benfluorex withdrawal, fully presented and discussed in media, several women who had taken benfluorex during pregnancy have questioned the Midi-Pyrenees Centre of Pharmacovigilance to know if benfluorex intake could be associated to malformations (mainly cardiovascular) for the newborn. No clinical data have been published yet on benfluorex and pregnancy.
Objective: Determine the relationship between benfluorex exposure in utero and malformation.
Method: We performed a case-control study in EFEMERIS. EFEMERIS is a French database including prescribed and delivered drugs during pregnancy (data from Caisse Primaire d'Assurance Maladie of Haute-Garonne) and outcomes (data from Maternal and Infant Protection Service and from Antenatal diagnostic Centre). At the time of the present study, 40 355 women who were delivered from 2004 to 2008 in Haute-Garonne and registered into the French Health Insurance Service were included into EFEMERIS database. Benfluorex prescriptions during organogenesis were compared between children with congenital anomalies (cases) and children without congenital anomalies (controls). Benfluorex prescriptions during organogenesis were compared between children with congenital anomalies (cases) and children without congenital anomalies (controls).
Results: During the study period, 59 women registered in EFEMERIS had at least one prescription of benfluorex during pregnancy including 52 during the two first months. Seven women only had an associated prescription of an hypoglycemic medication. From the group with congenital anomalies (943 cases), two babies (0.2%) have been exposed to benfluorex during the two first months of pregnancy vs. 50 (0.1%) among the 39, 412 controls (OR = 1.6 [0.4–6.7], $P = 0.5$ after adjustment on mother age). Malformations in benfluorex exposed babies concerned one urinary tract malformation and one heart defect (ventricular septal defect).
Conclusion: Benfluorex was a fenfluramine derivative. Data on animals and human have suggested a potential association between cardiovascular malformations and in utero exposure to amphetamine during organogenesis.
The present study was unable to show any significant association between a teratogenic risk and benfluorex exposure in early pregnancy. However, the dataset in EFEMERIS would have only been appropriate to detect at least a fivefold increase in the teratogenic risk. Thus, it would be interesting to perform further retrospective studies concerning a larger number of exposed women.

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Acute hepatitis potentially induced by lomustine

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Introduction: Fotemustine and carmustine, two nistrosourea compounds, are well known to disturb liver function while lomustine hepatotoxicity is sparsely described in humans.
We report here the case of a man who presented acute hepatitis potentially induced by lomustine.
Observation: A 56-year-old man was hospitalized in internal medical department for an anicteric acute hepatitis. At the entrance, serum alanine aminotransferase level was 35N, serum aspartate aminotransferase level was 46N, and gamma glutamyltransferase level was 396 UI/L. Renal function and hepatitis scan were normal. The patient didn't report alcohol abuse and viral etiology were excluded. He was treated during 5 months with lomustine and 1 month with bevacizumab for a parietal left cerebral tumor. He received his last dose 4 weeks before hepatitis was diagnosed. After 1 week of hospitalization, liver enzyme levels were slowly decreasing.
The tumor was diagnosed 3 years ago and treated with multiple line of chemotherapy such as temozolomide, fotemustine, bevacizumab alone during 6 months and finally lomustine combined with bevacizumab. One year ago, the patient had already presented acute hepatitis while he was treated with fotemustine. Fotemustine was stopped and hepatitis rapidly regressed.
Discussion: Hepatotoxicity with lomustine in animals, and most particularly in dogs, has been widely described in literature. Common biochemical abnormalities were high serum liver enzyme activities and hypoalbuminemia. However, lomustine human hepatotoxicity is not described and lomustine prescribing informations do not mention hepatotoxicity. Up to now, literature reports only one case of hepatitis in human induced by lomustine and only one case of acute hepatitis was found in the French Pharmacovigilance Database. Furthermore, bevacizumab is not known to induce hepatotoxicity and, its good safety during several months in this patient can exclude its responsibility in this acute hepatitis.
In conclusion, even if data are scarce, in light of his anterior hepatic disease induced by another nitrosourea, and literature data in animals, there are strong arguments to suspect lomustine in the occurrence of this acute hepatitis.

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TNF-alpha antagonists and alopecia: a review of the French pharmacovigilance database

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Introduction: In a few months, the Lille Regional Pharmacovigilance Center has received three cases of alopecia induced by TNF-α antagonists. If drug intake such as immunosuppressive agents is well known to induce alopecia, the role of other drugs as TNF-α antagonists is poorly described in literature. Thus, we studied the cases of alopecia induced by TNF-α antagonists present in the French Pharmacovigilance Database (FPVD).
Methods: We selected all the cases of alopecia induced by TNF-α antagonist (adalimumab, certolizumab, etanercept, golimumab, and infliximab) recorded in the FPVD from January 2000 to December 2011. Cases were defined by 'alopecia' according to the MedDRA terminology. Drug exposition was defined by the presence in the report of a TNF-α antagonist considered as 'suspect' in the development of alopecia.
Results: Among the 1104 cases of alopecia registered in the FPVD, 45 (4.1%) were induced by a TNF-α antagonist. The sex ratio of the patients in those reports was 6/39 and median age was 35 years (range 15–68 years). Fifteen cases were induced by infliximab, 15 by adalimumab, 13 by etanercept, two by certolizumab and none by golimumab. The time between the introduction of the drug and alopecia varied from 4 days to 4 years. Imputability was scored 'possible' in three cases, 'likely' in three cases and the others cases were scored 'dubious'. Positive rechallenge were described in three cases (two cases with infliximab and one case with etanercept).
Discussion: Up to now, the role of TNF-α antagonists in alopecia in not clear. If physiopathology suggests a beneficial effect of those drugs, because of the growth inhibitor effect of TNF-α which could inhibits hair growth, the effectiveness of TNF-α antagonists in alopecia has not been proved. These cases of alopecia during TNF-α antagonist therapy can therefore be considered as a paradoxical effect of these drugs such as psoriasis or vascularitis. Moreover, autoimmunity and genetic factors must be considered.
This study reports the descriptive data about cases of alopecia potentially induced by anti-TNF-α in the FPVD. More studies should be conducted to confirm this adverse drug reaction with TNF-α antagonist therapy.

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Comparison of Adverse Drug Reactions in children between prescription and OTC drugs

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Introduction: In France, drugs are registered in two categories. Some drugs are only delivered with a prescription and the other ones, also called 'over the counter' (OTC) drugs, may be sold directly to a consumer without a prescription from a healthcare professional. Prescription drugs have a higher toxicity or teratogenicity than OTC drugs. However, recently, some 'serious' adverse drug reactions (ADRs) have also been reported with OTC drugs as cough and cold medicines. Our study compared ADRs occurred in children with nonprescription and prescription drugs and registered in the French Pharmacovigilance Database.

Methods: We used the French Pharmacovigilance Database (FPVD) to select ADR occurred in children (0–18 years) and registered between January 2009 and December 2010 by the Midi-Pyrenees Pharmacovigilance Center. We compared gender and age of children, drugs, ADR and ADR seriousness between prescription and OTC drugs.

Results: A total of 232 ADR reports were included. It involved 296 suspected drugs divided into 245 prescription drugs, mainly anti-infective agents (44.1%) and 51 OTC drugs, mainly analgesics (53.1%). ADRs in girls were more frequently occurred with OTC drugs than with prescription drugs (62.7% vs. 45.7%, $P = 0.030$). Age (0–2, 3–9 and 10–18 years) was not significantly associated with the status of drug (prescription/OTC drugs) ($P = 0.465$). Frequencies of 'serious' ADRs was comparable in both groups (56.9% vs. 54.3%, $P = 0.737$). General disorders ($n = 87$; 22.1%), dermatological ($n = 59$; 15%) and neurological ($n = 53$; 13.5%) ADRs were the most frequent in both groups. Neurological ADRs were significantly more frequently reported with OTC drugs than with prescription drugs (24.7% vs. 13.3%, $P = 0.017$).

Conclusion: Most of ADRs in children were 'serious'. ADRs with prescription and OTC drugs in children were different for gender of patients and nature of ADRs. ADRs with OTC drugs were more frequently neurological and more reported in females than ADRs with prescription drugs.

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Drug-induced acute liver failure: a single center survey

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Introduction: Pharmaceuticals account for approximately 10–15% of acute liver failure cases after excluding acute paracetamol overdose. As the ranking of drugs inducing severe hepatotoxicity evolves over time in parallel with changes in drug consumption, it is important to have regularly updated surveys of drug-induced acute liver failure.

Methods: All cases of fulminant or subfulminant hepatitis defined as the occurrence of acute cytolytic hepatitis associated with coagulation disorders (prothrombin time < 50% or INR > 1.7) and hepatic encephalopathy, and notified to Lyon Pharmacovigilance Center since 1998 were reviewed. Reports with evidence or strong likelihood of another cause (e.g. ischemic hepatitis, viral infection, metabolic disorder) and those associated with acute paracetamol overdose were excluded.

Results: Twenty-one cases met these criteria: 18 adults (11 women and seven men; mean age: 52.8 ± 18.2 years) and three children under 16 years of age, including an 8-month-old infant. Only one drug was suspected in 12. Based on the time course of events, the suspected drugs ($n = 30$) were NSAIDs in six cases (nimesulide: 2, diclofenac: 2, celecoxib: 1, piroxicam: 1); antidepressants in 4 (venlafaxine: 2, paroxetine: 1, iproniazid: 1); antiepileptics in 3 (lamotrigine, topiramate, valproate); hypolipidemic agents in 2 (atorvastatin), phytotherapy in 2, and miscellaneous single drugs in the other cases. Six patients had also also exposed to therapeutic doses of paracetamol. The mean duration of the suspected treatment before the onset of first symptoms was 85 days. In 6 (29%) patients, the drug was continued during a mean of 18.5 days (6–40 days) after the onset of first symptoms, of whom four died and two underwent liver transplantation. Overall, 10 patients died within 8 weeks, eight received liver transplantation (four died subsequently) and only three fully recovered.

Conclusion: NSAIDs and antidepressants are the most common causes of acute liver failure in our series, but this should be balanced against the wide use of these drugs. Our data also confirm that late discontinuation of the suspected drug after occurrence of the first symptoms is consistently associated with a very severe outcome.

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Cutaneous eruption after the first administration of Montelukast

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Background: Immuno-allergic reactions are rarely described with montelukast. Their mechanisms suppose a prior sensitization contact with drug. Those reactions appeared commonly after a second contact with drug. In this case we describe maculopapular rash in man who does not received montelaukast before.

Method: An enquiry of Pharmacovigilance has been realized according to French imputation method.

Result: A 48-year-old man had generalized maculopapular rash, 30 min after receiving the first tablet and for the first time Singulair* (10 mg/day) for chronic treatment of asthma. The patient was also treated for allergic rhinitis. The lesions cleared completely within 24 h after discontinuation of this drug and reappeared after its rechallenge few days after the first event with the same chronology. All non drug aetiologies were eliminated. Imputability was evaluated as probable (C3S2: I3, B3).

Discussion and conclusion: Montelukast sodium, the active ingredient in Singulair*, is a selective and orally active leukotriene receptor antagonist that inhibits the cysteinyl leukotriene CysLT1 receptor. Immuno-allergic reactions are described with montelukast. Those reactions appeared commonly after a prior sensitization contact with drug. In this case we describe maculopapular rash in man who does not received montelukast before. So the physiopathology of this adverse effect is discussed. It's related to montelukast or to excipient and the mechanism it is immunologic or pharmacologic?

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Interaction between levothyroxine and ritonavir-boosted amprenavir in human immunodeficiency virus infected patients

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Introduction Protease Inhibitors (PIs), fosamprenavir and ritonavir are metabolized by cytochrome P450 3A4, involving these products in numerous drug interactions. We report two cases of hypothyroidism associated with the coadministration of fosamprenavir/ritonavir and levothyroxine.

Observations A 50-year-old man with HIV infection since 1989 was first treated with pentamidine diisethionate, zidovudine, and zalcitabine. Some immunological failures have been diagnosed in 1996, involving alternative treatments. In March 2006, he was treated with ritonavir, fosamprenavir and nevirapine. At the same time, for Basedow disease, an increase treatment of levothyroxine (until 325 µg) was ineffective and the patient has developed a hypothyroidism.

The second case concerns a 42-year-old woman patient with HIV infection since 1995. She was treated with ritonavir, fosamprenavir, and nevirapine since March 2005. She was also treated with levothyroxine since November 2006, for thyroid cyst. This patient underwent a thyroidectomy for a multinodular goiter, on February 2008, but TSH serum level increased to 65 µUI/mL, with 100 µg of levothyroxine.

The stop of fosamprenavir and/or ritonavir (in one case, fosamprenavir was substituted for darunavir) and an adapted dosage of levothyroxine allowed to obtain TSH serum level normalization, 1 month later, in the two cases.

Discussion Ritonavir reduces concentrations of other glucuronidated agents like ethinyl estradiol and zidovudine. Since thyroxine undergoes conjugation with glucuronic acid, thyroxine concentrations may have been reduced secondary to induction of glucuronosyl transferases by ritonavir and this effect have been significantly increased with the addition of fosamprenavir in our cases.

Fosamprenavir can also be an inducer of glucuronyl transferase and the coadministration with ritonavir results in addition of pharmacological effects. An increased induction of glucuronyl transferase with ritonavir can not be rule out. We have not found other notification of hypothyroidism in the French National Pharmacovigilance database but perturbation of hormonal tests results in HIV patients treated with ritonavir and lopinavir has already been reported in the international literature. The addition of fosamprenavir with ritonavir increased the metabolism of levothyroxine and leads to its inefficacy. The lack of data should not be interpreted as a lack of interaction.

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Tamoxifen-duloxetine: risk of reduced therapeutic response of tamoxifen

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Introduction Tamoxifen, a selective estrogen receptor modulator has been approved for the treatment of advanced estrogen receptor positive breast cancer in pre- and post-menopausal women and for the prevention of breast cancer. Tamoxifen is a prodrug, the formation of active metabolite endoxifen, is predominantly mediated by the cytochrome P450 CYP2D6. Plasma endoxifen levels are influenced by CYP2D6 polymorphisms and by potent CYP2D6 inhibitors use. To report a case of a discreased efficience of tamoxifen associated with the use of duloxetine.

Observation The case report concerns a 48-year-old woman patient with a medical history of breast cancer. She has been treated with tamoxifen since 01/06/2007. She has tolerated tamoxifen relatively well except for moderately hot flashes. She started duloxetine, a serotonin norepinephrine reuptake inhibitor (SNRI) used for depressive disorder. Three months later she observed a decrease tamoxifen adverse effects with hot flashes decreased. In October 2010, a breast cancer recurrence was detected and tamoxifen was stopped. A surgery has been planned with a radical mastectomy and an ovariectomy. Duloxetine is continued with lower doses and a treatment with exemestane was started.

Discussion Concomitant use of a CYP2D6 inhibitor has been reported in up to 30% of patients with breast cancer. The selective serotonin reuptake inhibitors (SSRIs) and SNRIs are treatments for concurrent hot flashes (65% among women with breast cancer treated by hormone therapy) and depression (10–25% in women with breast cancer). Some SSRIs are effective therapeutics for hot flashes and are strong inhibitors of CYP2D6. Their use is contraindicate with tamoxifen: the studies show a decreased of plasma levels endoxifen. Duloxetine is considered as a moderate CYP2D6 inhibitor.

To our knowledge, this is the first case who suggered a discreased efficience of tamoxifen with duloxetine and this interaction is not listed in the Summary of Product Characteristics of duloxetine or tamoxifen and not found in the French National Pharmacovigilance database.

The conflicting results of studies do not suggest a reduction of effectiveness of tamoxifen with the co-administration of CYP2D6 inhibitors. However, as a reduced effect of tamoxifen cannot be excluded, co-administration with potent CYP2D6 inhibitors should be avoid whenever possible.

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Major eosinophilia induced by enoxaparin

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Introduction Hypersensitivity reactions with heparins [unfractionated heparin (UFH) or low molecular weight heparin (LMWH)] are rare, the most dangerous is heparin-induced thrombocytopenia (HIT). Another hypersensitivity reaction has been also rarely reported: it is an isolated reactive peripheral eosinophilia. This adverse effect is already listed in the Summary of Product Characteristics but in our knowledge we report the most important eosinophilia described with the use of enoxaparin.

Observation A 76-year-old man was hospitalized after a 48-h history of fever with tightness of the chest. Physical examination revealed mediastinal adenopathy, polyserositis and atrial fibrillation.

August 22nd, 2011, enoxaparin sodium (LOVENOX®) was introduced and August 26th, bisoprolol (CARDENSIEL® and BISOCE GE®) a cardioselective beta-1 adrenergic blocker.

The laboratory data revealed an increased eosinophil count from August 26th with the upper limit 20.25 G/L (normal range: 0.04–0.5 G/L) on 07 September, without cardiac disorders.

Laboratory and radiology examinations have ruled out another diagnoses. The blood eosinophil count had decreased to 2.41 G/L after discontinuation of enoxaparin September 6th, and bisoprolol September 8th. A treatment with an UFH (heparin calcium CALCIPARINE®) is instaured without problems.

Discussion In our case, two news drugs has been introduced during the hospitalization. For bisoprolol no data are available with eosinophila, for enoxaparin there are data and the French National Pharmacovigilance database reports 16 cases of eosinophilia involving enoxaparin but not so high as in the case of our patient. The possible causative effect of treatment with enoxaparin has to be considered.

Eosinophilia results from a delayed-type hypersensitivity and the mechanism by which heparin cause this effect, could be a stimulation of CD4 cells. Activated CD4 cells are source of interleukins 3 and 5 which induce growth and activation of eosinophils.

In reports of HIT, there were cross-reactivity among the heparins, but for our patient the use of CALCIPARINE® is well tolerated. However, cross-reaction cannot be excluded and the use of an allergy test is necessary to ensure the absence of risk because heparins are important emergency drugs

Physicians must bear in mind the possibility of eosinophilia induced by enoxaparin, before using unnecessary medical examinations.

P395

Drug-induced hepatotoxicity with aliskiren, a renin inhibitor: a new adverse effect?

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Introduction: Aliskiren is the first drug of a new class of antihypertensive agents called direct renin inhibitor, licensed since March 2007 for essential hypertension. Foreseeable adverse effects are hyperkaliemia, hypotension, renal dysfunction, peripheral oedema and angioedema. Drug-induced liver injury is a rare but major health problem. Its predominant forms include acute hepatitis, cholestasis, and a mixed pattern. Such an adverse effect is rarely seen during clinical trials and the first cases of liver injuries are usually reported after approval. We report here on a case of cytolytic hepatitis with aliskiren.

Observation: a 61 year-old women with epilepsy, drug-induced osteomalacia, type II diabetes and hypertension presented with cytolytic hepatitis 1 month after the introduction of aliskiren (RASILEZ). Increased serum transaminases were found in routine blood tests (ASAT: 1631 UI/L and ALAT: 987 UI/L) without clinical disorder. Prothrombin ratio was 68%. Aliskiren, recently introduced, was stopped and serum transaminases rapidly decreased. The patient had no previous history of liver disorders. A few months prior to the therapy, liver function tests were normal. Differential diagnoses were ruled out (viral hepatitis, auto-immune hepatitis, metabolic disease…).

Discussion: In this case, dechallenge after drug interruption and the lack of other etiologies support the diagnosis of drug-induced cytolytic hepatitis. The mecanism remains unknown. To our knowledge, a few cases of hepatic dysfunction have been notified in pharmacovigilance databases (FDA-AERS, Afssaps-banque nationale de pharmacovigilance) but have never been published. Aliskiren is a new drug and so clinicians should be aware of the risk of hepatotoxicity with this drug.

P401

Drug-induced eosinophilic colitis with zonisamide: a case-report

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Introduction: Zonisamide is an antiepileptic drug used in partial epilepsy. We report on a patient who developed diarrhea while on zonisamide and was diagnosed with drug-induced eosinophilic colitis.

Results: a 58-year old man taking zonisamide for epilepsy in March 2011. He had already been on valproate, lamotrigine and propranolol for a long time. Few weeks after introduction of zonisamide a watery non-bloody diarrhea without abdominal pain appeared. As the patient reported this adverse event to his physician several months later, investigations were performed rather late. Infectious, autoimmune etiologies were ruled out. Blood tests as well as the thyroid function were normal. Thyroid function was normal. Histological explorations were performed and histopathology showed eosinophilic infiltrate compatible with eosinophilic colitis. Zonisamide was discontinued and symptomatic treatment with 5-ASA was started. Epilepsy was well balanced with a new antiepileptic drug and the symptoms of colitis disappeared.

Discussion: Drug-induced eosinophilic colitis is a rare adverse effect witch has been reported with other anti-epileptic drug such as carbamazepine, phenytoin or other drugs such as rifampicine or clozapine. Diarrhea is a known adverse effect of zonisamide, but to our knowledge zonisamide-induced eosinophilic colitis has never been published. No case was found on the French Pharmacovigilance database.

P402

Six days of coma with baclofen overdose

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Introduction Baclofen, a derivative of gamma-aminobutyric acid specifically binds to GABA beta receptors, is used for symptomatic relief of skeletal muscle spasm and spasticity. Currently this drug seems to be a safe and effective medication to treat alcohol dependence. Adverse effects at usual doses include drowsiness, headache, dizziness and orthostatic hypotension. We describe a case of massive baclofen overdose.

Observation A 50-year-old woman suffered from adrenoneuroleukodystrophy discovery in 2004, she had been receiving baclofen 60 mg/day to relieve spastic muscular contracture ever since. She was admitted to hospital upon having taken baclofen overdoses by intention. The ingested dose was 1800 mg i.e. 20 times the upper therapeutic dose. Physical examination revealed a deeply comatose with a Glasgow Coma Scale score of 3, bradypnea (6/mn), desaturation (89%), hypotension and bradycardia. Baclofen serum level from a blood sample obtained 3 days after the onset of her symptoms was 275 µg/L (normal limit 80–400 µg/L). After 6 days, the patient had a dramatic improvement in consciousness with adequate supportive care.

Discussion No specific antidote to baclofen exists. Profound central nervous system depression is a recurring finding in patients who have baclofen poisoning. In our patient with her chronic treatment, a massive overdose may have resulted in a saturation of plasma protein and tissue-binding sites. There were case reports in which plasma elimination half-life of baclofen in patient with surdosage was much longer (34.6 h) than that observed following a single oral dose (40 mg/day, 3–4 h). The elimination pharmacokinetics and toxicokinetics for baclofen is not fully known. The prolongation of half-life might be due to the fact that baclofen is a moderate lipophilic drug and may subsequently be released from lipid stores. Management of baclofen overdose is primarily supportive.

Baclofen is not routinely detected in toxicology screens but healthcare personnel should be ever vigilant for patients when the drug is prescribed for spasticity and all the more reason if an extension of indications was approved in the treatment of alcohol-dependent patients.

P403

Management of obesity and Pharmacovigilance

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Obesity is defined according to World Health Organization (WHO) as an excess of body fat that causes adverse health consequences. When obesity is said morbid or clinically serious, the risk of complications is more frequent and the risk of death increases dramatically. In this way, the WHO defines measures to encourage people having a healthy diet and getting regular exercise. However, this strategy is not sufficient for obese or overweight patients and use of drug therapies is often necessary for lose weight faster.

The objective was to assess the treatments used in the covering of obesity and their real place in terms of benefit/risk ratio.

Firstly, from literature, we listed drugs that have demonstrated or have potential interest in obesity. Secondly, for each drug, we have researched adverse drug reactions (ADR) reported in the French Pharmacovigilance Database, in the literature and risk management plans (RMP).

Seven drugs seem to be currently used in this indication. On one hand, orlistat, the only drug approved, exposes to many risks of misuse, pancreatic or liver disorders. On the other hand, six others molecules are used off-label:

Two anticonvulsant drugs, topiramate and zonisamide, for which congenital defects have been reported.

Two oral anti-diabetics, exenatide with risk of pancreatitis, cancer, misuse and liraglutide at risk of thyroid cancer and pancreatitis. Both have a RMP.

Bupropion, used in tobacco withdrawal, mostly induced convulsions and suicidal ideation and has a pharmacovigilance follow-up for convulsions, abuse, dependence.

One antidepressant, fluoxetine for which the most serious effects are suicide risk and delayed sexual maturation reported in children.

This study shows that, in spite of weight loss observed, these drugs increase risk of potentially serious ADR and highlights the widespread use of off-label drugs because of lack of effective and validated drugs. More generally, the presence of these serious ADR and sometimes of deaths can lead to the market withdrawal of drugs. This was the case of benfluorex indicated for the treatment of mellitus diabetes, and used as 'appetite suppressant'.

As far as possible, the management of obesity should be non-drug with a diet and physical exercise.

P404

Is pharmacovigilance ready to take a look at itself? Results from a quality auto-challenge

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The benefits of quality management systems (accreditation), are improving confidence in our public services or help to demonstrate that our analysis process is fit for purpose. Little has been implemented in pharmacovigilance, although its Centers might some day undergo a certification process to assess the quality of their task and effectiveness.

Aims: To ensure the reliability of our pharmacovigilance activities, we adapted current guidelines of existing accreditation processes to the Centre Regional de Pharmacovigilance de Nice.

Methods: The CRPV recruited a quality assessment engineer for 6 months (April–September 2011) in order to audit our daily practice, assess a necessary qualifications framework and identify which urgent tasks should be undertaken. We urgently had to:

Adapt to the CRPV the document managing software (validation groups, addition of follow up criteria and indicators, Ennov®) of the Nice Medical Center

Evaluate our 'customer' degree of satisfaction (or complaint): all of our regional medical referees (>2800 health professionals from departments 06, 05 and 83) have been contacted for a survey (e-mail).

Conducting and internal Management Review in order to evaluate the improvement in predefined chosen indicators as compared to the end of the 6 months period.

Results: By October, 70% of operating procedures have been created or implemented (according to French end European directives), transferred onto the hospital managing software, and validated.

13% of our survey correspondents replied within a week. From this small but representative sample, the answers were overwhelmingly satisfied (> 90%: concerning rapidity, quality specificity and relevance of the responses provided by the CRPV). The Management Review gathered all causes of non conformities, took corrective actions to avoid their recurrence, pointed out new areas for improvement, and solutions to complete the remaining procedures.

Discussion and conclusion: Quality management is a new process that must be adopted in Pharmacovigilance which still is under intense public scrutinization. Such an independent process can easily be adapted from what exists in laboratories undergoing ISO 15 189 accreditation process. Continually monitoring, measuring and improving the effectiveness of its quality management system should help current problems to occur again.

P405

Pathological gambling worsened after aripiprazole adjunction: case report and pharmacological considerations

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Introduction: Pathological gambling is an impulse control disorder with noxious social consequences, sometimes associated with schizophrenia and often associated with bipolar disorder. More recently, the amplifying role of dopamine agonists have been reported. Aripiprazole exhibits D1 and D2 receptors partial agonist properties. We report a case of pathological gambling dramatically worsened after the drug has been introduced in a patient's treatment.

Case report: Mr J. began gambling at the age of 18. He has a medical history of cerebral aneurysm rupture at 24, in 1996, followed by the loss of his lorry driver work. It was the starting point of several addictive disorders: alcohol abuse, tobacco dependence with considerable damages (debts, divorce, major depression). He was first admitted for a suicide attempt in October 2000. Psychosis was diagnosed and olanzapine, cyamemazine, oxazepam and a hypnotic were started. Only occasional gambling was noted. The treatment remained unchanged until April 2008. Doluxetine was then added for rise of the depressive component of the recognized bipolar depression. Increased gambling, but always under control, was observed. The patient stopped alcohol consumption but failed to stop tobacco. In November 2009 olanzapine and doluxetine were replaced by aripiprazole and mirtazapine. The aripiprazole dosage was increased from 10 to 20 mg/day in March 2010. Gambling became pathological from May-June 2010. Aripiprazole was stopped in April 2011 and the patient was referred to an addiction unit. The outcome is in progress.

Discussion: This patient presented post cerebral damage social and psychiatric liabilities and had medical history of compulsive behaviour for alcohol and tobacco. The link between the compulsive disorders and dopamine dysregulation is suspected from the examination of Parkinson's disease patients treated with dopamine agonist. Doluxetine is a mixed serotinine and noradrenaline reuptake inhibitor with only little effect on reuptake of dopamine. Aripiprazole with its partial agonist activity on the dopamine receptors may enhance the dopamine activity in the brain, especially in a patient previously treated with a dopamine receptor antagonist as olanzapine. The etiologic hypothesis are discussed with regard on the pharmacological effect of the drugs and their relationships with the different receptors.

P406

Neurological side effects associated with métoclopramide in pediatric population

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Introduction: Metoclopramide is an antiemetic drug widely used in hospital by physicians. It's a central and peripheral acting dopamine antagonist whose main side effects are related to the central nervous system action. The aim of this study is to describethe prevalence and evolution of neurological adverse events associated with the use of métoclopramide in the pediatric population.

Patients and method: A transversal study was carried out in two pediatric department of university hospital Ibnou Rochd of Casablanca between July and October 2007 regarding the neurological adverse reactionsassociated with the métoclopramide administration.

Results: On1 063 hospitalizations, 73 poisoned including eight cases of pediatric poisoning. The mean age was 5.9 years with a male predominance (sex ratio M/F = 1.6). The routes of administration were oral (five cases), rectal (two cases), intramuscular (one case). The median daily dose was 20 mg [10–30 mg]. The acute extrapyramidal dystonia appeared in the first 24 h of treatment in 87.5% of cases. Others neurological symptoms were acute dyskinesia (five cases), tonic-clonics seizures (two cases), generalized hypertonia (two cases), localized hypertension (one case), oculogyric movements (one case), drowsiness (one case), opisthotonos (one case), non reactive mydriasis (one case) and difficulty swallowing (one case). The evolution, for all children was favorable without drug treatment. The median duration of hospitalization was 24 h.

Discussions: The frequency of occurrence of extrapyramidal syndrome in children is relatively rare, with a favorable outcome even without treatment. Hospitalization being advocated as toxic dose.

P407

Blood derived drugs: a law for which data recorded in the French pharmacovigilance database?

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Introduction: The activity of the Regional Pharmacovigilance Centres bases essentially on the spontaneous notifications within health care establishments about drugs with post-marketing authorization. Blood derived drugs (BDD) belongs to our scope since they have the status of drugs, with the particularity to be manufactured from human blood. They are subjected to the same rules of pharmacovigilance (PV) as the others compounds, but all spontaneous notifications must be recorded in our national data base in the 24 h following the event, accompanied with traceability.

Materials and Methods: the aim of this study was to get a picture of all the BDD's data recorded in our national PV database over 1 year. We choose the 2009 year in order to have data with a good informativity, because our data base was modified in 2007.

Results: Four hundred and twenty-six spontaneous notifications (SN) have been recorded in 2009 implicating BDD, corresponding to 13.74 SN/regional centre. The mean age of the population was 47 years [08–89]. The distribution was as follow: 346 SN for immunoglobulin, 42 for albumin, 28 for coagulation factors, 10 for inhibitor of coagulation and two for biological glue. The adverse effects described were in accordance with the summary of product characteristic. The notion of seriousness was reported between 30% and 60% of cases. But there was a lack of information about the final evolution for all BDD between 14% and 40% of the cases. 0.5% of the death were considered as secondary to the effect. In details, the information about the modality of administration was always lacking as well as the number of the treatment course.

Conclusion: The study clearly shows that even with a strict regulation, under notification for BDD was observed and a lack of monitoring from pharmacovigilant in order to complete these cases. There are certainly some improvements to do with healthcare professionals to have more spontaneous notifications and with pharmacovigilants to ameliorate the informativity of the cases.

P408

Childhood bullous pemphigoid developed after rotavirus vaccin

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Introduction Bullous pemphigoid is the most frequent acquired autoimmune subepidermal blistering disorder which predominantly affects the elderly and rarely occurs in childhood. Bullous pemphigoid is characterized by the presence of homogeneous linear depositions of C3 and immunoglobulins at the dermoepidermal junction.

Observation An Algerian 3-month-old boy, with no medical history, living in Germany with nonconsanguineous parents, was vaccinated with a rotavirus live oral vaccin on the 28th of June 2011. One month later, he developed a blistering eruption initially affecting the extremities.

The infant was hospitalized on the 7th of August 2011 for severe diarrhea with vomiting and worsening of cutaneous state with tense bullae with clear contents on erythemic bottom. There where no infection notion, serology (HSV, VZV, and parvovirus) and bullae cultures where negative.

On the digestive aspect, stool virology showed the presence of rotavirus. The results of laboratory tests revealed an important eosinophilia of 3500/mm^3 with thrombocytosis, without inflammatory syndrome. The elevated serum level of anti-PB 180 antibody and skin biopsy confirmed the diagnosis of bullous pemphigoid.

The infant was initially treated with topical corticosteroids because of the context of infection tract. On the 12th of August 2011, the patient was transferred to Germany, where he was treated by systemic corticosteroids (1 mg/kg). Initial improvement of symptoms and relapse when steroids treatment decrease was followed by the introduction of treatment with dapsone, mycophenolate mofetil and immunoglobulins, without relapse at 3 months.

Discussion Infections, medications or vaccinations are suspected in the occurrence of bullous pemphigoid. The clinical presentation with acral initial lesions is typical in childhood bullous pemphigoid. The general trend is rapidly favorable under oral corticosteroid therapy without relapse. This case describes a very severe skin disorder occurring 1 month after a vaccination to Rotavirus and unusual evolution, requiring concomitant administration of antibiotic treatment, immunosuppressant and immunoglobulin.

P409

Differential safety profiles of loratadine and its active metabolite, desloratadine

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Objectif: Can two structurally related molecules cause different adverse effects? This is the question we addressed by studying the iatrogenic profile of loratadine and its metabolite, descarboethoxyloratadine, more widely known as desloratadine. These two compounds are prescribed for the symptomatic treatment of allergic rhinitis and chronic idiopathic urticaria.

Methods: This was a retrospective analysis comparing the adverse effects of loratadine and desloratadine notified to 31 Regional Pharmacovigilance Centres between the dates they were put on the market and 22 September 2011, and collated via the National Pharmacovigilance Database. After sorting the data by organ class (SOC), the percentages of adverse effects with each drug were calculated and compared according to $P \leq 0.05$. Cases where only loratadine and desloratadine were considered to be suspect were included whatever their imputability.

Results: This study involved 231 patients and 350 adverse events. In the loratadine group (L), 178 adverse events were notified in 120 patients (72 women and 48 men) with a mean age of 34 years (0–94 years). In the desloratadine group (D), 172 adverse events were notified in 111 patients (69 women and 40 men) with a mean age of 39 years (3–91 years).

Some adverse effects were more frequently described with (L) than with (D) and included palpitations, tachycardia, allergic oedema, blood pressure changes, weight gain, hyperprolactinemia and psychiatric disorders. Conversely, cases of hepatitis, abdominal pain, myalgia, dyspnoea, headache and drowsiness were more often notified with (D) than with (L).

Discussion-conclusion: This study revealed differences in the safety profiles of desloratadine and loratadine. Desloratadine is 10–20 times more potent vs. H1 receptors than loratadine, which may explain the headaches and drowsiness observed with this compound. In the absence of bibliographical data, it is only possible to hypotheses regarding the presence of a carboethoxy- group to explain the allergic oedema observed with loratadine. The differential pharmacodynamic activities of the two drugs on muscarinic, histamine, serotonin and dopamine receptors may explain some of the other adverse effects. Comparative studies in humans of the pharmacodynamics of desloratadine vs. loratadine would provide an answer to these questions.

P410

Myocarditis and/or pericarditis under mesalazine treatment: a review of the French pharmacovigilance database

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Objective: Mesalazine is a common derivate of five aminosalicylic acid used in Crohn's disease and hemorrhagic rectocolitis (HRC). This intestinal anti-inflammatory is associated with a rare incidence of heart adverse reactions. The aim of this study was to analyze myocarditis and/or pericarditis cases under mesalazine treatment recorded in the French Pharmacovigilance Database (FPVD).

Methods: We used the FPVD to select all the reports of 'Cardiac disorders' (MedDRA terminology) in which mesalazine was suspected, from January 1984 to November 2011. We collected type and severity of cardiac disease, other associated suspected drugs, time to onset, evolution and characteristics of patients (age, gender).

Results: Among the 38 case reports retained for analysis, there were 16 (42%) pericarditis, 10 (26%) myopericarditis, 8 (21%) myocarditis and four cardiomyopathies not detailed. Mean age of patients was 34 (15–77) and sex ratio was 1. Thirty-six (95%) cases were recorded as 'serious', leading to hospitalization or being life threatening. Concomitant disorders (fever, hypereosinophilia, pulmonary disease) were reported for several cases. There were other associated suspected drugs in only four cases. Time to onset was documented in 27 (71%) cases and varied from 1 day to 8 years. For 21 (78%) of these cases, cardiac diseases had appeared in the two first months of treatment. Evolution of cardiac diseases, known for 36 (95%) cases, was most often full recovery (spontaneous or under corticosteroids). We found two cases of positive rechallenge.

Discussion: Several cases of mesalazine-induced pericarditis and/or myocarditis were described in the literature, generally during the first weeks of treatment but on occasion, following treatment extending over years. Analysis of cases serie extracted from the FPVD allows to precise the characteristics of these adverse reactions and their potential seriousness. Similarities between reports of the FPVD and literature cases can be underlined: type of mesalazine-induced cardiac disease (myocarditis, pericarditis), time to onset, clear improvemet following the discontinuation of the drug and potential mechanism of hypersensibility.

Conclusion: Physicians must be aware of these rare but serious adverse reactions, even after a chronic treatment with mesalazine.

P411

Validity of a risk scale for clinical trials

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Introduction: A risk scale for clinical trials (CTs) sponsored by our teaching hospitals has been developed. This scale classifies CTs into four categories of increasing risk from I to IV according to the type of CT (medicine, medical device, medical imaging, etc.). This scale is used to evaluate a priori the workload of each CT and thus the budget needed for the CT safety part. The objective of this study was to assess the validity of this risk scale.

Method: A retrospective study on the risk of CTs conducted in our teaching hospitals between January 1, 2007 and June 30, 2010 was carried out. The scale validity was assessed by the agreement between the estimated risk according to the risk scale and the observed risk, represented by the rate of serious adverse events (SAEs) using Kappa coefficient. The inter-observer reproducibility was also tested by five observers. The results were compared to those obtained with the Optimon scale, a grid designed to evaluate the patient risk during CT and to adapt the monitoring.

Results: Eighty-two CTs were included with an average number of subjects included of 152.6, a mean duration of 1.7 years and a mean rate of SAEs of 7.3/100 patients/year. For the physiological CTs, the agreement between the estimated and the observed risks was rather good (30 CTs well classified over 56). For the CTs on medicines (six CTs well classified over 20) or medical devices (two CTs well classified over 12), the agreement was poor. For all CTs, the Kappa agreement between the estimated and the observed risks was 0.24, whereas it was 0.26 with the Optimon scale. For the inter-observer reproducibility, the Kappa agreement was high (0.63) with our risk scale and moderate (0.24) with the Optimon scale.

Discussion: Our risk scale is more user-friendly than the Optimon scale, with a greater reproducibility and an almost identical validity but it could be improved for CTs on medicines and medical devices. The next step will be to evaluate a new version of the risk scale on a greater number of CTs, within the Interregional Clinical Research Directorate.

P412

NADIS, a tool to reduce the under-reporting rate of Adverse Drug Reactions induced by Anti- Retroviral drugs: Experience in Midi-Pyrénées area

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Introduction: Anti-Retroviral Drugs (ARD) are well-known to induce Adverse Drugs (ADRs), often under-reported. NADIS is a national database used for monitoring patients affected by HIV (Human immunodeficiency Virus), allowing physicians to share biological and medical data and to report ADRs related to ARD. For patients followed in Midi-Pyrénées, each notification is collected by the Regional Center of Pharmacovigilance of Toulouse. The aim of the study was to describe ADRs collected via Nadis.

Materials and methods: ADRs reported by physicians via NADIS were collected during 1 year from November 2010 to November 2011. For each report, data about demographic data, type of ADRs according to System Organ Class, their severity, duration and evolution, suspected HAART taken and the delay of occurrence of ADRs were collected.

Results: A total of 90 ADRs were reported in 82 patients. Mean age was 48.2 years (SD = 9.6; min = 27; max = 80) the sex ratio was 2.2 (H/F). In 4 (4.4%) cases, ADRs were 'unexpected' and 43 cases (47.8%) were classed as 'serious'. ADRs were: gastrointestinal (24.4%), renal (20.0%), neuro-psychiatric (15.6%), liver (13.3%), cutaneous (11.1%) and others (15.6%). Drugs suspected were classed as following: Nucleoside (33.2%) and Nucleotide (14.3%) Reverse Transcriptase Inhibitors (NRTIs), Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) (9.2%), Protease Inhibitors (39.3%) (PIs), Integrase Inhibitors (3.5%) and CCR5 Inhibitors (0.5%).

Discussion: ADRs reported with HAART were mostly 'non-serious' (52.2%) and 'expected' (95.6%) but they could affect the quality of life of the patient. Compliance is a major factor in the effectiveness of HAART. The diagnosis and management of ADRs could contribute to better monitoring of HIV patients. NADIS could be an interesting tool to reduce under-reporting of ADRs.

P413

Cutaneous reactions associated with aromatic anti-epileptic drugs notified in regional center of pharmacovigilance of Sfax during 4 years

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Introduction: Reports of serious cutaneous reactions (DRESS syndrome, Stevens-Johnson syndrome) continue to be reported in patients treated with the aromatic anticonvulsants, such as phenytoin, phenobarbital, and carbamazepine.

Methods: The French imputation method was used to conclude the responsibility of drugs. The aim of our study was to analyse cutaneous side effects of aromatic anti-epileptic drugs notified in the regional pharmacovigilance center of Sfax from January 2008 to November 2011.

Result: Eight hundred and sixty-five cases of drug side effects had been notified in our regional center during 4 years. In this period, 23 cases of cutaneous reactions were associated with aromatic anti-epileptic drugs (2.6%). The responsibility of these drugs was retained in all these cases. The average age of these patients was 43 ± 15 years with a sex ratio of 0.64 (9 H, 14 F). DRESS syndrome was reported in nine cases (39%), maculopapular rash in eight cases (43.7%), Stevens-Johnson syndrome in three cases (13%), erythrodermia in two cases (9%) and fixed pigmented erythema in one case (4.3%). Carbamazepine was the most causative aromatic anti-epileptic drug (15 cases), followed by phenobarbital (eight cases).

Discussion: Some cutaneous reactions related to the administration of aromatic antiepileptic drugs can be severe. Therefore, physicians must be aware of such side effects and inform patients of this potential risk.

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Acute hepatitis associated with microvesicular steatosis induced by Fenofibrate

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Introduction: Fibrates are used for the treatment of dyslipidemia and its actions are believed to be mediated by the activation of peroxisome proliferator-activated receptor α (PPARα). Fenofibrate-induced acute or chronic hepatitis is rare. We report a rare case of severe acute hepatitis following exposure to fenofibrate.

Case report: A 47-year-old woman was admitted on Marsh 08, 2011 for itching, dark urine and jaundice. She had a 2-year history of hypertension treated with nifedipine. Fenofibrate was prescribed due to hyperlipidemia 45 days prior to the admission. Laboratory profile on admission showed serum total bilirubin (TB) 311 μM (reference range: 2–17 μM), direct bilirubin (DB) 184 μM (2–5 μM), alanine aminotransferase (ALT) 240 IU/L (0–40 IU/L), aspartate aminotransferase (AST) 180 IU/L (5–45 IU/L), alkaline phosphatase (ALK-P) 200 IU/L (10–100 IU/L), gammaglutamyl transpeptidase (GGT) 1100 IU/L (4–61 IU/L). Liver histology showed hepatocellular damage with inflammatory infiltration and microvesicular steatosis without fibrosis. Fenofibrate was discontinued due to the suspected etiology of acute hepatitis. Her clinical manifestations and liver function tests improved gradually and returned to nearly normal in 2 months.

Discussion: It has been hypothesized that fibrate-induced hepatotoxicity could be caused by mitochondrial dysfunction and ATP deficiency rather than direct interaction of fibrates PPARα. Liver function tests should be monitored in patients receiving fenofibrate therapy.

P415

Spontaneous reporting with methylphenidate: off-label use in growth?

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Introduction: Methylphenidate is a piperidine derivative structurally and pharmacologically similar to amphetamin. Methylphenidate is usually prescribed for children aged over 6 years affected by ADHD (Attention Deficit Hyperactivity Disorder). In adults, its indication, except in narcolepsy, is not defined. Methylphenidate received its regulatory approval 50 years ago with a first registration in Switzerland in October 1954. In France, methylphenidate was launched in 1995 with restriction conditions of prescription and delivery.

Objective: To evaluate data obtained by spontaneous reporting in patients under methylphenidate treatment.

Methods: This study analysed data from the French Pharmacovigilance Database of adverse drug reactions spontaneously reported by health professionals from 1985 until December 2010. Off-label use was evaluated with respect to age, dose, indication and frequency of administration.

Results: Methylphenidate is suspected in 133 observations. One hundred and six children are concerned, 87 boys and 19 girls (mean age: 10 ± 3 years), and 27 adults, 18 men and nine women. Neuropsychiatric effects were the most frequent adverse effects reported (n = 55, 41.35%) followed by cardiovascular and cutaneous side effects (15.04% and 15% respectively). Off-label use is frequently observed (37.7%).

Discussion-conclusion: Methylphenidate has been marketed for more than 50 years. However, we have no information related to long term outcome as decreased rate of growth, effect on final height, delayed sexual maturity, cerebrovascular toxicity. The pharmacovigilance based on the spontaneous adverse drug reactions reporting is not appropriated to resolve these questions. Additional long term exposures and independent clinical studies are necessary to establish the long term profile safety of methylphenidate. ADHD diagnostic and methylphenidate indication should be more respected and guidelines prescription, handling status controlled. A proactive information of the different stakeholders is needed.

P416

Renal papillary necrosis following therapy with both ciprofloxacin and niflumic acid: case report

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Introduction: Renal papillary necrosis (RPN) is a significant problem in human beings. Many compounds, including non-steroidal antiinflammatory drugs (NSAID) and quinolones have been linked to RPN. We report a case of RPN following therapy with both ciprofloxacin and niflumic acid.

Case report: A 16-year-old girl was admitted to the nephrology department for acute renal failure. There was nothing significant in her past medical history, except the diagnosis of renal infection 15 days prior to admission treated with both oral ciprofloxacin and intrarectal niflumic acid during 7 days. At admission, investigations revealed serum sodium 144 mM, potassium 5.3 mM, serum creatinine 907 μM, urine pH 6 with no proteinuria or glucosuria. The blood pressure was 112/88 mmHg and temperature 36.7°C. She presented no clinical signs of dehydration. Ultrasonography examination revealed normal kidneys. Kidney biopsy revealed RPN with birefringent crystals in some tubuli.

A single dialysis session and intravenous hydration were performed for this patient. During hospitalization, the serum creatinine levels decreased to 156 μM on the 10th days after admission.

Discussion: The mechanism of this RPN is not completely understood. A possibly direct local toxic effect of niflumic acid and ciprofloxacin may cause papillary necrosis exacerbed by a decrease synthesis of renal prostaglandins by NSAID.

The report highlights the need for caution while using NSAID or ciprofloxacin, even for a short duration.

P417

Severe pulmonary arterial hypertension associated with interferon beta 1a therapy for multiple Sclerosis: first case report

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Introduction: Until now, a few cases of pulmonary arterial hypertension (PAH) linked to an interferon (IF) therapy have been reported. Only one case has been described with an β1b IF treatment for multiple sclerosis (MS). We reported the first case of the occurrence of PAH in a woman with MS during treatment with β1a IF.

Observation: A 48 year old woman with a relapsing-remitting course of MS from 2002 had been treated during 7 years until end of 2009 without any trouble with β1a IF (44 μg subcutaneously three times a week). The ECG in 2007 was normal. In January 2010, an exertional dyspnoea appeared (NYHA III). ECG showed: right axis deviation, complete right-bundle-branch block. Echocardiography showed: right ventricle and atrium dilated, grade IV tricuspid valve insufficiency and severe PAH (95 mmHg) confirmed by catheterisation. No history of pulmonary embolism or appetite suppressant drugs intake was found. HIV tests were negative. The lung scintigraphy was normal. β1a IF was withdrawn in May 2010.

A treatment with diuretics, bosentan and sildenafil was begun. Until now, the dyspnea has been markedly improved with no relapse of the MS. The patient has partially responded to the treatment. Echocardiography in June 2011 showed: decrease of the dilatation of the right ventricle, grade I tricuspid valve insufficiency and improved PAH (60 mmHg).

Discussion: In animal studies, the infusion of α IF into the pulmonary vessels induced PAH by stimulating the thromboxane cascade and the secretion of mediators of inflammation.

A possible role of microangiopathy induced by α IF (or IFs in general) cannot be ruled out.

Despite the very rare occurrence of cases of PAH linked to β1 IF (one with β1b IF in 2009 and the present case with β1a IF), we consider that given the seriousness of the clinical symptoms, the slightest exertion dyspnoea in a patient treated with IF should prompt the physician to check for this rare complication even though the β1 IF treatment had been well tolerated for several years. Cardiac echography should at least be performed (followed perhaps by right catheterization) if there is no other explanation for dyspnoea in MS patients.

P418

Pseudoephedrine-induced pigmented purpuric dermatosis: a case report

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Introduction: We report an exceptional case of pigmented purpuric dermatosis (PPD) induced by pseudoephedrine.

Case report: A 70-year-old man has presented a generalized macular eruption with erythema and oedema on the lower limbs, 15 h after starting self-medication with paracetamol 500 mg and pseudoephedrine 30 mg, for a catarrhal infection. Two days later, a petechial eruption at all the lower limbs and at the umbilical region was observed with multiple and confluent purpuric macules on his legs and purpuric papules eruption on his fingers. The reaction was resolved within 7 days with an oral anti-histamine drug and was followed by desquamation of the affected areas with residual melanotic pigmentation. Two months later, patch tests to pseudoephedrine and paracetamol were positive only to pseudoephedrine. A biopsy of this positive reaction, revealed a lymphocytic perivascular infiltration with extravasation of erythrocytes. The patient refused the application of patch tests to other sympathomimetic drugs.

Conclusion: The diagnosis of PPD due to pseudoephedrine was retained. Therefore, he was instructed to ovoid all products containing pseudoephedrine and ephedrine.

P419

Reconcialiation, a key against an iatrogenic hospital?

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Contexte: Data suggest that medication errors are a significant, and often preventable, contributor to errors in the Emergency Departement (ED).

Objective: Analyse the continuity of treatments for unscheduled patients from admission via the ED to discharge, identify and prevent eventual iatrogenic effects.

Method: In collaboration with ED, the pharmacovigilance center led a Professional Practice Evaluation during a 6 weeks prospective study in randomized selected patients. The outpatient prescriptions, treatments prescribed and administered during the first 72 h, as appropriate, and the output prescriptions were documented.

A Prescription Drug Review (PDR) was achieved with the outpatient prescription forms and the patients interviews. Patients' pharmacists or physicians were also contacted. All discrepancies between the collected data and inpatients prescriptions were analysed and justifications for changes collected. We also recorded the hospitalisation motives.

Results: Two hundred and twenty-four patients were enrolled in the study, mean age 58.8, [16–100], 93 patients over 65. Hospitalisation was due to a drug's adverse effect (AE) for 8% of them. On admission, 193 patients had a treatment (86%, and 98% after 65 years) but <33% provided a prescription form. Patients interviews was impossible due to medical condition in 79 cases (35%). The outpatient treatments were unchanged in 32 cases (17%) and intentionally modified with justification in 70%: some unjustified discrepancies involved insulin, antihypertensive and oral anticancer drugs. The PDR also showed that 20% of patients brought their own medications without the knowledge of the teams.

Discussion – Conclusion: this study sensitized the professional to difficulties, inherent potential risks, of medication recollection and necessity to improve their practices. The PDR and the medication reconciliation, allow detection and prevention of eventual AE. It is thus a key element to improve medical care, but the sustainability of this approach could be facilitated by access to shared folder such as the outpatient Pharmaceutical File or the implication of pharmacists in the ED work.

Reference:

1. Ruth Mills P, Crawford McGuffie A. Formal medicine reconciliation within the emergency department reduces the medication error rates for emergency admissions. Emerg Med J 2010;27:911–915.

P420

Cost approach to iatrogenic drug

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Introduction: The consequences of iatrogenic effects in terme of public health are important given the frequency of the problem. Its impact in terms of morbidity, functional decline and mortality as well as economic and social repercusions.

Object: Our study was conducted to quantify the overhead of managements of iatrogenic drug within the university hospital, identify presdisposing factors and therapeutic classes implicated in order to make suggestions for prevention, warning and advice on good prescribing practicies. We can thus reduce in terms of preventable iatrogenic side effects.

Methods: The notification was requested through our collection of adverse events of various departements of the hospital, with sheets of reporting side effects, incuding a section for estimating the cost of treatement effect. Only the direct cost was calculated.

Results: The overral average cost of 182 cases collected during the study periode (8 months) was about 25 340 Euros. This cost is largely underestimated at first by the under-reporting of adverse events and a lack of support for these side effects whatever in terme of diagnosis and monitoring. Liver side effects alone have gernerated an average cost of about 12 236 Euros, followed by skin disorders (that exceed the frequency of occurence 30%) wich cost reached 5589 and over 3000 Euros for neuroligical and hematological side effects. Therapeutic classes accounting for more than iatrogenic cost are antibiotics, antineoplasics, corticoides and antipsychotics.

Discuss: A reconciliation of the reality of the impact of the extra cost and no side effect requires an awarness of the health professionals in the interest of the reporting of adverse events or misuse and collaboration with pharmacovigilance unit to preventable cases and reduce unnecessary expenditures.

P421

Methysergide associated valvular heart disease. A case of mitral regurgitation diagnosed after long-term treatment of a patient with cluster headaches

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Introduction: Methysergide, an hemisynthetic ergot derivative, was the first drug reported in the literature to be associated with valvular heart disease (first cases reported in 1966). We report a case recently notified of such a valvular disease described after a long-term methysergide treatment. In this case, clinical, echocardiographic, macroscopic and histological findings were very similar to those described with various drugs with 5-HT_{2b} agonistic properties.

Observation: A 48-year-old woman was referred for a severe and progressively increasing exertion dyspnea, palpitations and progressive need to reduce her physical activity. Clinical examination revealed apical holosystolic III/IV mitral murmur associated diastolic murmur. Echocardiographic findings were thickened mitral valve leaflets with restriction in motion and involvement of subvalvular apparatus. These alterations resulted in grade III mitral regurgitation associated with mild mitral stenosis. A grade II tricuspid regurgitation was also found with pulmonary artery systolic pressure estimated to be of 65 mmHg. These findings were confirmed by ventriculography and right cardiac catheterism. The patient underwent mitral valve replacement. Macroscopic examination showed mitral valve fibrosis with thickening of chordae tendinae. Histopathological examination of the valve demonstrated valvular fibrosis with fibromyxoïd dystrophy and nodular hyaline deposits of extracellular matrix.

Discussion: The valvular heart disease (mitral regurgitation necessitating valve replacement associated with pulmonary arterial hypertension) reported here had the same characteristics than those described with fenfluramines and other ergot derivatives. This case was observed in a patient treated since 10 years by methysergide (for cluster headaches) without any period of wash-out ('drug holidays') as recommended. Valvular heart diseases in this setting may be isolated as in this patient or may be associated with other fibrotic processes such as pericardial, pleuro-pulmonary and retroperitoneal fibrosis. Such adverse effects may be taken into account in the evaluation of the risk-benefit ratio of the drug.

P422

Delayed cutaneous allergy reactions to iodinated contrast media:to simplify drug skin testing

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Introduction: Late adverse reactions to radio contrast media (RCM) are not yet well documented. There are no guidelines concerning drug skin testing.

Study and results: We have studied retrospectively the files of 22 patients with delayed reaction after enhanced radiologic examination. For each patient RCM was the only drug imputable. The main clinical feature was maculopapular rash. We diagnosed acute generalized exanthematous pustulosis (AGEP) in three cases. Patch tests, prick tests and intra dermal tests (IDT) with immediate and delayed readings, were performed with the incriminated RCM and a series of five different RCM. Distant from skin testing, a RCM with negative IDT and different from the one imputable was intravenously administered under hospital surveillance.

Skin tests were positive in 11 cases: prick tests at delayed reading in two cases, delayed IDT in 10 cases, and patch tests for four patients. Cross allergies were diagnosed in four cases. Skin tests were always positive in AGEP, and in half of the maculopapular rashes. Twenty-nine patients underwent another RCM administration after skin testing. No eruption's relapse occurred.

Conclusion: To simplify skin testing in delayed adverse reaction to RCM we propose to stop performing prick tests and patch tests. IDT have to be done with delayed reading.

We put forward the idea that the intravenous administration of an alternative RCM with negative IDT in cases of benign eruption could be carried out directly by the radiologist.

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Spontaneous reporting of drug-induced osteoporosis or osteomalacia: review of the French pharmacovigilance database

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Introducton: Long-term exposure to some drugs like corticosteroids can induce osteoporosis or other bone disorders. The aim of this study was to identify drugs and risk factors more frequently involved in the development of these adverse drug reactions (ADR) in clinical practice.

Methods: In the French National Pharmacovigilance database, we identified spontaneous case reports of drug-induced bone disorders, according to the System and Organ Classification of MedDRA 14.0 dictionary and registered until the 15th of september 2011. In these reports, we analyzed suspected drugs, main features of cases, differences between osteomalacia and osteoporosis and association or not to corticosteroids.

Results: We found 256 cases (217 osteoporosis and 39 osteomalacia). 60.2% were female. Median age was 64 years (8 months–95 years) and mean body mass index was: 21.8 ± 0.7 kg/m^2 (n = 54). There was no other risk factor in 63.7% of cases. The main ADRs reported were osteomalacia (15.2%) and osteoporosis (84.8%). Mean latency period to both ADRs was 1077.2 ± 107.1 days, clearly longer in the osteomalacia reports (1933.2 ± 395.5 days) than in the osteoporosis reports (859.7 ± 84.0 days). Nearly half (46.5%) of the reports analysed did not include corticosteroids. Absence of corticosteroids was more frequent in osteomalacia than in osteoporosis reports (92.3% vs. 38.2%). Fracture was reported in 47.7 of cases (41.0% with osteomalacia vs. 48.8% with osteoporosis). The mean of suspected drugs included in each report was 2.6 ± 0.1. Apart from corticosteroids, the more common suspected drugs were anti-retroviral (23.4%), antiepileptic (5.6%), immunosuppressant (3.4%), proton pump inhibitors and other drugs for acid related disorders (3.2%) and antithrombotic (3.2%) drugs.

Conclusion: In a high number of cases, osteomalacia or osteoporosis were not associated with corticosteroids. Anti-retroviral and antiepileptics drugs were the most frequent non-corticosteroid drugs involved. The possibility of development of bone disorders should be kept in mind during long-term treatment with these drugs.

P424

Safety of statins in pregnancy

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Introduction: In France, 19.9% of 18–24 years old women and 26.8% of the 30–54 years old women present a dyslipidemia. This high incidence and pharmaceutical company efforts to promote statins for primary and secondary cardiovascular disease prevention are leading to increased pregnant women exposition.

Although statins have been identified as potential teratogens on the basis of theoretical considerations concerning the role of cholesterol in embryo development and small case series, few data exist on their safety in human gestation. A retrospective cases series analyse from the FDA lists 22 cases of major birth defects and in particular a VACTERL syndrome. Which advice can we give to the pregnant patients treated with statins?

Methods: To estimate the safety of statins, we use the French Terappel Database (which records since 1984 requests from health professionals to Regional Centres of PharmacoVigilance about women exposed to drugs during pregnancy). Cases were all the pregnancies between 1984 and 2010 exposed to statins (simvastatin, fluvastatin, atorvastatin, pravastatin, rosuvastatin) during the first trimester and registered in TERAPPEL*.

Results: In TERAPPEL, we have located 172 pregnancies exposed to statins with 56 lost to follow up, 84 babies born without malformations, seven babies with major birth defects, two deaths in utero, 16 miscarriages and five medical termination of pregnancy. Any specific pattern of birth defects has been detected.

Conclusion: Terappel does not reveal an increased risk of malformation with statins in pregnancy. Others prospective studies (Wolgang et al 2004; Taguchi et al 2008; Winterfeld et al. 2010) counting respectively 44, 64 and 249 women treated with statins don't detected a teratogenic effect. But today, data are insufficient to stay that there is no risk and more controlled studies are needed to investigate the teratogenicity of individual drugs in this class. However in case of inadvertent exposure, we can be reassuring.

*The data until 2007 from Lyon and Fernand Widal have in the course of publication by Winterfeld U et al.

P425

Acetaminophen-induced fixed drug eruption

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Introduction: Fixed drug eruption (FDE) is a common cutaneous adverse drug reaction characterized by one or few round, sharply demarcated erythematous and edematous plaques, sometimes with a central blister. Lesions recur at the same sites with rechallenge with the causative drug. The drugs the most frequently associated with FDE are tetracyclines, sulfonamides and carbamazepine. Few cases of acetaminophen-induced FDE have been reported. We describe a new case.

Case report: A 62-year-old man with a medical history of hypertension, venous insufficiency and pollinosis, started a treatment for a cold with Actifed rhume jour et nuit® (acetaminophen-pseudoephedrine, acetaminophen-diphenhydramine). Three days after treatment initiation, the patient presented pruriginous lesions localized on his back, trunk, and lower and upper limbs. Medication was stopped. Skin lesions resolved within a month under topical corticosteroids. Eight months later, patch-tests were performed with Perfalgan® (acetaminophen), Actifed rhume jour et nuit® and allergens of the European Standard Series with negative results. A few days later, a rechallenge test with acetaminophen was conducted. The patient developed a delayed macular, erythematous rash on the trunk and back with some pigmented areas. A skin biopsy of a pigmented lesion was carried out and revealed a FDE. A complete remission was observed within 2 months. Acetaminophen administration was definitively stopped.

Discussion: Acetaminophen-induced FDE is relatively rare. It has already been described in the literature and seems to affect children. In a recent European multicentric study on FDE in a hospital setting over 3 years, the most common causative drug among 59 reported cases of FDE was acetaminophen. Moreover, 3 years ago, a team reported that among the 307 cases of FDE (with positive rechallenge) of the French pharmacovigilance database, acetaminophen was implicated in 15% of the observations. Nowadays the database reports 71 cases of FDE where acetaminophen is implicated. In 46% of these reports, acetaminophen is the only suspected drug. As acetaminophen is widely prescribed and used over the counter, clinicians should be aware of this type of adverse reaction, especially since it is not mentioned in the core data sheet.

P427

Dipeptidyl peptidase IV inhibitors and skin reactions: a typical DRESS syndrome

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Introduction: Dipeptidyl peptidase IV (DPP-4) is ubiquitously expressed in various tissues and is upregulated during functional activation of different cell lines, including T-cells and endothelia. In the skin, many cell types including keratinocytes constitutionally express DPP-4. Some reports of adverse skin reactions in patients treated with DPP-4 (gliptins) have increased awareness towards skin-targeting side-effects of these anti-hyperglycaemic drugs. Herein we report a typical case of DRESS syndrome.

Case report: A 67-year-old female patient with morbid obesity (BMI 43), T2DM, hypertension and asthma was treated with metformin (2 550 mg *tid*), nebivolol (5 mg od) candesartan/hydrochlorothiazide (15 mg/12.5 mg od), lercanidipine (20 mg od) and budesonide/formoterol (400 µg/12 mg) for several years. She started vildagliptin/metformin 50 mg/1000 mg (*bid*) in June 2011. In September, she presented a trunk rash and severe pruritus treated by topical corticosteroids and antihistaminics. One month later, her general condition impaired with fever and dysphagia. Vildagliptin/metformin and nebivolol were stopped. Laboratory tests showed eosinophilia (2 400/mm3) and creatininemia at 378 µM. She was hospitalized in intensive care unit with oligoanuria and 80% skin necrolysis with positive Nikolsky sign, suggesting a toxic epidermal necrolysis. Following lactic acidosis, she received insulin, albumin and systemic corticosteroids. A course of IV immunoglobulin was performed without disease improvement. At this time, laboratory tests showed an increase of ASAT (219 UI/L) and ALAT (128 UI/L), monocytosis (1842/mm^3), atypical lymphocytes and HSV6 positivity. Histological examination revealed moderate edema with infiltration of eosinophils, in favor of DRESS syndrome. The patient condition was aggravated with pleuropulmonary damage. Despite multiple organ failure and two others episodes of skin exfoliation, her condition improved progressively under corrective treatment.

Conclusion: Drug eruptions induced by DPP-4 inhibitors (gliptins) are not rare. It is worth noting that in the preclinical setting, gliptins induced 'blistering-necrotic' skin lesions in cynomolgus monkeys. The development of bullous/blistering dermatoses in T2DM patients on treatment with gliptins might be considered as a drug class adverse effect. The precise role of the increase of physiological incretins and the inhibition of DDP-4 in skin reactions remains to be defined.

P428

Hepatic adverse drug reactions: data from the hospital database in a teaching hospital

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Objective: to identify cases of hepatic adverse drug reactions (ADRs) in a hospital database; to compare these data to those reported to our pharmacovigilance centre.

Method: a cross-sectional retrospective study was conducted within the hospital database of our hospital, from January 1 to October 31, 2010. Selection criteria were hospital stays with preselected ICD-codes on general or specific hepatic diseases and on medicines. Exclusion criteria were codes evoking another explanation. Electronic discharge summaries and, when required medical records, were used to validate ADR cases. These data were compared to the cases reported to the pharmacovigilance centre.

Results: out of the 90 906 patients hospitalized, 648 cases were extracted from the hospital database. Of these, 61 were excluded. From the remaining 587 suspicions of cases, 553 (94.2%) were not validated as hepatic ADRs (cancer: 29%, alcohol: 14%, infection: 11%, another cause: 16%, past history of hepatic disease: 5%, insufficient data: 18%, unavailable discharge summary: 14%). Finally, only 32 cases (7%) were validated as hepatic ADR cases. Patients included had a mean age of 63 years (range: 22–95) and the *sex ratio* M/F was 0.6. More than 3/4 of cases (78.1%) were serious, with one hepatic transplant and one fatal outcome. There were 15 cases of cholestatic (mostly due to parenteral nutrition), seven of hepatocellular and 10 of mixed hepatitis. Suspected medicines (n = 38) belonged to the following organ-classes: blood and haematopoietic organs (26%), anti-infectives for systemic use (24%), nervous system (21%) cardiovascular system (8%) and others (21%).

A total of 37 hepatic ADR cases were reported to the pharmacovigilance centre: only seven cases were common to both systems (among which the transplanted and the fatal cases). The type of hepatitis in reported cases was less frequently cholestasis, the number of suspected medicines was 83 and their distribution different (anti-infectives for systemic use representing 43%, followed by nervous system medicines with 21%).

Discussion: Hepatic ADR cases, not easily identified in the hospital database, were well-known and underreported. A further study is needed to assess if a search focused on selected medical units could be more efficient.

P429

First report of clinical consequences of a triple-drug association: methylprednisolone, itraconazole and azithromycin

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Introduction: An interaction exists between azole antifungal drugs and dexamethasone, and is mentioned in the prescribing information of the latter because of clinical consequences, not observed with the other glucocorticoids. We report here a case of clinically significant interaction between itraconazole and methylprednisolone.

Observation: A 17-year old girl, suffering from mucoviscidosis, has been long-term treated with itraconazole, azithromycin and salbutamol. Hospitalized for the management of an acute respiratory distress syndrome, she was given a single intravenous dose of methylprednisolone. High blood pressure and bradycardia gradually appeared overnight, at first intermittently, becoming continuous after a few hours. An antihypertensive treatment was then started, and the dose of methylprednisolone reduced. Blood pressure and heart rate were back to normal on the third day. A drug interaction involving methylprednisolone was therefore envisaged.

Discussion: Itraconazole is, as well as all the azole antifungal drugs, a potent inhibitor of cytochromes P450 3A4 and 3A5 and so potentially modifies the metabolism of numerous drugs. Glucocorticoids, including methylprednisolone, are mainly metabolized by CYP3A4/5. Pharmacokinetics studies have shown that the plasmatic concentrations and the bioavailability of the corticoids can be dramatically increased when co-administered with itraconazole (1). Here, itraconazole was associated with azithromycin which is also a potent inhibitor of CYP3A4/5. The effects of an interaction involving enzymatic inhibition can theoretically be seen rapidly, sometimes as early as a few hours after the administration of the inhibitor, exposing the patient to a greater risk of adverse effects. In the literature, there are only isolated reports of Cushing's syndrome following several months of treatment with itraconazole and budesonide (2), and we found no reports of acute or subacute adverse effects similar to our patient's. This case is, to our knowledge, the first report describing an acute adverse effect of methylprednisolone in a patient treated with itraconazole and possibly potentiated with azithromycin.

References:

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2. Main KM et al. Acta Paediatr 2002; 91: 1008–11.

P430

A case report of enoxaparin induced skin necrosis and thrombocytosis with positive antibodies heparin-PF4

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Introduction: Skin necrosis is a rare side effect of low-molecular-weight-heparin (LMWH) and is mainly related to heparin induced thrombocytopenia (HIT).

Presentation: We report the case of a 47-year-old patient who has presented a skin necrosis with thrombocytosis during enoxaparin therapy. The patient was hospitalized for deliberate self poisoning. Preventive anticoagulation by enoxaparin was prescribed on January 3rd. On that day, the platelet count was 169 G/L. The next day, a decreased platelet count began, to reach a minimal of 33 G/L on January 7th. Nevertheless, enoxaparin was continued. On January 14th, an hematoma at the injection site was notified. In the same time, an increase of platelet count was detected at 702G/L. The next day, a skin necrosis was observed and enoxaparin therapy was stopped. Platelet count reached its maximum (1163 G/L) on January 21th before decreasing. After essential and secondary thrombocytosis have been eliminated (infection, cancer, chronic inflammatory disorders, acute hemorrhage, iron deficiency, drugs), HIT was suspected and antibodies heparin-platelet-factor 4 (PF4) complex were positive. Skin lesions improved in the same time.

Discussion: This cases emphases the difficulty to diagnose LMWH-induced skin necrosis in combination with elevation of platelets and positive heparin-dependent antibodies. Heparin can induce skin necrosis not only by inducing intravascular platelet thrombi in a context of HIT, but also through type III hypersensitivity or by calcium salt precipitation. But in our case, the presence of heparin-PF4 antibodies is in favour of HIT. However, when patient presented thrombocytopenia at the beginning of enoxaparin therapy, it's difficult to confirm the diagnosis of type 1 or type 2 TIH. Indeed, no sign of thrombosis or renal insufficiency were present and previous patient's exposition to heparin remains unknown. Atypical reaction with thrombocytosis and skin necrosis should be known by clinicians. Moreover, contrary to thrombocytopenia, thrombocytosis induced by heparin is rarely reported in the literature with a mechanism not clearly known (interaction between heparin and PF4 complex, which inhibits thrombocytopoiesis). During heparinotherapy, in a context of thrombocytosis with clinical signs suggestive of thrombosis, clinicians should keep in mind to measure heparin-PF4 antibodies.

P431

Can the analysis of the french pharmacovigilance database provide the same conclusion as the analysis of the fda database? Muscle and tendon side effects related to statins

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Introduction: Statins inhibit HMG-CoA reductase, an enzyme involved in cholesterol synthesis. It is widely prescribed to prevent cardiovascular diseases. Muscle and tendon damage is a significant side effect of this class of drugs. A retrospective analysis of adverse drug reaction (ADR) related to statins and reported to the Food and Drug Administration (FDA) from January 2004 to March 2011 has been published in Reactions in November 2011 [1]. This publication concluded that there is a difference in frequency of muscle side effects function of the statin drug considered. Compared with the others statins, rosuvastatin has a relatively higher risk.

Methods: All muscle and tendons ADRs related to statin were collected in the French pharmacovigilance database (FPDB) from January 2004 to March 2011. This research concerned the following drugs: pravastatin, rosuvastatin, simvastatin, fluvastatin, atorvastatin. ADR specification was muscle damage, muscle and tendon lesion and elevated creatine phosphokinase as preferred term.

Percentage of ADRs for each statin was compared using Chi-square statistical test. All the cases collected were analyzed and results were compared with those of the FDA study.

Results Seven hundred and forty-four cases of muscle and tendon disorders related to statins have been recorded in the FPDB from January 2004 to March 2011, with 321 serious cases.

This side effect is linked to pravastatin intake in 17.2% of cases, 20.8% with atorvastatin, 22.1% with fluvastatin, 22.2% with simvastatin and 23.3% with rosuvastatin. There is no statistically significant difference between rosuvastatin, simvastatin, fluvastatin and atorvastatin. Anyway, we found a statistically significant difference between pravastatin and rosuvastatin ($P = 0.01$) and other statins ($P = 0.05$). Same statistically difference is found with a selection of serious cases only.

Conclusion: Identifying this specific side effect risks is important for the rechallenge of patients who have experienced muscle or tendon disorders with statins. Previous publication over FDA database concluded that rosuvastatin was at higher risk. Analysis of the FPDB provide the conclusion that pravastatin is at lower risk of muscle ADR.

Reference:

1. AdversEvents Inc. Results of an extensive safety review of the $19 billion U.S. statin drug class. Media release: 20 October 2011. Available from http://www.adverseevents.com.

P432

Drug-induced fever: analysis of data recorded in the national database of pharmacovigilance in 2010

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Objective: Drug-induced fever (DIF) is a febrile response that coincides with the administration of a drug and disappears after its discontinuation. DIF is frequently misdiagnosed and corresponds to a diagnosis of exclusion. The aim of this work is to determine the pharmacological classes most often implicated in the occurrence of these fevers, based on spontaneous notifications (SN) recorded in the french database of pharmacovigilance.

Method: This work focused on spontaneous reporting recorded during 2010. The search was made with the preferred terms 'fever' and 'mild fever', drugs considered as 'suspect' (WHO imputability). We used the second level of ATC classification to determinate pharmacological classes. Two hundred and sixty-six SN were identified. Cases concerning vaccines were excluded.

Results: Ninety-five notifications were studied. The mean age was 54.5 [0.5; 80] years, with a large predominance of male. In 25% of cases, patients presented benign/malignant tumour as principal medical history, 12% with vascular, 9% with nervous or with musculoskeletal and systemic diseases. Sixty percent of SN was considered as serious, but the evolution was 'without sequel' in 86% of cases. Fever was registered alone in 37% of cases and for all the others, it mainly associated with symptoms related to hypersensibility reactions or biological disorders. Concerning drugs implicated to fever, we have found 42 pharmacological classes with intrinsic imputability (II) equal to one in 68% of reporting. Irrespective of the II, only four classes were responsible for 60% of reporting: systemic antibacterial drug, antineoplastic agent, immune globulins and antifungal.

Discussion: This preliminary work realised during 1 year of registration shows that DIF are difficult to identify. Moreover these fevers are often accompanied with other adverse drug reactions and it is difficult to rechallenge the suspect drug. The literature is scarce on this subject. This work allows to highlight the majority responsibility of four pharmacological classes, but much more can be implicated in drug fevers, with a low intrinsic imputability. This study will be continued on all of the records of the national database of pharmacovigilance in order to prove or disprove the results.

P433

Actions to achieve continuous improvements in quality at the regional pharmacovigilance centre in Angers: impact measurements

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Objective: Internal audits carried out in 2009 in the Regional Pharmacovigilance Centre in Angers led to an evaluation of professional practices within this Centre. This evaluation identified several areas for improvement, such as the introduction of monthly meetings to review sensitive files within a lag time of 2 months. The objective of this study is to determine the impact of this internal control measure.

Methods: This was a descriptive study of changes to case reviews during monthly meetings. The files studied during this evaluation were cases reported to Angers of a particularly serious nature and/or with an unknown or ongoing outcome, between 01/01/2009 and 30/06/2011. These monthly meetings enabled decisions on changes to be made to a file or a need for long-term follow-up.

Results: Between 01/01/2009 and 30/06/2011, 1700 cases were reported to the Regional Pharmacovigilance Centre in Angers (708 in 2009, 663 in 2010 and 329 between 01/01/2011 and 30/06/2011). Of these cases, 549 (32.39%) were reviewed during monthly meetings (197 in 2009, 215 in 2010 and 137 between 01/01/2011 and 30/06/2011). Changes were made regarding 181/549 cases, which usually concerned data on adverse reactions (notably their outcome) and on medicinal products (particularly their intrinsic causal relationships). This resulted in modifications to 170 files (93.92%). Follow-up was decided upon for 140 cases (25.50%) and was assured in 108 of them (77.14%).

Discussion: These formalised internal controls supplemented the external controls ensured by the French Agency for the Safety of Healthcare Products (AFSSAPS) and by pharmaceutical companies with respect to serious adverse event. They enabled:
An improvement in the quality of computerized files in terms of global consistency, the coding of adverse events, medicinal products and causal relationships;
The long-term follow-up of cases and modifications resulting from this procedure.

P434

Pancreatitis and acute renal failures associated with incretin mimetics

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Introduction: GLP-1 analogues (GLP1A) and DPP-4 inhibitors (DPP4I), called incretin mimetics, are relatively new treatments of type 2 diabetes mellitus. We present here a description of acute renal failures and pancreatitis occurred under these treatments.

Methods: We reviewed all the serious adverse effects (SAE) associated with GLP1A (exenatide, liraglutide) and DPP4I (saxagliptine, sitagliptine, vildagliptine) reported in the French national pharmacovigilance database (Afssaps BNPV) since 2006. We focused on characteristics associated with acute renal failure and pancreatitis.

Results: Overall, 108 SAE were associated with GLP1A and 136 with DPP4I. The most reported SAE with GLP1A and DPP4I were gastrointestinal and hepatobiliary disorders (46.3% and 33.1% respectively) followed by renal disorders (14.8% and 15.4% respectively).

Fifteen and 20 acute renal failures were reported with GLP1A and DPP4I respectively: 6 (40.0%) and 12 (60.0%) were over 65 years of age, median age was 63 and 66.5, median treatment duration was 18 days and 1 month, 4 (26.7%) and 5 (25.0%) patients showed an history of chronic renal failure, 7 (46.7%) and 7 (35.0%) patients were also treated with angiotensin-converting enzyme inhibitors (ACEI), 7 (46.7%) and 9 (45.0%) with angiotensin II receptor blockers (ARB) and 7 (46.7%) and 8 (40.0%) with diuretics.

Nineteen and 22 pancreatitis were reported with GLP1A and DPP4I respectively: 4 (21.1%) and 3 (13.6%) were over 65 years of age, median age was 59 and 62, median treatment duration was 3 and 3.5 months, 1 (5.3%) and 5 (22.7%) patient showed an history of pancreatitis, 3 (15.8%) and 4 (18.2%) dyslipidaemia, 7 (36.8%) and 9 (40.9%) patients were also treated with statins, 10 (52.6%) and 7 (31.8%) with ACEI, 3 (15.8%) and 5 (22.7%) with ARB, 3 (15.8%) and 3 (13.6%) with diuretics. We noted that five patients (26.3%) showed a recent increase in GLP1A posology.

Discussion: These adverse effects not only happen in elderly and polymedicated patients. However, practitioners should be careful when patients show hazardous comedications and comorbidities and when a dose increase is scheduled. The precise mechanisms involved in renal failures and/or pancreatitis induced by incretin mimetics remains to be defined.

P435

Drug consumption caracteristics and outcomes of one hundred pregnancy notified to the Centre National of Pharmacovigilance of Tunis

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Reproductive studies on animals are insufficient to conclude to the absence of risks on the human foetus. The aim of this study is to describe the main characteristics of the first one hundred cases of women who have taken drugs during pregnancy and who were notified to the Centre National Of Pharmacovigilance of Tunisia during the year 2008 and to determine the outcomes of those pregnancies.

Patients and Methods: We led a retrospective study implying 100 women who have taken drugs during pregnancy. All our cases were notified at the Centre National of Pharmacovigilance during the year 2008. Data was collected from the medical file and transcribed in information cards including the follow parameters:

age, number of drugs intake, international common denomination, pharmaceutical class, exposition period. The outcomes of pregnancies were documented with phone calls to the patients.
Results: The median age of the pregnant women was 33 years (min/max19/43 years). The number of drugs varied from 1 to 10. Women have taken one drug in 18%, two drugs in 21% three drugs in 23% and more than three drugs in 38%. The three first pharmaceutical class that have been used by pregnant women were the analgesics, the anti-infectious, and the central nervous system drugs. We have obtained 67 responses by phone calls and 33 couldn't be reached to precise the pregnancy outcome. Fourty nine per cent of the parturients have carried their pregnancy to end. Sixteen per cent have undergone an abortion, 6% have miscarriages, and only one case of cardiac malformation has been noted. This case dealed with patent ductus arteriosis, the parturient was 14 years old and has taken apoamytriptillin, maprotyllin, and alprazolam.
Conclusion: The most frequent drugs consumed were analgesics including antispasmodics, anti-inflammatory drugs and muscle relaxant. The phone calls have précised the pregnancies outcome in 67% of the cases

P436

Pholcodine hypersensitivity reactions in a patient with previous history of neuromuscular blocking drugs allergy, a case report

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Introduction: Neuromuscular blocking agents, mostly suxamethonium, are the main cause of IgE-mediated anaphylaxis during anaesthesia. The presence of quaternary ammonium ion, in neuromuscular blocking drugs (NMBD) is responsible for the pharmacologic activity, as well as the allergenic potential in those drugs. Pholcodine also contains this quaternary ammonium ion and so is suspected to be linked to allergic sensitization to NMBD. Allergic reactions to pholcodine have been very rarely reported. Although only one case of facial and laryngeal angioedema has been published, we found numerous cases of hypersensitivity reactions involving pholcodine in the French pharmacovigilance database. Moreover the SPC mentions 'cutaneous allergic reactions'.
Observation: Here we report an original case of a patient who presented an allergic reaction after taking a syrup containing pholcodine. Specific IgE were detected for both pholcodine and suxamethonium. Only at this time, the notion of a perioperative anaphylactic reaction was found. It happened 2 years earlier without any investigation. No main anaphylaxis biomarkers had been researched and no allergy tests performed. Moreover, the patient and his physician had not been told of this adverse effect.
Discussion: This case report reminds us of the necessity for adequate medical care in hypersensitivity events, as the SFAR recently recommended it (2011). Communication between all medical actors is an absolute necessity. Therefore an allergologic evaluation is required, including specific IgE assay for both pholcodine and NMBD. The originality of this case report resides in described hypersensitivity symptoms with those two different drugs for the same patient.

P437

Cytolitic hepatitis following methylprednisolone bolus

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Pulsed methylprednisolone treatment for multiple sclerosis is well accepted since years. Acute liver damage following intravenous methlprednisolone therapy is rare and has been reported in sporadic cases. We report a case of cytolytic hepatitis after a methylprednisolone pulsed treatment notified to the Centre National of Pharmacovigilance of Tunis on august 2011.
Case report: HC is 45 year-old women without any history of atopy. A multiple sclerosis was diagnosed in May 2010. She underwent a therapy with Vitamine E®, Tri B® (vitamin B1, vitamin B12, vitamin B6), Vitamine C® and Tonicalcium® (calcium). On May 2010, she began to receive her pulsed monthly methylprednisolone therapy. Her laboratory tests especially hepatic ones were normal until april 2011. On May 2011, transaminases abnormalities started and ranged between 2 and 3N. She received her last bolus on June 2011. Transaminases values increased with continuing the bolus of methylprednisolone up to 5N. On July 2011, they started to improve, whereas she continued her vitamin therapy with calcium. Serology of Hepatitis B, C and serology of VIH, Immunological tests were negatives. An abdominal ultrasound found an hepatic aspect of steatosis.
Discussion: The responsability of methylpednisolone bolus was suspected in front of a compatible chronology (delay of onset 1 year, worsening at the pursuit of the methylprednisolone treatment and regression of the abnormalities in about 2 months after the discontinuation of the treatment), the lack of another etiology (negative etiological tests), and the improve of the hepatic tests despite the pursuit of vitamins. In literature, mild elevation in transaminases and even fatal liver failure were associated with methylprednisolone bolus therapy.

P438

Venlafaxine induced liver injury: a case report

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Introduction: Venlafaxine is an antidepressant significantly inhibits reuptake of serotonin and norepinephrine. His usual side effects are headache, nausea and profuse sweating. Hepatitis associated with this drug is rare. We report a case of hepatotoxicity caused by venlafaxine notified to the Centre National of Pharmacovigilance of Tunisia on July 2011.
Case report: KH is a 57-years- old woman with a history of agoraphobia treated since April 2010 with Prazin (Alprazolam): one tablet daily, Lysanxia (prazepam): two tablet daily and Effexor Lp 37.5 mg (Venlafaxine): one tablet daily. She tooks occasionally, by automedication, Panadol® (paracetamol) for arthrosic pain. The dose of venlafaxine was increased to two tablets (75 mg) on January 2011. The liver tests were normal on March 2011. On the 18th of July 2011, the patient presented pruritis, and jaundice. Liver tests noted an elevation of liver enzymes up to 4–6 N for the transaminases, and the rate of bilirubin were 2.5N/7N for total bilirubin/conjugated bilirubin. Abdominal ultrasonography showed steatosis without signs or other alterations. Serology of hepatitis B and C was negative. The evolution was marked by the worsening of the values at the pursuing of the treatment. The Venlafaxine was stopped on the 30th of July 2011, whereas all the other drugs were pursued (Prazin®, Lysanxia®, and Panadol®. All liver tests normalized within on the 29th of august 2011 (a month after the withdrawal of venfalaxine).
Discussion: The role of venfalaxine was strongly suspected in front of the temporal relation (a compatible delay of onset (15 months), the worsening of the liver values at the pursued of venlafaxine and the normalization of those values after the withdrawal of this medication), the unlikely role of the other medications in front of the regression of hepatitis despite their continuation.
In literature, both cytolytic and cholestatic hepatitis were associated with venlafaxine in some rare cases. A toxic mechanism is evocated when the drug is used with a dose ≥50 mg daily. In our case the dose was increased to 75 mg daily, 6 months before hepatitis.

P439

Antiretroviral induced adverse drug reactions in Malian human immunodeficiency virus positive patients

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Purpose: To our knowledge, there is no report regarding antiretroviral induced adverse drug reactions (ADRs) in Malian patients who infected with human immunodeficiency virus (HIV). We have evaluated the frequency of antiretroviral therapy (ART) induced ADRs in this population and assessed some risk factors of these reactions.
Methods: This is a prospective cohort study that was performed in Malian Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome Research Center (The CESAC) during years 2010–2011. Adult patients who infected with HIV and newly started on ART were included in this study and followed laboratory and clinically for the development of any ADRs for at least 6 months. Narnajo scale side's effects classification has been used to characterize the side effects
Results: During this study 94.6% of patients showed at least one ADR and 5.3% at two ADR. Prevalence of ADRs based on affected organ was: gastrointestinal (GI) (3.1%), hematological (15.4%), neurological (45.9%), cutaneous (10.6%), hepatic (1.4%), and metabolic adverse effects (20.4%). Adverse events were highly probable according to the Naranjo score (83.7%)
Conclusions: Side effect was frequently encountered in our study. The nature of these adverse events were mostly peripheral neuropathy, lipoatrophy, lipodystrophy, and anemia. The link between the use of antiretroviral drugs and adverse events were highly probable according to the Naranjo probability scale. We recommend an active clinical and laboratory monitoring of antiretroviral therapy to strengthen the Malian Pharmacovigilance.

P440

Lupus erythematosus due to anti-tuberculosis drugs: a report of two cases and review of the literature

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An estimated incidence of drug-induced lupus erythematosus caused by all drugs is 15 000–20 000 cases a year, and represents approximately 5–10% of the total number of patients with systemic lupus erythematosus.
Objective: Drug-induced lupus is well known. Through this work; we recall the principle clinical, biological and immunological characteristics. The existing literature on the subject is reviewed.
Observations: We report two cases of drug-induced lupus erythematosus caused by anti-tuberculosis drugs. The patients were under isoniazid and rifampicin to treat pulmonary tuberculosis. Both were onset cases (3 and 4 months) that occurred in women (37 and 58 years old). The common features were fever, arthralgia, myalgia, a malar rash, and the presence of anti-histone autoantibodies. The treatment consisted of the interruption of the anti-tuberculosis drugs and a bolus of methyl-prednisolone during 3 days relayed by an oral corticosteroid. The evolution was favourable after few months of corticosteroids.
Discussion: The suspicion of drug induced SLE (D-SLE) can be made if the drug was given prior to the occurrence of syndrome; the syndrome regresses on it's withdraw and if recurrence occurs on rechallenging with the suspected drug. Such rechallenge is always dangerous and also non-ethical. These drugs could act in two ways: (i) Due to their peculiar pharmacological properties which are dose related e.g. hydralazine, procainamide, anticonvulsants and isoniazid etc. and (ii) as a hypersensitivity reaction which is rare and not dose related e.g. sulfonamide, methyldopa and oral contraceptives etc. The mechanism of D-SLE is hypothesized in multiple reports. The overall prognosis remains favourable although occasional life-threatening cases have been reported in the literature. Constant pharmacovigilance is crucial for prompt diagnosis and cessation of offending therapy offers the best outcome.

P442

Steroid-induced diabetes: about 18 cases

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Introduction: Corticostroids are steroid hormones used primarily for their anti-inflammatory effects. Among their metabolic side effects, diabetes occurs with a frequency ranging between 1% and 50%. The objective of this work is to take stock of the factors favoring steroid-induced diabetes.

Materials and methods: Retrospective analysis of 18 cases collected in pharmacovigilance department of Clinical Pharmacology Hospital Ibn Rochd Casablanca, over 3 years from 2008 to 2011.

Results: Eighteen patients (7M/11W), the average age of 47.54 (between 18A and 65Y), nine patients (82%) suffering from systemic disease. Seventy-three percent of patients received prednisone alone and in 36% the methylprednisolone relayed by prednisone using a higher dose than 20 mg in nine patients over a longer period than 3 months in seven patients. Diabetes was diagnosed by a fasting blood glucose was >2.5 g/L in 8 patients (72%) and between 1.8 and 2, 5 g/L in three patients (27%).

Steroid diabetes was retained by a period >3 months suggestive and improved after discontinuation of treatment or dose reduction.

Steroids have been stopped in three patients, doses reduced in two patients and treatment with insulin or oral antidiabetic agent in 10 patients. The evolution was marked by improvement in seven patients or 74% of cases.

Discussion and conclusion: Our study confirms the high prevalence in adults with a history of defects, and taking high doses of steroids over 20 g for an extended period over 3 months

P443

Amoxicillin-induced meningitis

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Introduction: Recurrent aseptic meningitis is not frequent and the identification of its cause is often difficult. Numerous drugs may induce aseptic meningitis among which non-steroidal anti-inflammatory drugs, intravenous immunoglobulins, anti-CD3 monoclonal antibodies vaccines, and intrathecal agents. Among antibiotics, cephalosporin, penicillin, and cotrimoxazole can be involved. We describe herein a case probably induced by amoxicillin.

Case report: A 78-year-old man developed fever and confusion 3 days after the start of a treatment with amoxicillin for bronchopneumopathy. Cerebrospinal fluid (CSF) examination showed pleiocytosis and increased protein concentration. The patient recovered a few days after amoxicillin withdrawal. Five months later, he was admitted to the hospital in emergency because of confusion, behavioral disorders, aphasia, apraxia and fever. The day before, a treatment with amoxicillin for bronchopneumopathy had been started. CSF examination showed pleiocytosis and increased protein concentration again. Six months later, he was re-admitted to the hospital in emergency with loss of consciousness followed by loss of urines. CSF examination, which had normalized meanwhile, showed pleiocytosis. One day before, amoxicillin had been initiated to treat bronchopneumopathy. The patient spontaneously recovered after amoxicillin withdrawal. No bacterial microorganism, serological sign of viral infection or arguments for autoimmun or granulomatous diseases was ever found.

Discussion: Amoxicillin-induced meningitis (DIAM) or meningoencephalitis is an uncommon adverse drug reaction. To our knowledge, only seven cases have been reported in the English literature. These cases describe more often headhache, fever and neck stiffness, 6 h to 7 days after amoxicillin beginning, with lymphocytic or monocytosic pleiocytosis in CSF examination. The diagnosis of DIAM, which is usually a diagnosis of exclusion, has several features: temporal relationship with drug initiation, recurrence on rechallenge, prompt clinical and CSF improvement on withdrawal of the drug, absence of other cause. As this observation meets the criteria of DIAM, we believe that amoxicillin was the etiology of these three episodes of aseptic meningitis.

Conclusion: The incidence of DIAM is probably underestimated because clinical features can't be differentiated from those of any other type of meningitis. Clinicians should be aware of the risk of DIAM and be careful to drug history.

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Therapy related leukemia after a prior breast cancer diagnosis, a descriptive study

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Objective: In breast cancer survivors, an increased risk of a second primary malignancy at other sites has been observed, compared to the general population (1). Therapy-related myeloid leukemia (t-AML/t-MDS), divided into subsequent acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS), is a clinical syndrome occurring after exposure to cytotoxic therapy (CT) (2).

We aimed to describe the t-AML/t-MDS after a prior breast cancer diagnosis recorded in the French Pharmacovigilance Database, classified by age, treatment, and time interval since breast cancer diagnosis.

Methods: Data were collected from the French Pharmacovigilance Database. First, we selected all adverse reaction notifications recorded from 1988 to 2011 with 'leukemia' adverse effect in the MedDRA dictionary as High-Level Group Terms (HLGT). Second, we selected women with a prior breast cancer diagnosis with commentaries data.

Results: We collected 554 patients with leukemia adverse effect, and included in our analysis 161 women with a prior breast cancer diagnosis. The median age was 53 years (35–100 years) at primary diagnosis and 57 years (31–98 years) at secondary diagnosis. The latency between primary diagnosis and therapy-related disease ranged from 5 to 156 months, with a median of 28 months. Patients received various cytotoxic agents including alkylating agents (n = 143, 89%), topoisomerase-II inhibitors (n = 149, 92%), antimetabolite (n = 131, 81%) and spindle poison (n = 44, 28%). Fifty patients (31%) received CT alone, 62 (39%) received CT and hormonotherapy, 89 patients (55%) received CT and radiotherapy (RT), and 49 patients (30%) received CT, RT and hormonotherapy. Eight patients (5%) did not receive CT, and were treated by hormonotherapy and RT. Karyotypes data were available for 38 patients, including 33 (87%) patients with an abnormal karyotype.

Conclusion: T-AML/t-MDS after a breast cancer diagnosis is likely to be associated with CT for breast cancer. It can occur after a breast cancer without CT, suggesting involvement of others factors, like genetic or environmental risk factors.

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Palmar-planter erythrodysthesia associated to nail changes induced by docetaxel therapy

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Introduction: Docetaxel is a taxane chemotherapy agent used habitually in the treatment of breast cancer. Adverse effects of this drug are frequently skin reactions, especially palmar-planter erythrodysthesia (PPE) or nail changes. PPE associated to nail changes is rarely described in literature. We report a case of PPE associated with nail pigmentation induced by docetaxel.

Case report: A 54-year-old woman had received in September 2008 four cycles of chemotherapy: docetaxel (Taxotere®) and adriamycine (Epiadria®), for infiltrating ductal carcinoma of the left breast, with a good tolerance. She was treated with simple mastectomy, axillar unbridling, and chemotherapy with three cycles of docetaxel. In July 2011, for gastric and bone metastases she had received three cycles of cisplatin (50 mg/m^2), docetaxel (60 mg/m^2) and zoledronate. Each cycle include three injections (one injection per week) followed by cessation for 2 weeks. At the end of the third cycle, she developed a dysthesia and tingling in the palms, fingers and soles of feet with erythema witch progressed to burning pain with dryness, cracking and desquamation. She presented also subungual haemorrhages and nails hyperpigmentation without onycholysis. The diagnosis of PPE associated with nail changes was retained.

This case was notified to the Tunisian National Centre of Pharmacovigilance and analyzed according to the Begaud's et al method of imputation (1).

Discussion: The responsibility of docetaxel in this case was retained in front of:

1 Chronologic data: lesions have been appeared after the third cycle of chemotherapy. PPE appears to be dose dependent and its occurrence appears to be determined by peak drugs concentration and total cumulative dose.

2 Semiologic data: the type of lesions evoked a Palmar-planter erythrodystesia syndrome associated to nail changes.

3 Bibliographic data: PPE was descriped with the cisplatin and docetaxel but never with zoledronate. Nail toxicity is only known with docetaxel.

Reference:

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Interferon beta-1a-induced acute pancreatitis

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Introduction: Interferon β-1a is used in the treatment of multiple sclerosis. The most common side effects of this drug are flu-like syndrome, fatigue, headache and injection site reactions. Acute pancreatitis induced by interferon β-1a is an exceptional side effect.

We report in this work a case of Interferon β-1a-induced acute pancreatitis notified in July 2011 to the Tunisian National Centre of Pharmacovigilance.

Case report: A 20-year-old girl, followed for multiple sclerosis since October 2002, was treated, initially, by boli of methylprednisolone. In February 2007, she began treatment with interferon β-1a 30 μg per week. She experienced abscess at injection site, in May 2010, with a favorable outcome. In June 2011, she presented epigastric pain, vomiting, an abdominal tenderness with hyperleukocytosis. The diagnosis of acute appendicitis was initially evoked, and the patient underwent a coelioscopy for appendicectomy. During the surgery, sero-hematic effusion, infiltration of mesocolon and a normal appendix were discrovered. The assay of lipase showed a high rate (82 N) and the abdominal CT scan showed an acute pancreatitis grade 'D' of Balthazar score. Etiological investigation was negative, in particular no gallstones, no pancreatic malformations, no alcoholism, no dyslipidemia and no hypercalcaemia. The treatment with interferon β-1a was interrupted. The patient was treated with diet and analgesics. The outcome was favorable in 10 days.

Discussion: The responsibility of interferon β-1a in the genesis of acute pancreatitis was retained because of:

A delay of 4 years compatible with iatrogenic origin.

A favorable outcome after cessation of this drug.

Negative etiological investigations.

In the literature, one case of acute pancreatitis induced by interferon β-1a was reported in 2005 in a 53-years-old man followed for multiple sclerosis[1].

Reference:

1. Life-threatening acute pancreatitis associated with interferon beta-1a treatment in multiple sclerosis. Neurology 2005;65(1):170–1.

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Vasculitis induced by ceftazidime: about a case

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Introduction: Vasculitis induced by drugs is a hypersensitivity vasculitis. This is a rather common cause of about 22% of all causes of vasculitis. It can be observed in both sexes and all ages. It is characterized by the predominance or exclusivity of the skin disease, affecting mainly small vessels.

The characteristic histological appearance is 'leukocytoclastic veinulite' characteristic of the drug-induced.
Materials and methods: We report the case of Mr. S. B 35 years old, admitted to the hospital for chemotherapy treatment (first treatment) of acute myelogenous leukemia. The patient presented symptoms of vasculitis drug in cutaneous expression (vascular purpura infiltrated necrotic). The study of accountability and review of literature incriminating mainly antibiotics (cephalosporin third generation) prescribed for a febrile episode.
Results and analysis: Our patient presented 11 days after starting ceftazidime, purpura of the face and leg and fever without stiffness or other meningeal signs associated. Before the negativity of paraclinical (PL, blood cultures, urine culture ...), the biopsy showed a moderately acanthotic epidermis seat of a neutrophilic exocytosis. The Drug seemed the most likely first incriminating ceftazidime which was immediately replaced by ciprofloxacin. The change has been towards the total regression of lesions in 2 weeks as corrective treatment due to bolus solumedrol.
Accountability by the method of the WHO found an intrinsic score I3 likely to ceftazidime. The effect is well known as described in reference books (Vidal, Meyler's side effects).
Conclusion: The betalactam antibiotics are the most implicated in the occurrence of hypersensitivity vasculitis. The incidence is 1/10 000 to 1/1000. Treatment is based on final adoption of the offending drug and administering symptomatic treatment (antihistamines and anti-inflammatory drugs). Skin allergy tests should be performed to confirm the allergy drug offending
Faced with this iatrogenic drug, the reintroduction of a drug in the same class should be prohibited and will if necessary (no alternative treatment) under close supervision, close to an intensive care unit. The patient may need to carry a card stating the drug and other proscribed drugs in the same chemical class.

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Stevens-Johnson syndrome after intake allopurinol: report of nine cases

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Introduction: Stevens-Johnson syndrome (SJS) is a complex immunological syndrome with high morbidity and mortality and characterized by acute blistering affecting the skin and at least two mucous membranes. The aim was to collect cases of SJS with allopurinol use in the database of Pharmacovigilance unit of the University hospital IBN ROCHD of Casablanca and describe epidemiologic, clinical, therapeutic and evolutive patterns.
Patients and methods: Since January 2008 and December 2010; nine patients with SJS were identified. The average incidence was three cases per year. There were six women and three men with a mean age of 66 years. The onset of the SJS occurred at a mean and median of 36 (range 15–90) days after initiation of allopurinol. Total skin injury area reached from 7% to 9%. Mucosal and viscera injury occurred in respectively 9/9 and 4/9 patients. The average time before hospitalization was 11.4 days. Intrinsic and extrinsic imputability criteria lead to the diagnosis of allopurinol-induced Stevens-Johnson syndrome.
Result and discussion: Stevens-Johnson syndrome is a rare but severe blistering mucocutaneous disease with a high rate of morbidity and mortality. Mortality rates are below 5% for Stevens-Johnson syndrome. The typical interval from beginning of drug therapy to onset of reaction is 1–3 weeks, but is shorter with rechallenge. SJS can be induced by drugs, but also by infections and probably other risk factors not yet identified. Identification of the cause is important for the individual patient and in cases of drug-induced disease withdrawal of the inducing drug(s) has an impact on the patient's prognosis. Allopurinol, which is most often administered for long periods, is frequently cited as a cause of Stevens-Johnson syndrome. The risk is not constant over time. Old age, impaired renal function and concomitant intake of diuretics (especially thiazides) may be predisposing factors. Patients who undergo Allopurinol therapy should be informed about possible cutaneous adverse effects and treatment should be discontinued immediately if any rash appears.

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Arterial hypertension associated with coxibs and non-selective non-steroidal anti-inflammatory drugs: a case/non-case study in the french pharmacovigilance database

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Introduction: Both COX-2 selective inhibitors (coxibs) and non-selective Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are associated with an increase in blood pressure (BP) and a risk of arterial hypertension (AH) in normotensive and hypertensive individuals. The aim of this study is to assess the risk of AH associated with coxibs and non-selective NSAIDs.
Methods: We collected from the French pharmacovigilance database cases with the following MedDRA preferred terms: hypertension, essential hypertension, hypertensive crisis, accelerated hypertension, malignant hypertension, systolic hypertension, diastolic hypertension, hypertensive emergency, labile hypertension and secondary hypertension. Patients <18 years and cases involving more than one NSAID were excluded. We used the case/non-case methodology (OR estimation and 95% confidence interval) with reports of AH as cases and all other reports as comparators. Amoxicillin and bevacizumab were used as negative and positive controls, respectively.
Results: From 1985 to November 2011, 117 AH cases associated with NSAIDs were identified (sex ratio H/F = 47/70) and the NSAID was the only suspected drug in 68%. The mean age was 57.3 ± 16.8 years. Forty-two cases (36%) were serious. The mean systolic and diastolic BP were 190 ± 30 and 105 ± 16 mmHg. The median time of onset was 6 days (IQR [3;12]). Isolated AH occurred in 36%. Pre-existent AH was noted in 34%. Complete recovery was obtained in 77%. Drugs associated with AH were coxibs in 31% (n = 36), OR 3.4, CI 95% [2.5–4.8] (rofecoxib, n = 21, OR 7.2 [4.6–11]; celecoxib, n = 11, OR 1.5 [0.8–2.7]; etoricoxib, n = 4, OR 16 [5.7–45]), non-selective NSAIDs in 69% (n = 81), OR 0.7, CI 95% [0.5–0.8] (nimesulide, n = 6, OR 2.6 [1.2–5.8]; indomethacin, n = 10, OR 2 [1.1–3.8]; ibuprofen, n = 11, OR 0.6 [0.3–1.0]; naproxen, n = 2, OR 0.3 [0.1–1.2]; diclofenac, n = 13, OR 0.7 [0.4–1.2]). The OR for bevacizumab and amoxicillin were 13 [10–18] and 0.07 [0.03–0.20], respectively.
Conclusion: AH seems to be associated with coxibs but not with non-selective NSAIDs, except nimesulide and indomethacin. Concerning non-selective NSAIDs, results are not completely consistent with the existing literature: this may be explained by either under-reporting or a lack of iatrogenic AH identification. The hypertensive effect of NSAIDs, and especially coxibs, should be taken into account by clinicians and monitored in normotensive and hypertensive patients.

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Acute generalized exanthematous pustulosis metronidazole related: A case report

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Introduction: Acute generalized exanthematous pustulosis (AGEP) is almost specific medication. It is exceptionally due to a topical medication. We report an AGEP after using vaginal suppositories of metronidazole.
Observation: This is a woman of 39 years, hospitalized for the second time for AGEP as metronidazole vaginal.
Clinical examination found a: diffuse erythroderma on the body with the presence of non-follicular pustules, some of which coalesced bubble. In biology, a leukocytosis to 23 910 cells/mm^3 with neutrophil predominance. Skin biopsy is in favor of AGEP.
The score of imputability I3 is likely intrinsic and extrinsic B2 is not well known.
The patient progressed well after stopping of treatment and installation of corrective treatment based on a double histaminotherapy.
Discussion: The AGEP is a drug-induced eruption in more than 90% of cases. Antibiotics are the most incriminated but the list continues to grow. Cases of AGEP induced by topical are exceptional and that we present is the first to our knowledge involving metronidazole vaginal.
Conclusion The AGEP is a rash of serious drug etiology in most cases. This side effect deserves to be known as the reintroduction leads to a more severe.

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Amiodarone-induced steatohepatitis

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Introduction: Amiodarone is effective in treating both ventricular and supraventricular dysrythmias. Its hepatic side effects are isolated and moderate elevation of transaminases, acute hepatitis and, rarely, chronic hepatitis in prolonged treatment.
We report one case of steatohepatitis induced by amiodarone, notified in October 2011 to the Tunisian National Centre of Pharmacovigilance.
Case report: A 67-year-old man was treated with amiodarone 200 mg/day, 5 days a week, and acenocoumarol 2 mg/day since 2003 for a complete atrial arrhythmia, ramipril 5 mg/day since 2007 for an arterial hypertension and alfuzosine 10 mg/day since 2010 for a prostatic adenoma. In October 2009, liver test abnormalities were detected: SGPT = 1.7 N and SGOT = 2 N. No other biologic hepatic abnormalities were detected. Liver test abnormalities persisted during 2 years. In October 20, 2011, in front of the rise of transaminase levels (SGOT = 2.96 N and SGPT = 2.9 N), amiodarone was withdrawal and the other medications were continued. Transaminase levels improved in 1 week.
Abdominal ultrasound showed fatty liver. The diagnosis of non alcoholic steatohepatitis was suspected. The liver biopsy could not be performed since the patient was under anticoagulant.
The rest of explorations showed normal endoscopic ultrasound, negative B and C viral serologies, and hypertriglyceridemia.
Discussion: The role of amiodarone was retained in the genesis of the steatohepatitis because of chronological data:
A delay of 4 years compatible with iatrogenic origin.
Persistence of liver test abnormalities in pursuit of drugs.
Normalization of transaminase levels 1 week after cessation of the drug.
In the literature, amiodarone is the drug most commonly implicated as causing steatohepatitis.
Steatohepatitis (NASH) is a rare form of drug induced liver diseases, and drugs account for fewer than 2% of cases of NASH [1]. Liver biopsy interpretation continues to be considered the 'gold standard' for the diagnosis [1,2].
References:
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P452

Celecoxib induced anaphylaxis

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Introduction: Celecoxib is a selective inhibitor of the cyclooxygenase-2 (COX-2) enzyme. It has been developed as a powerful, nonsteroidal anti-inflammatory drug with a good gastric tolerance. Very few hypersensitivity reactions have been described with this drug, particularly anaphylactic reactions. We report one case of celecoxib anaphylaxis, notified to the Tunisian National Centre of Pharmacovigilance.
Case report: A 62-year-old woman with no history of atopy or allergic drug reaction, had a gastric ulcer, dyslipidemia and diabetes under pioglitazone. In August 2010 she had taken one pill of celecoxib$_{100}$ for arthralgia. Two hours later,

she developed pruriginous edema on the face and hands. Then, she recovered fully within 1 h with corticosteroid. One month later, she developed 1 h after readministration of celecoxib in association with thiocolchicoside, a generalized oedema, a loss of consciousness, a facial erythema, dyspnea, and hypotension. A diagnosis of an anaphylactic shock was retained and the patient has been admitted in an intensive care unit. All medications have been withdrawn. She received a symptomatic treatment and she recovered 4 days later.
On November 2011, Pioglitazone was substituted by gliclazide (Diamicron) without incidents. The score of imputability of celecoxib was evaluated as likely and coxibs were contraindicated.
Discussion: In our case, the responsibility of celecoxib was retained because a suggestive delay of occurrence (2 h), favorable evolution after drug withdrawn and the positive readministration of this drug. Cases of anaphylaxis have been rarely described with celecoxib. The mechanism of celecoxib induced anaphylaxis is not associated only with an immunoglobulin E related reaction but can also be linked to a non-specific histamine release.

P453
Generalized erythema and fever associated with oxybutynin
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Introduction: Oxybutynin is an anticholinergic drug. It is indicated for enuresis and for overactive bladder. Its most reported adverse effects are usually related atropine like effects: dizziness, mucosal dryness, constipation and micturition disorders. Cutaneous effects are rare.
We report a case of generalized erythema associated with fever in a child treated by oxybutynin with positive rechallenge notified notified to the Tunisian National Centre of Pharmacovigilance.
Case report: A 5-year-old girl was treated for recurrent urinary infections due to overactive bladder with cefuroxime. Two years later, in february 2011, oxybutynin was introduced. One week later, she developed a mild fever and generalized erythema which occured daily, 1 h after drug intake and resolved about 3 h later. The oxybutynin was interrupted for a month in March 2011, while cefuroxime was continued. The symptomatology disappeared completely.
In April 2011, oxybutynin was reintroduced. The same symptomatology reoccurred as described previously. Oxybutynin and cefuroxime were continued until August 2011, with persistence of the event.
In August 2011, there was an exacerbation of fever, leading to drug withdrawal. Infectious etiological investigations were negative. The outcome was marked by disappearing of fever and erythema.
Discussion: The role of oxybutynin in the genesis of this event was suspected because of chronological data: delay of 1 week compatible with the iatrogenic origin, a favorable outcome after the cessation of the drug and the positive rechallenge. Our case reported a generalized erythema with fever which occurred in a child treated with normal doses of oxybutynin. In literature, in case of surdosage, oxybutynin can induce local facial flush and fever. The fever can be important especially in case of extreme heat. The risk of developping such side effect increases in children and in the elderly.

P454
Stevens-Johnson syndrome associated with ciprofloxacin
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Introduction: Stevens-Johnson syndrome (SJS) is a severe mucocutaneous reaction, which can be elicited by various drugs. This syndrome has been rarely reported with ciprofloxacin. We present one case of Stevens-Johnson syndrome associated with ciprofloxacin notified to the National Centre of Pharmacovigilance of Tunis on September 2011.
Case report: A 28-year-old woman with no allergic or medical history received on September 8, 2011 for acute pyelonephritis ciprofloxacin 750 mg two times daily. Twelve days after drug initiation, she developed erosions on the nasal, buccal and genital mucous membranes, conjonctival hyperaemia and a multiple pruritic erythematous maculopapules with central hemorrhagic and necrotic features located over the face, trunk and arms. Nicholsky's sign was negative. The ciprofloxacin was withdrawn and a corticosteroid treatment was initiated. The patient recovered 15 days later with no sequelae.
The score of imputability was evaluated as plausible. Ciprofloxacin was contraindicated and patch-tests with the others fluoroquinolones was recommended.
Discussion: The responsibility of ciprofloxacin was retained in front of a suggestive delay of occurrence (12 days) and a favorable evolution after drug withdrawn.
The most common adverse cutaneous events were exanthema, photosensitivity, urticaria, angioedema, and pruritus. SJS, Toxic Epidermal Necrolysis (TEN), or erythema multiform have also been reported with this drug but are less common.

P455
Bisphosphonates and cardiac side effects
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Introduction: Bisphosphonates are classical medicines used to prevent and to treat osteoporosis, malignant hypercalcemia, tumor-induced osteolysis and Paget's desease. Eight drugs are currently marketed (Alendronate, Clodronate, Etidronate, Ibandronate, Pamidronate, Risedronate, Tiludronate and Zoledronate). Cardiac adverse effects (AE) have been reported only with Zoledronate. The aim of this work is to evaluate the potential involvement of bisphosphonates in proarrhythmic effects.
Material and methods: We investigate case reports from the french national pharmacovigilance database (AFSSAPS BNPV). The selection criteria were the High Level Term (from MedDRA) hearth rhythm disorder, and the High Group Level Term: sudden death.
Results: We found 38 case reports of heart disturbancies. Among them, 27 were female (71.1%). Median age was 75.5. Seven over eight bisphosphonates were concerned: Zoledronate (44.7%), Pamidronate (26.3%), Risedronate (10.5%), Alendronate (5.3%), Clodronate (5.3%), Ibandronate (5.3%) and Etidronate (2.6%). Fifty percent of cardiac AE were atrial pathology (atrial fibrillation, atrial extrasystole, atrial arrhythmia, atrial flutter), 23.7% heart rhythm disorder (bradycardia or tachycardia), 13.2% sudden death, 10.5% ventricular disease (torsade de pointe, ventricular extrasystole, ventricular tachycardia) and 2.6% of atrio ventricular bloc (one case report).
Discussion and conclusion: Our review shows that cardiac AE are mainly observed with intravenous bisphosphonates (73.7%). This is consistent with the literature data. In our study, half of the patients presented a supraventricular arrhythmia as reported by Camm in 2010[1]. The precise mechanism of that effect is not elucidated even if we know that arrhythmic disease increased with age[2]. Three over five sudden deaths described here occured within 24 h. However, no significative difference between bisphosphonates and placebo in cardiovascular mortality was described by Camm[1]. In conclusion, because of a potent cardiovascular risk, we recommand a special management of elderly (more than 75 years old) and patients with history of cardiac disease.
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P458
Vaginal route drugs and misuse
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Introduction: Therapeutic mistakes represent a large part of pharmacovigilance activity. Some drugs are more frequently misused. Drugs administrated by vaginal route are numerous. We retrieved 22 different drugs, with different presentation (soft gel capsule, vaginal suppository, capsule….).
Method: We performed a research in the french Pharmacovigilance Database concerning report of adverse drug reactions involving vaginal route drugs declared to PACA pharmacovigilance center from 1996 to 2011.
Results: Eighty-eight cases were retrieved.
Some drugs are more often declared as misused in our pharmacovigilance center:
Colposeptine: was involved in 20 cases (including 15 cases of misuse) without seriousness.
14 route errors (oral intake). Consequences were digestive discomfort epigastralgia diarrhea.
Colpotrophine: was involved in four cases (including two cases of route error) without seriousness.
Consequence was diarrhea in one case.
Florgynal: was involved in 25 cases (including 23 cases of route error) without seriousness.
14 route errors. Consequences were digestive troubles (mainly nausea).
Trophigil: was involved in 32 cases (including 24 cases of route error) without seriousness.
Consequences were digestive troubles (nausea, epigastralgia).
Lomexin: was involved in six cases (all are route errors) without seriousness.
Consequences were digestive troubles (nausea, abdominal pain).
Polyginax: One route error without consequences.
Discussion: Even if those route errors were free of medical consequences, they perturb patients. It outlights existence of poor communication between patient, physician and chemist. It can also be explained by the absence or impossibility for the patient to read drug instructions. It is also linked according to some patients to the similarity between oral route forms and vaginal routes form.

P459
Drug-induced melanomas: analyse of the AFSSAPS Pharmacovigilance Database
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Introduction: we know that skin cancers increase with age, sun exposure and drugs use. The aim of this study is to determine drug classes involved in the most severe disease: melanoma.
Material and method: first we analysed all cases reported in the AFSSAPS pharmacovigilance database (BNPV). For selection criteria we used the high level term 'skin malignancies' and focused on melanomas then restricted on drugs considered as 'suspect'. Secondly we compared the results to literature data.
Results: among 247 skin malignancies, 81 melanomas (33%) are found concerning 49 female (F) and 29 male (M), three not documented (ND). Average age was 55.4 years old (10% ND). Cases concerning patients under 40 years old were 18% (67% F), between 40 and 50 12% (60% F), between 50 and 60 28% (61% F), between 60 and 70 14% (73% F), between 70 and 80 14% (55% F), and more than 80 4% (67% F). Drugs suspected in the occurrence of melanomas were as fallows: immunosuppressive agents 66%, immunosuppressive and/or toxic skin agents 16%, photosensitizing agents 5%, and various classes 13%. Among immunosuppressive drugs, there were TNFα inhibitors/antagonists (48%), monoclonal antibodies against other targets (7%), antimetabolites (20%), corticosteroids (6%), antineoplasic drugs (4%) and all drugs classes (15%). Underlying diseases were: rheumatoid arthritis (26%), ankylosing spondylarthritis (11%), Parkinson disease (9%), psoriasis (7%), Crohn disease (6%) and multiple sclerosis (6%).
Discussion and conclusion: TNFα inhibitors/antagonists are widely represented but a bias can be evocated because reporting adverse effects of these drugs were promoted. Melanomas concern more female patients probably in relation with high

F/M ratio of autoimmune diseases. In literature, most common skin cancers related to drugs are melanomas and cutaneous epidermoid cancers. Immunosuppressive drugs are first involved followed by photosensitizing agents. Some cases of melanomas are also reported with L-Dopa, the melanin's precursor. This mechanism could explain melanomas cases reported in Parkinson disease. Relationship between drugs and melanomas remain difficult to assess and necessitate further investigations.

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Drug-induced acute pancreatitis: a report of four cases

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Introduction: Drug-induced acute pancreatitis (AP) is approximately 2% of the acute pancreatitis. Its incidence is increasing with more than 260 incriminated drugs and which are indexed and classified on the base of pancreatox. A right diagnosis should eliminate the other causes mainly pancreatitis induced by alcoholism or biliary stone, and should also include a study of an intrinsic and extrinsic imputability.

Observations: We report observations of four cases of acute pancreatitis, three women and one man; their ages are between 18 and 38. In their background, three patients were HIV positive. As therapy, they were receiving antiretroviral therapy, associated in one patient with an antibiotic and in another one an antidepressant and an anticonvulsant. A fourth patient was under antibiotic, antispasmodic and anti-ulcer. The deadline was between 2 days and 2 months. For symptoms, three patients had vomiting accompanied by epigastric pain, one patient had cutaneous icteria. For all patients the therapeutic was a digestive rest and rehydration; two patients were transfused and two others were given analgesic. All patients show high level of serum lipase, and for one patient, Abdominal ultrasound revealed a swollen pancreas. The evolution was unfavorable for two patients. The intrinsic imputability was plausible in three patients and probable in a patient, and for the biobibliography antiretroviral drugs, valproate of sodium, trimethoprim of sulfamethoxazole were the most incriminated.

Discussion: Causality assessment between drug intake and development of acute pancreatitis is based on the temporal relationship, effect of dechallenge, rechallenge, the presence of other established causes (gallstones or alcohol abuse) and the presence of cases in the literature described above.

Some patients are more likely to develop drug-induced acute pancreatitis same as the elderly, poly medication, the HIV-positive, those with cancer and those treated with immunosuppressors.

Conclusion: The greatest challenge to understanding the cause of drug-induced AP is the ability to rule out other causes. Patients diagnosed with a drug-induced AP may have unknown underlying risk factors, and initiation of the medication could exacerbate the patient's risk. It is prudent for clinicians to consider high-risk medications associated with AP in HIV-positive.

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Bleomycin-induced interstitial pulmonary: a five case report

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Introduction Bleomycin is a potent antitumor agent that is particularly effective for the treatment of squamous cell and testicular tumors. However, its usefulness is limited by the potentially life- threatening pulmonary toxicity that has mortality between 2% and 10% of affected patients.

Observations We report five cases which developed interstitial pulmonary, collected at the pharmacovigilance unit of University Hospital in Casablanca, four women and one man, aged 23, 42, 48, 48 and 50 years, all treated for Hodgkin's lymphoma with chemotherapy with antineoplastic bleomycin. The deadline of appearance of respiratory distress was 2, 4, 5, 6 and 8 months. Chest radiograph was abnormal for four patients. The therapeutics was to stop bleomycin, maintain other anticancer and administering oral corticosteroids. The result was favourable in three cases.

Discussion The number of patients with cancer who receive bleomycin is significant. These patients are at risk of developing acute respiratory distress syndrome postoperatively. Because of lack of the bleomycin- inactivating enzyme, bleomycin hydrolase, in the lungs and the skin, bleomycin- induced toxicity occurs predominantly in these organs. There are no pathognomonic signs or symptoms of bleomycin related pulmonary damage. In the preoperative evaluation of such patients, a history of dyspnea or dry cough, and the presence of rales on physical examination are significant. Pulmonary function tests may be helpful in determining the extent of damage in known pulmonary fibrosis, but they are not predictive to subclinical disease.

Several factors, including age, drug dose, route of administration, renal function, concomitant administration of oxygen, radiation therapy and a smoking history, may increase the risk of developing bleomycin drug toxicity.

Conclusion: Knowing the occurrence of pulmonary toxicity by bleomycin and predisposing factors, we can conclude that the goal of clinical and radiological follow-up and functioning of this adverse effect and the proposed preventive measures to decrease the frequency of occurrence of this toxicity.

P462

Adverse reactions associated with paracetamol

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Introduction: Paracetamol is an analgesic and antipyretic drug usually used in the symptomatic management of pain and fever in Tunisia and all over the world. This drug is generally well tolerated. Adverse reactions to this drug were rarely reported and usually moderate.

The aim of this study was to assess adverse reactions related to paracetamol and notified to the Tunisian National Centre of Pharmacovigilance.

Methods: It was a retrospective study involving all reports of adverse reactions associated with paracetamol from 2009 to 2010. We considered the cases where the paracetamol has the most important imputation score alone or in association with other drugs. We collected age, sex, medical history, type of adverse reactions, delay, outcome and imputation score. The cases were analyzed according to Begaud method of imputation.

Results: There were 16 cases including 12 women and four men. The age varied from 7 months to 80 years. In two cases paracetamol were administered intravenously, in the other 12 cases paracetamol was administered orally. Only seven patients had a history of allergy (one case: allergic rhinitis; six cases: drug allergy). Paracetamol is administrate alone in six cases and associated with other drugs in 10 cases. Adverse reaction were skin lesions in 13 cases: 81.25% (including two cases of fixed drug eruption, two cases of skin rash, six cases of urticaria, one case of Toxic Epidermal Necrolysis, one case of labial edema and one case of urticaria, labial edema and dyspnea); anaphylactic choc in one case: 6.25% and liver test abnormalities in two cases: 12.5%. In eight cases, Paracetamol were implicated with an imputation score of I3 (Likely), in seven cases I2 (possible), in only one case I1 (Doubtful).

Discussion: In our study adverse reactions to paracetamol is not an exceptional event. Skin reactions are the most observed reactions (81.25%). In the literature, hepatic toxicity of this drug is well known. We noted two serious life-threatening adverse reactions (Toxic Epidermal Necrolysis, anaphylactic shock). In the literature, despite the frequent use of paracetamol there are only few cases of Toxic Epidermal Necrolysis and anaphylactic shock.

P463

Erlotinib-induced interstitial lung disease: study in the French pharmacovigilance database

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Introduction: Erlotinib is an oral human epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor used in the treatment of non-small cell lung cancer and pancreatic cancer. The aim of the study was to review all the observations of erlotinib-induced interstitial lung disease (ILD), reported to the French Pharmaco-Vigilance Database (FPVD).

Methods: We collected all spontaneous reports of erlotinib-induced ILD notified in the FPVD and reviewed the literature.

Results: Fifteen cases of erlotinib-induced ILD, all serious, were found in the FPVD which one was associated to gemcitabine. Subjects were mainly male (67%) with mean age 64 years old (49–78). Fourteen patients were treated for lung cancer and one for pancreatic cancer. Five patients had history of smoking (unknown in 10 cases) and five radiotherapy (unknown in 10 cases). Onset of symptoms ranged from 4 to 150 days (on average 55 days). All patients had dyspnea which was associated with fever (20%), cough (27%) or hemoptysis (13%). ILD diagnosis is based on clinical findings and mainly by CT-scan. Erlotinib was discontinued in all cases. The treatment of ILD is mentioned in nine cases: corticotherapy (78%), antibiotics (56%). Four patients recovered (one patient died 3 months later for progression); six patients died (50% had a history of radiotherapy). Outcomes were unknown in five patients.

A literature search find 20 cases, mainly male (75%), with mean age of 59 years old (43–79). Eleven patients with history of smoking (unknown in four cases) and eight radiotherapy (unknown in 12 cases). Main symptom was dyspnea with or without fever or cough. Onset of symptoms ranged from 2 days to 1 year. Erlotinib was discontinued in 65% of cases. The treatment of ILD was a corticotherapy (95%), antibiotics (50%). Twelve patients died. Outcomes were non fatal in eight patients.

Conclusion: Respiratory symptoms, especially a worsening of dyspnea, in a patient under erlotinib should suggest erlotinib-induced ILD, rare but potentially fatal ADR. The exact mechanisms remain unknown. History of smoking or radiotherapy could be identified as a risk factor. Erlotinib should be withdrawn and a treatment by corticotherapy should be considered.

P464

Cardiac ischemia and anti-TNF: spontaneous reports notified to the French Pharmacovigilance Database

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Introduction: Anti-TNF drugs, monoclonal antibodies or fusion protein, are supposed to have a beneficial cardiac effect by reducing inflammatory cytokine involved in atherogenesis. Cardiac ischemia cases in patients receiving these drugs are rarely reported.

Methods: Cases registered in the French Pharmacovigilance database about ischemic cardiac events where anti-TNF is 'suspect' were analyzed. Literature was studied.

Results: Forty-three cases reports of cardiac ischemic events during anti-TNF treatment were found (21 females, 22 males). Treatment: infliximab (24), adalimumab (5), etanercept (14). Median age: 57 years (23–86). Indications: rheumatoid arthritis (RA) (27/43), rheumatoid spondylarthritis (5/43), inflammatory bowel disease (IBD) (5/43), rheumatoid psoriasis (5/43) or ocular cicatricial pemphigoid (1/43). Cardiac events: myocardial infarction = 25 cases (58%), acute coronary syndrome = 10 cases (23%) and pectoral angina = eight cases (18%).

In seven on 37 documented cases, patients did not present cardiovascular risk factor (CRF): four females, three males; median age = 70 year (39–77). Thirty patients had >1 CRF: hypertension (n = 16), diabetes (n = 4), tabagism (n = 11), obesity (n = 4), dyslipidemia (n = 11), substitute hormonal treatment (n = 1) and cardio-vascular history (n = 11).

In 20 cases, Methotrexate and/or corticotherapy were associated. Median delay of the ischemic cardiac events: since the onset of the disease is 10 years (4–34), since the onset of the treatment is 18 months (0–168).

In seven on 34 documented cases, treatment by anti-TNF is continued.

Evolution: unknown: 9, favorable: 28, deaths: 5 (four females and one male, age >65 year, treated for RA, two cases without CRF) were observed.
Discussion: The increased risk of premature cardiovascular disease in RA and IBD may depend on traditional CRF, and also be attributable to the inflammatory disease specific risk factors.
Anti TNF alpha drugs is expected to reduce the progression of atherosclerosis and therefore cardiac ischemia; however, cases of ischemic cardiopathy are reported.
Regarding our cases, traditional CRF and/or long term of the disease are mainly reported. Cardiac evaluation before and during the treatment and additional studies are necessary to determine the role of anti TNF drugs in these events.

P465
Amoxicillin induced acute generalized exanthematous pustulosis confirmed by patch testing

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Introduction: Acute generalized exanthematous pustulosis (AGEP) is a severe cutaneous eruption. It mainly caused by antibiotics. We report a case of AGEP induced by amoxicillin and confirmed by a positive patch test.
Case report: A 49-year-old man was admitted to hospital with a generalized pustular eruption 2 days after starting amoxicillin prescribed for dental pain. He had no personal history of drug allergy or psoriasis. On examination, a widespread symmetric erythematous eruption, with numerous non follicular pinpoint pustules over the trunk and proximal limbs, was observed. The eruption was associated with oedema of the face and extremities. There was no mucosal involvement. The patient was apyretic. Blood analysis showed hyperleukocytosis at 15×10^9/L with a neutrophil count of 13.2×10^9/L and normal eosinophilia. All blood biochemistry results were normal. Swabs from the pustules were sterile. Serology was negative for acute viral infections (cytomegalovirus, Epstein Barr virus, hepatitis B, C and Parvovirus B19). A skin biopsy showed subcorneal and intraepidermal spongiform pustules. The dermis was oedematous, with perivascular mixed inflammatory infiltration, including numerous eosinophils.
Based on the clinicopathologic features, AGEP secondary to amoxicillin was diagnosed. After discontinuation of the medication, the acute eruption improved within 8 days, followed by postinflammatory desquamation.
Patch testing with amoxicillin was performed 5 weeks later and was positive.
Discussion: Drugs are incriminated in more than 90% of cases of AGEP. Medications from many different pharmacologic classes, especially antibiotics, have been suspected in the development of AGEP.
AGEP related to amoxicillin was reported in literature. Patch tests were not usually performed. In our case, a clear temporal relationship was observed between amoxicillin administration and the eruption, the remission of symptoms after amoxicillin withdrawal and the positif patch test to amoxicillin. According to the objective causality assessment by the Naranjo probability scale, amoxicillin-induced AGPE was probable.
Conclusion: Clinicians should be aware of the possibility of AGEP induced by amoxicillin. Skin tests may be useful in recognizing the implicated drug.

P466
Reversible acute hepatotoxicity related to olanzapine

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Introduction: Olanzapine, a thienobenzodiazepine derivative, is an atypical antipsychotic agent indicated for the treatment of schizophrenia and bipolar disorder.
We report two cases of olanzapine induced hepatotoxicity.
Case reports: A 29-year-old woman, diagnosed with Bipolar Disorder Type I, according to DSM-IV criteria, was hospitalized for maniac episode. On admission, liver function tests were within normal limits. The patient was treated with lithium, lorazepam, and olanzapine. Few days after starting olanzapine the alanine aminotransferase (ALAT) level increased to 192 U/L (reference range <37), the aspartate aminotransferase (ASAT) level increased to 98 U/L (reference range <37), bilirubin level, alkaline phosphatase level and prothrombin time were normal. There were no history of alcohol or drug abuse. Recent viral hepatitis was excluded as well as autoimmune hepatitis. Ultrasonography of his hepatobiliary system was normal. A diagnosis of hepatotoxicity related to olanzapine was made and the drug was stopped, other medications were unchanged. Serum transaminases normalized 2 weeks later.
In the second case, a 29-year-old female with no significant medical history, was admitted to department of psychiatry for maniac episode. She had been on 10 mg daily of olanzapine. Eight days later, her liver biochemistry tests showed an elevated alanine aminotransferase at 181 U/L and aspartate aminotransferase at 124 U/L. All other hepatic parameters were within normal limits. Serum transaminases performed on admission were normal. Olanzapine was discontinued and the enzyme levels reached 312 U/L (ALAT) and 124 U/L (ASAT), 5 days later and then gradually normalized after 1 week.
Discussion: In our two cases, the diagnosis of olanzapine induced hepatotoxicity was based on a temporal relationship between drug challenge and onset of liver disorders, the resolution of biological signes after drug discontinuation, and elimination of other etiologies for hepatitis. According to the objective causality assessment by the Naranjo probability scale, olanzapine-induced hepatotoxicity was probable in tow patients.
Hepatotoxicity is a known but infrequent complication of conventional antipsychotics as well as second-generation antipsychotic.
Olanzapine-associated hepatitis is believed to be transient, and asymptomatic or sub-clinical.
Conclusion: Because of the risk hepatotoxicity induced by olanzapine, we suggest that patients treated with this drug undergo periodic assessment of liver function.

P467
Osteomalacia in a patient receiving adefovir for hepatitis B

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Introduction: Adefovir, a nucleotide analogue, was approved in 2002 for the treatment of chronic hepatitis B at the daily dose of 10 mg. We report a case of severe bone disorders probably related to adefovir.
Observation: In January 2007, a 58-year-old Cambodian patient treated with adefovir 10 mg/day for 5 years for chronic hepatitis B, complained of asthenia and back pain with osteoarthritis. In April 2007, he had a spontaneous upper left femoral metaphyseal fracture. A thoracic scan showed recent and ancient multiple rib fractures. Biological investigations revealed hypophosphoremia, hypophosphaturemia, and tubular acidosis. Bone biopsy was consistent with osteomalacia and hyperparathyroidism. Kidney biopsy evidenced interstitial fibrosis. Adefovir was replaced by entecavir in July 2008. In November 2008, mineral metabolism improved, bone pain decreased and he gradually recovered his autonomy.
Discussion: Two cases of osteomalacia involving patients treated with adefovir for hepatitis B have been recorded in the French pharmacovigilance database and seven other cases have been published. Overall, these were seven men and two women aged 40–68 years. They had received adefovir (10 mg daily) for 18–64 months before the onset of symptoms (1–6). Interestingly, our patient and four from others previously published cases were Asian, suggesting a possible increased sensitivity in this population. In conclusion, healthcare professionals should be aware of this long-term and potentially severe toxicity induced by low-dose of adefovir therapy.
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P468
Opsoclonus myoclonus syndrome in a young adult after a measles, mumps and rubella vaccine

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Introduction: Opsoclonus myoclonus syndrome (OMS) is a rare syndrome characterized by chaotic eye movements and myoclonic jerks. The pathogenesis remains unknown but is probably immune-related. In adult, it is mostly associated to neoplasic, infectious or inflammatory diseases. We report here a case of OMS with cerebellar ataxia after an MMR vaccine.
Observation: A 25-year-old male received the MMR vaccine (M-M-RVaxPro®) on 29/09/2011. Eleven days later, he was admitted to hospital for urinary retention, ataxia and involuntary eye movements. Electroencephalogram showed diffuse brain suffering despite a normal magnetic resonance imaging. Lumbar picture showed a lymphocytic meningitis with hyperproteinorachia. PCR for human simplex virus (HSV), enteroviruses, measles and T. whipplei antibodies were negative in the cephalorachidian liquid. No antineuronal antibodies were found. Serology for Lyme disease, *Chlamydia*, *Mycoplasma*, enteroviruses, HSV, Varicella Zoster Virus, arboviruses antibodies were negative. Anti-measles IgG, anti-rubella IgG and anti-mumps IgG were detected.
The patient was treated with intravenous immunoglobulin and the symptoms improved in a few days. However, on the 25/11/2011, the patient has still not completely recovered.
Discussion: Two cases of post-vaccinal OMS have been published (1,2). The first one occurred in a 30-year old female after an anti-rubella vaccination and the other in an 11-year old female after a human papilloma virus vaccine. A case-report of OMS after MMR vaccine in a 10-month old male was recorded in the French Pharmacovigilance Database. As in our patient, the delay between the vaccination and the first clinical signs was 15 days. The recovery is delayed in time (about 10 months). No pathological explanations were found except for the vaccination. Despite those cases of OMS, benefits of vaccination outweigh this risk and the vaccination policy against MMR remains important.
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P469
DRESS syndrome: diagnostic scores and suspected drugs

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Introduction: Many publications have focused on the definition of DRESS syndrome. Herein we compare three scores used to diagnose DRESS syndrome: Bocquet[(1)], Shiohara[(2)] and Mockenhaupt[(3)]. We focused on characteristics associated with DRESS syndrome according to Mockenhaupt.
Methods: We conducted a retrospective study from the French national pharmacovigilance database (Afssaps BNPV). We identified spontaneous reports of cutaneous serious adverse effects (SAE) notified in the Languedoc-Roussillon CRPV from January 1985 to May 2011. Then we selected from medical records all

reported cases (≥15 years old) with the following symptoms: rash and/or fever and/or eosinophilia and/or visceral injury. These cases were subsequently rated using the three scores. DRESS syndrome was considered as possible/certain score according to Shiohara and probable/certain score according to Mockenhaupt.
Results: Among the 1208 cutaneous SAE, 333 cases met the selection criteria. Among them, 26 patients (7.8%) with DRESS syndrome according to Bocquet were identified, 14 (4.2%) were rated as possible/certain according to Shiohara, 109 (32.7%), 29 (8.7%) and 6 (0.9%) patients were rated as possible, probable and certain respectively according to Mockenhaupt. Agreement was good between Bocquet and Mockenhaupt scores (κ = 0.77, 95% CI: 0.64–0.89) and moderate between Shiohara and Mockenhaupt scores (κ = 0.50, 95% CI: 0.33–0.67). Among the 35 patients with probable/certain DRESS syndrome according to Mockenhaupt, 17 were female (66.6%), 15 were over 65 years old (42.9%), median age was 60 (range: 15–86). Suspected drugs were: angiotensin II receptor antagonist or angiotensin-converting enzyme inhibitors (n = 10, 28.2%), benzodiazepines (n = 8, 22.9%), carbamazepine (n = 6, 17.1%), diuretics (n = 6, 17.1%), allopurinol (n = 5, 14.3%) and minocycline (n = 3, 8.6%).
Discussion: The different scores for DRESS syndrome diagnosis seem to be concordant. In addition to the well-known drugs inducing DRESS syndrome, we highlight the potential involvement of diuretics and renin-angiotensin system modifiers.
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P470

Uveitis associated with moxifloxacin therapy: report of two cases

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Introduction: Although well described for some drugs, ophthalmological complications of systemic medications are uncommonly reported by clinicians, sometimes ignored and expose to misdiagnosis. We report two cases of uveitis associated with moxifloxacin therapy.
Case description: These cases concern two adult patients, consulting with the same ophthalmological presentation appeared after systemic administration of moxifloxacin and initialy diagnosed as uveitis. Both patients suffered from severe ocular pain, pigmentar dispersion and severe and diffuse iris transillumination, complicating with intra-ocular hypertension. The affection was unilateral in one case, bilateral in the other one and occurred respectively between D11 and D14 and D11 after the beginning of the antibiotherapy, introduced for acute sinusitis.
Complementary investigations of this intra-ocular inflammation remained negative, including the research of HSV and VZV DNA in the anterior chamber.
Evolution was slowly favourable with antiglaucoma treatment and local corticotherapy. However, these patients are left with sequels, photophobia due an iris transillumination for one, nonreactive mydriasis for the other.
Discussion: Drug induced uveitis are relatively infrequent. A recent retrospective case series, database study and literature review presented 40 potential cases of fluoroquinolones-induced uveitis, among which 25 implicating moxifloxacin with causal relationship considered as 'possible' and a clinical presentation associating a very specific aspect of transilluminable iris and a diffuse pigmentary dispersion. Nevertheless, clinicians still doesn't seem to be informed of this possible adverse effect.
Conclusion: It then seems very important to inform clinicians of these potential complications of moxifloxacin therapy. These adverse effect have a very specific clinical presentation and the ophtalmologists' awareness, as well as all health professionnals' vigilance, should allow an early recognition of this complication that could avoid unnecessary invasive investigations for patient. In addition, as the recent publication reports cases with other fluoroquinolones, and although no signal has emerged at this time, we have to pay close attention to eventual ocular complication occuring during those treatments.
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Albendazole-induced severe pancytopenia

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Introduction: Albendazole is widely used in parasitic infections. Albendazole-induced pancytopenia is rarely reported but may have lethal consequences.
Herein, we report the case of albendazole-induced severe pancytopenia.
Case report: A 62-year old man, with history of liver cyst hydatid operated in 1996, was applied to hospital with chief complaint of fatigue and abdominal pain. A computed tomography of the abdomen showed cirrhosis with multiple small disseminated liver hydatid cysts. The diagnosis of liver cyst hydatid recurrence was retained and albendazole therapy was initiated.
Two weeks later, the patient presented with fever at 40°C. Laboratory investigations revealed a total white blood cell count (WBC) of $2.3 \times 10^3/mm^3$ with hypereosinophilia (eosinophil count $1.2 \times 10^9/L$), haemoglobin (Hb) of 6.7 g/dL and platelets: $21 \times 10^3/mm^3$.
Viral serology was negative for cytomegalovirus, Epstein-Barr virus, hepatitis B and C, HIV, parvovirus B19, and human herpes virus 6. Antinuclear antibody, anti-mitochondrial antibody and anti-smooth muscle antibody were also negative. In previous laboratory tests of the patient, 1 week prior to albendazole treatment, Hb was 8.3 g/dL, WBC was 5.93×10^3, and platelets was 118×10^3 mm^{-3}. Albendazole-induced pancytopenia was suspected and the treatment was stopped.
One month following albendazole withdrawal, Hb was 9 g/dL, WBC was 3.8×10^3 mm^{-3}, and platelet was 196×10^3 mm^{-3}.
According to the Naranjo probability scale, albendazole-induced pancytopenia was probable.
Discussion: Albendazole is an important broad spectrum antihelminthic drug widely used in parasitic infections. Side effects to albendazole are generally well-tolerated including nausea, vomiting, constipation, thirst, dizziness, headache, hair loss and pruritus. Albendazole-induced pancytopenia is rare but may lead to death if not recognized earlier. Albendazole acts by detoriating microtubular formation of the parasite. High levels of inhibition of tubulin polymerization, result in a greater inhibition of microtubule-dependent processes such as cell division. This could result in bone marrow toxicity.
Conclusion: Clinicians should proceed to frequent serial monitoring of blood counts and immediate cessation of albendazole with any evidence of marrow toxicity to avoid a fatal outcome.

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Institutional Clinical trials vigilance: situation at University Hospital of Montpellier

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Introduction: French regulation concerning the clinical trials vigilance has changed recently since in 1988 has been introduced the Huriet-Serusclat Law. Then in August 2006, this law has been modified by the transposition of European Directive 2001/20/CE and resulted in the law of Public Health. The scope of clinical trials vigilance is large as to be reported, analyzed and monitored the adverse events, new facts and the annual safety reports, in research involving a drug, a medical device or others.
Methods: A summary of data on institutional clinical trials vigilance conducted by the University Hospital of Montpellier between 1996 (date of first declaration of a serious adverse event) and 2011 has allowed us to identify different indicators of this increasing institutional activity.
Results: The number of serious adverse events (SAE) reported by the investigators has increased significantly over time since we've gone from nine SAE in 1998 to a maximum of 188 in 2011, with a total of 1159 SAE during the study period. Three hundred and seventy-six serious adverse effects were reported to the competent authorities since 1998. In addition, the number of SAE by trial varies according to the type of research. Indeed, it takes 16 SAE per trial for a research concerning a drug, against 0.6 for research on a medical device or research on out of health product. Regarding the accountability of SAE, we count 90% SAE unrelated and 10% related in 2011. Concerning the diffusion of annual safety reports (ASR) introduced in 2006, to date 60 ASR were written with a very large increase in 2011 (55 in 2011 vs. two in 2008). Finally, the workload on the activity of vigilance in clinical trials has increased dramatically since the same competent person analyzed 100 SAE in 2011 vs. 34 in 2007.
Discussion: Vigilance in clinical trials is growing and structuring is critical to standardize practices. Indeed, there is a significant difference between the institution processes. Unfortunately, the vigilance of institutional clinical trials will increase even more that there is a disengagement of pharmaceutical companies in the post-marketing survey.

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Sulfasalazine-induced DRESS syndrome in a child

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Introduction: Drug Rash with Eosinophilia and Systemic Symptoms or DRESS syndrome is a severe drug-induced hypersensitivity reaction associated with multisystem involvement. It is poorly known by pediatricians and rarely reported in children. Sulfasalazine-induced DRESS syndrome is rarely reported.
Herein, we report a case of sulfasalazine-induced DRESS syndrome in a 10-year-old girl.
Case report: A 10-year-old girl was treated with sulfasalazine (150 mg/kg daily) for psoriatic eruption. Five days later, she presented with generalized erythematous skin eruption and fever. Examination revealed multiple disseminated lymph nodes. Biological test revealed leukocytosis with eosinophilia and elevated liver enzymes. Hepatitis B, C, Epstein Barr virus serology were negative. HHV6 and CMV were also negatives. No antinuclear antibodies were detected. Sulfasalazine-induced DRESS syndrome was suspected and the drug was immediately withdrawn. Prednisolone therapy at the doses of 30 mg daily was started.
The patient was discharged home within 2 weeks.
Discussion: DRESS-syndrome is a rare drug-induced hypersensitivity reaction. It is mainly associated with antibacterial sulphonamides and anticonvulsivants. Sulfasalazine may rarely induce hypersensitivity reactions including DRESS syndrome.
DRESS syndrome is characterized by diffuse maculopapular rash, lymphadenopathy, multivisceral involvement, eosinophilia and/or atypical lymphocytes.
In children, DRESS syndrome may mimic infectious, neoplastic and immunologic conditions. This may delay the diagnosis and the prompt management.
The pathophysiology of DRESS syndrome remains unclear. It has been recently classified under a delayed type IVb hypersensitivity reaction where T-helper type 2 cells play a significant role.
The treatment of DRESS syndrome remains empirical. The suspected drug should be stopped immediately. Corticosteroid treatment with gradual withdrawal is proposed in severe forms.
Conclusion: In children, increase in awareness enables early recognition of sulfasalazine-induced DRESS syndrome so as to reduce morbidity and mortality.

P475

Pharmacokinetics of ertapenem in burn patients

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The aim of this study is to evaluate pharmacokinetic parameters of total and unbound ertapenem in burn patients since burn injury may be associated with damages which alter significantly the pharmacokinetic parameters of drugs. Ertapenem total and free plasma concentrations after separation of unbound fraction by ultracentrifugation were measured in burn patients (n = 8) who received a 0.5 h infusion of ertapenem (1000 mg) every 24 h. Pharmacokinetic parameters [mean ± standard deviation] of total ertapenem which were estimated according to a two-compartment model are consistent with results in healthy volunteers (1) and lower than results in critically ill patients (2) [Clearance (mL/min) = 21.3 ± 3.5 vs. 29.5 ± 3.4 (1) and 200.5 ± 306.9 (2). Volume of distribution (L) = 8.1 ± 2.4 vs. 8.2 ± 1.5 (1) and 59.4 ± 85.7 (2)]. This discordance could be explained by a higher unbound fraction of ertapenem in this previous study (2) [mean of area under the curve of unbound ertapenem concentration – time curve from 0 to infinity = 84 in our study vs. 180 mg/L/h in patients treated with 1000 mg of ertapenem once daily (2)] according to lower values of albuminaemia and leading likely to an increase of total ertapenem clearance and volume of distribution. These results suggest the main influence of hypoalbumineamia on ertapenem pharmacokinetics.

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P476

Effect of early reassessment of antimicrobial prescription on antibiotic use in lower respiratory tract infections

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Objective: Inappropriate antibiotic use is one of the major health problems worldwide, especially in lower respiratory tract infections (LRTI). In the Emergency Department (ED), the physician is often left with diagnostic uncertainty because of the lack of specificity of clinical signs, symptoms and laboratory markers, responsible for the overuse of antibiotics in LRTI. As the diagnosis is difficult, early reassessment of the diagnosis may help to reduce antibiotic consumption. Thus, we set up a prospective interventional study to evaluate a new strategy: early reassessment of initial antibiotic prescription for inpatients with suspected LRTI.

Methods: Prospective, interventional study, based on an intention-to-treat analysis. All consecutive patients aged 18 or older who presented to the ED with a LRTI and were prescribed antibiotics by an ED physician were eligible for inclusion. All the included patients were examined by a senior pneumologist during the first 24 h of the hospitalisation for a reassessment of the initial ED diagnostic. Based on the reassessment of the diagnosis, initial ED antibiotic prescriptions were classified as appropriate or inappropriate. Antibiotics were immediately withdrawn in the case of patients with antibiotic prescriptions classified as inappropriate. The main objective of our study was to evaluate the effectiveness of the intervention. The main endpoint was a composite of overall adverse events occurring within a 30-day period following ED admission.

Results: During a 1-year study period, a total of 63 patients were included. Based on the reassessment of the diagnosis, antibiotic prescriptions were considered inappropriate and withdrawn in the case of 15 patients (24%). All the 15 patients were free of any adverse event related to the initial diagnosis within a 30-day follow-up.

Discussion: Our data indicate that antibiotic prescriptions in the ED were relatively inappropriate. Our findings support the fact that early reassessment of initial ED antibiotic prescription is a promising strategy to develop in LRTI.

P477

Population pharmacokinetic model and Bayesian estimator of a generic formulation of cyclosporine in Tunisian renal transplant patients

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Background and objective: Equoral® is a generic formulation of cyclosporine widely used as an alternative treatment to Neoral® in Tunisian renal transplant patients. The purposes of this study were (i) to explore the pharmacokinetic variability of Equoral® in Tunisian renal transplant patients, (ii) to develop a population pharmacokinetic (popPK) model and a Bayesian estimator (MAP-BE) for the estimation of Equoral® PK parameters and global exposure (ie, AUC).

Material and methods: Full-PK profiles were obtained from 17 renal transplant patients given Equoral® twice-daily. Measurements were performed using an FPIA technique (Axsym; Abbott). To estimate popPK parameters of cyclosporine, a non linear mixed effects approach was used (NONMEM program). Different covariates (age, weight, hematocrit, AST, ALT, albumin) were investigated. In a second step, the popPK parameters were used as priors to develop a MAP-BE for estimation of Equoral® PK parameters and AUC using three blood concentrations. Predictive performances were tested by calculating mean predicted error (bias) between estimated and reference AUC (trapezoidal rule). The validation of these PK tools was performed using jackknife approach, visual predictive check (VPC) and bootstrapping method.

Results: Equoral® exhibited a high inter-patient variability: trough concentration = 90 ± 57 µg/L, C_{max} = 762 ± 343 µg/L and AUC_{0-12} = 2460 ± 1240 µg/h/L. A two compartment model with Erlang distribution best described the data: residual proportional error was 26.8% and imprecision parameter estimate was <26% (8.7–25.6%). None of the tested covariates significantly affected cyclosporine pharmacokinetics. Using this model and cyclosporine concentrations measured at 0, 30 and 180 min post-dose, MAP-BE could accurately estimate cyclosporine AUC: mean bias between estimated and reference AUC was 5.47 ± 29% with 82% of the patients with AUC bias < 20%. The doses proposed by the BE were similar to those proposed using all concentrations in 14 out of 17 patients.

Conclusion: We report a popPK model for Equoral® developed in Tunisian kidney recipients. A MAP-BE using only three blood concentrations that estimates accurately Equoral® exposure in these patients was developed and could allow dose adjustments based on the AUC in clinical setting.

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Modeling plasma virions and CD4+ lymphocytes kinetics in HIV-1 infected children related to three drug exposures: efavirenz-didanosine-lamivudine

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Background: Modeling of viral dynamics in HIV-1 infected patients has played an important role to describe and understand the mechanism of HIV-1 infection. Although highly active antiretroviral therapy is often used for the treatment of HIV infection, HIV dynamic models have only considered the effect of the most potent drug among the multidrug therapy. Based on an open phase II trial (BURKINAME – ANRS 12103), the relative effects of efavirenz, didanosine and lamivudine were determined from the simultaneous modeling of plasma virions and CD4+ lymphocytes in HIV-1 infected children. Pharmacokinetic targets for efavirenz, lamivudine and didanosine were deducted from this model.

Methods: Forty-nine children aged from 2.5 to 15 years were administered once daily dose of lamivudine, didanosine and efavirenz. Virological and hematological measurements were performed, before treatment and every trimester up to 1 year after the beginning of the treatment. A total of 285 plasma virions concentrations and 287 for CD4+ lymphocytes counts were available. A model with three differential equations representing three compartments was established: uninfected target cells (TC), infected cells (IC), and free virions (VL). The three drugs effect was then characterized by a single equation combining the effect of each drug, according to their site and mechanism of action.

Results: Efavirenz was the most potent antiretroviral and was responsible for 64% of the total effect, then didanosine for 23% and lamivudine was the less potent with 13% of the total observed effect. An EC_{90} for efavirenz was determined (2.9 mg/L). The current recommendation for efavirenz is to obtain a C_{min} from 1 to 4 mg/L then it appears from our model that this range guarantees an optimal efficacy for this drug. From the EC_{90} estimated for lamivudine and didanosine, corresponding AUC_{90} were derived: 6 and 1.4 mg/h/L respectively. These values could be taken as minimum pharmacokinetic targets to ensure optimal efficacy for these drugs.

Conclusion: The relative contributions of three combined drugs were assessed thanks to a population modeling of their effects on plasma virions and CD4+ lymphocyte counts kinetics in HIV-1 infected children. New pharmacokinetics targets have been also proposed for lamivudine and didanosine.

P479

Tacrolimus population pharmacokinetics and bayesian estimation in tunisian renal transplant recipients

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Background and objective: The inter-dose area-under-the-curve (AUC) has been described as the best marker of exposure to tacrolimus (TAC), suggesting its use for dose adjustment. In a population of Tunisian renal transplant patients, this study aimed (i) at building a population pharmacokinetic (popPK) model for tacrolimus, (ii) at identifying factors that explain interpatient variability, and (iii) at developing a Bayesian estimator (MAP-BE) enabling the estimation of individual AUC.

Material and methods: Full-PK profiles were obtained from 20 stable renal transplant recipients given Prograf® and tacrolimus blood concentrations were measured by a CMIA technique (Architect, ABBOTT). PopPK analysis was performed using nonlinear mixed effects approach (NONMEM program). The following covariates were tested: age, weight, hematocrit, AST, ALT, albumin. PopPK parameters where then used as priors to develop a MAP-BE for the estimation of tacrolimus AUC using a limited sampling strategy. The predictive performance of the MAP-BE were tested by (i) comparing the estimated AUC to that obtained by the trapezoidal rule; and (ii) its ability to provide similar dose adjustments to those obtained using all the available time-points. Validation was performed by both jackknife and bootstrapping methods.

Results: TAC pharmacokinetics were well described by a two-compartment model combined with an Erlang distribution to describe the absorption phase: residual proportional error was 16% and imprecision parameter estimate was <15% (9.3–14.4%). Body weight was identified as a covariate influencing the apparent central volume of distribution (inter-patient variability decreased from 28% to 7.7%). MAP-BE based on three blood concentrations measured at 0, 30 and 180 min post-dose provided a good estimation of AUC with a mean bias −1 + 13.2% (−22% to 22%) with 85% of the patients having an AUC bias < 20%. The BE proposed similar doses to those proposed using all concentrations in 16 out of 20 patients, with a maximum difference of 0.5 mg.

Conclusion: A popPK model and its associated Bayesian estimator providing good prediction of tacrolimus exposure have been developed in Tunisian renal transplant recipients. These tools allow us to individualize tacrolimus dosages based on the AUC using only three concentrations.

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Cholecalciferol optimal treatment during the first year after renal transplantation determined by a population pharmacokinetic model

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Aims: Because the time course of serum 25-hydroxy vitamin D (25(OH)D) concentration was not investigated, no information on optimal cholecalciferol dosing in kidney transplant patients are available. The aim of this study was to investigate 25(OH)D pharmacokinetics in kidney recipients receiving cholecalciferol and to simulate the optimal dosage scheme to maintain 25(OH)D concentrations between 30 and 100 ng/mL during the first year post-transplantation.

Methods: Four months after renal transplantation, 49 patients received four oral doses of 100 000 IU cholecalciferol every 2 weeks (intensive phase), then every 2 months until 1 year after transplantation (maintenance phase). Seventy-four samples were collected before the first cholecalciferol administration and 119 thereafter. Serum 25(OH)D concentrations were analyzed using a population approach. The turnover of 25(OH)D was modeled using a one compartment model with first order absorption and elimination and zero order production.

Results: Mean population parameter estimates were: absorption rate constant k_a 0.11/day, clearance CL/F 2.61 L/day (0.45), central volume of distribution V/F 239 L and basal concentration C_0 14 ng/mL (0.36). In order to maintain 25(OH)D concentrations between 30 and 100 ng/mL, cholecalciferol dosing should be: two successive administrations of 350 000 IU of cholecalciferol at 2 weeks intervals, then, 2 months later, 150 000 IU once a month until the end of the first year.

Conclusions: We propose an optimal and practical scheme for treatment of vitamin D insufficiency after renal transplantation. Considering the numerous effects of vitamin on health, this scheme could help clinicians to improve the care of kidney recipients.

P481

New sampling strategy to assess iohexol clearance in renal transplant recipients by Bayesian approach

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Objective: Glomerular filtration rate (GFR) measurement is a major issue in renal transplant recipients. GFR can be determined by estimating the plasma clearance of iohexol, a non radiolabeled exogenous compound. A single bolus injection of iohexol is administered and 4–6 blood samples are collected between 120 and 270 min in the terminal phase of elimination. For practical and convenient implementation for patients and caregivers, it is important that a minimal number of samples are drawn. The aim of this study was to develop and validate sampling strategies with fewer samples for reliable prediction of GFR in renal transplant recipients.

Patients and methods: Iohexol plasma concentration-time curves from 95 patients were divided into an index ($n = 63$) and a validation set ($n = 32$). Individual reference values of iohexol clearance ($CL_{iohexol}$) were calculated from k_e (elimination slope) and V (volume of distribution from intercept), measured from 4 to 6 samples, as $CL_{iohexol} = k_e.V$. Individual values were then introduced in the Bröchner-Mortensen equation to obtain the GFR [$GFR = 0.990778.\ CL_{iohexol} - 0.001218.\ (CL_{iohexol})^2$]. A population pharmacokinetic (PK-POP) model was developed in the index set and validated according to standard methods. For the validation set, Bayesian estimates of $CL_{iohexol}$ were obtained from population parameters (k_e and V) and concentrations at two or three sampling times. Individual GFR were calculated using the Bröchner-Mortensen equation and compared to individual reference values by analysis of bias (mean error) and precision (rmse).

Results: As expected, a one-compartment model best described our data. Covariate analysis showed that uremia, serum creatinine and age were significantly associated with k_e and weight with V. Standard plots and distribution of NPDE (normalized prediction distribution error) validated our PK-POP model. The strategy with samples drawn at times 120 and 270 min allowed accurate prediction of GFR (mean bias: −3.71%, mean imprecision: 7.77%).

Discussion: This Bayesian approach can help decreasing the number of samples required to calculate GFR with good accuracy. This strategy improves patient and caregivers comfort. To further reduce bias associated with the use of the Bröchner-Mortensen formula, it would be interesting to perform the same analysis on the full pharmacokinetics of iohexol.

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Population pharmacokinetics of 25-hydroxycholecalciferol in HIV-infected adults

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Background: Deficiency in serum levels of 25-hydroxycholecalciferol [25(OH)D, calcidiol] (levels < 10 ng/mL) is prevalent in HIV-infected patients, associated with osteopenia, rickets and HIV disease progression. Our aims were to investigate the 25(OH)D pharmacokinetics, the effect of antiretroviral treatment (ARV) and others factors that may influence pharmacokinetics, and to determine a dosing scheme to reach the 30 ng/mL threshold (defined as 25(OH)D sufficiency).

Methods: Calcidiol concentrations, age, bodyweight, body mass index, skin phototype, CD4 T-cell count, viral load and ARV were recorded in routine therapeutic drug monitoring in 422 HIV-infected patients (290 men, 132 women) aged from 16 to 85 years (median: 46). Among the 422 patients, 96% were on ARV and 135 received vitamin D3 supplementation with a median (min−max) number of samples per patient of 3 (2–6) for a median follow-up time of 11 months (2–31.7). A total of 723 25(OH)D concentrations were available for pharmacokinetic evaluation and a population pharmacokinetic model was developed with MONOLIX 3.2.

Results: Median 25(OH)D at baseline was 16 ng/mL (interquartile range 11–23) for the total population, 17% of patient had levels below 10 ng/mL, 68% between 10 and 30 ng/mL and 15% above 30 ng/mL. Calcidiol pharmacokinetics was best described by a one-compartment model with an additional endogenous production. The effects of summer and skin phototype were significant on production rate. The endogenous production was 20% lower in non-white skin phototype patients and is decreased by 16% during the autumn, winter and spring. No significant differences in 25(OH)D levels were related to ARV. To reach the 30 ng/mL threshold, adult should receive the following dosing scheme: 200 000 UI/2 weeks for 1.5 months then 300 000 UI/2 months for patients with a baseline below 20 ng/mL and 200 000 UI/2 weeks for 1.5 months, followed by 200 000 UI/2 months for patients with a baseline concentration between 20 and 30 ng/mL.

Conclusions: This 25(OH)D population showed that summer and skin phototype had an influence on the endogenous production of calcidiol. However no effect of ARV was found. A dosing scheme to reach sufficient 25(OH)D levels was proposed.

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Implication of flow cytometry in the development of a cellular model for the prediction of drug-induced idiosyncratic hepatotoxicity

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Idiosyncratic drug hepatotoxicity commonly results from drug-induced hypersensitivity which occurs rarely in susceptible individuals causing more than 13% of acute liver failure cases (Shaw et al. 2010). This type of toxicity represents a major obstacle in drug development due to inadequacy of preclinical screening assays (Cosgrove et al.2009). Our study aims at developing an in-vitro high throughput predictive model of idiosyncratic hepatotoxicity in which potential hepatotoxic drugs are administered to human HepG2 cell line within an inflammatory context. For this purpose we investigated drug–inflammation hepatotoxic synergies for multiple idiosyncratic hepatotoxicants in the presence of LPS and TNF by assaying the pro-apoptotic effect of these drugs on HepG2 cells using flow cytometry. Apoptosis was determined by double staining the cells with Annexin-V and PI which permits the discrimination between live cells, early apoptotic cells, late apoptotic cells and necrotic cells. The exact mechanisms underlying this synergistic hepatotoxicity remains unclear for the moment, however we are currently focusing on the modulatory effect of this synergy on important hepatic efflux transporters as a mean to explain its toxicity (Adams et al. 2010). Accordingly, we have studied the activity of MDR1 by evaluating the inhibitory potential of idiosyncratic hepatotoxicants on the efflux of Rhodamine 123 using flow cytometry. Our results demonstrate that idiosyncratic drug hepatotoxicity is noticeably potentiated by co-administrating some of the studied hepatotoxicants (trovafloxacin, taxol and nefazodone) with pro-inflammatory mediators confirming the inflammatory stress hypothesis which states that: 'An inflammatory episode occurring during drug therapy renders an otherwise non-toxic drug hepatotoxic' (Deng et al. 2009). Moreover telithromycin, nefazodone, cinnamaldehyde and thymoquinone have demonstrated an inhibitory potential on the activity of MDR1 when compared to verapamil; suggesting a tight correlation between cell death and drug induced cholestasis. The results attained so far suggest that this drug–cytokine co-treatment approach may provide a useful preclinical tool for investigating inflammation-associated idiosyncratic drug hepatotoxicity.

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Validation of the EMIT Methotrexate assay (SIEMENS) on the Cobas integra 400+ (ROCHE Diagnostics) system and comparison to the FPIA Methotrexate II assay (ABBOTT) on TDx/FLx system

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Background: Methotrexate (MTX) is used as a chemotherapeutic agent in the treatment of leukemia and lymphoma as well as of certain solid tumors. The monitoring of the treatments includes the control of MTX concentrations. There are currently two immunoassays on the market for this testing: Abbott® TDx® and SIEMENS® EMIT® assays. EMIT assay has a low-end sensitivity of 0.3 µM while TDx assay goes down to 0.02 µM. This appears to make the TDx assay a better choice for MTX monitoring. However, Abbott will stop marketing TDx test, leaving few technical solutions for MTX dosage. The aim of this study, in partnership with Roche Diagnostics, is to validate EMIT MTX assay on COBAS Integra 400+.

Methods: Reagents used were MTX SIEMENS® EMIT® assay. MTXHigh with EMIT kit standards (2; 1.5, 1; 0.5; 0.2 and 0 µM) and MTXLow with 10-fold to 100-fold dilutions of the standard 2 µM were developed on Cobas Integra 400+. Validation parameters recommended by COFRAC (French Committee for Accreditation) and their comparison to MTX-TDx assay were evaluated.

Results: Within- and between –day imprecision were <7.5%. MTXLow test linearity was validated down to 0.01 µM with a limit of detection (LOD) evaluated on 30 blanks of 0.013 µM and a limit of quantification (LOQ) of 0.03 µM (CV = 9.3%). MTXHigh test linearity was validated down to 0.1 µM with a LOQ of 0.2 µM (CV = 10%). Bland-Altman plot revealed a good correlation with no significant discrepancy for MTX concentrations between MTXlow test and MTX-TDx assay in range 0.03–0.2 µM but an overevaluation of 24% by MTXHigh test compared to MTX-TDx assay in range 0.2–20 µM. The overevaluation close to

100% for MTX concentrations below 0.2 μM when measured with MTXHigh test vs. MTX-TDx test lead us to use MTXLow test if MTX concentration obtained with MTXHigh test is below 0.4 μM.
Conclusions: the SIEMENS EMIT method application on COBAS Integra 400+ has been validated according to COFRAC guidelines. Discrepancies with MTX-TDx test exist only with concentrations above 0.2 μM. It constitutes a useful method for measuring MTX in the range of therapeutic concentrations expected in patients.

P485
Validation of a HPLC/UV method to determine daptomycin concentrations in bones

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Objectives: Daptomycin, a lipopeptide antibiotic, displays high activity against gram-positive bacteria such as *Staphylococcus aureus*. Currently, daptomycin is indicated for the treatment of complicated skin and soft tissues infections and for right-sided infective endocarditis. However, clinical data show that this concentration-dependent antibiotic may be effective against bone and joint infections. A pilot, phase one clinical study was performed in the CHRU of Besancon to assess daptomycin penetration in bones. For this purpose, a HPLC method was developed to determine daptomycin concentrations in bones fragments.
Material and method: Phosphate buffer (pH 6.0) was used for the extraction of daptomycin from bone fragments with vortex mixing, ultrasonication and centrifugation. This extractive step was repeated three times and final supernatants were kept for further process. Ethylparaben was used as Internal Standard and was added in theses samples just before SPE process (Bond Elut Plexa Agilent®; 30 mg; 1 mL). Chromatographic analysis was performed on a reverse phase C18 column (Omnispher Agilent®; 150 × 4.6; 5 μm) at room temperature. The flow rate was constant (1 mL/min) with a mobile phase: phosphate buffer (pH 3.8) and acetonitrile (65/35 v/v). Detection was by UV absorbance at 224 nm (PhotoDiode Array Spectra System Thermo®).
Results: Retention times were respectively of 6.2 and 8.2 min for ethylparaben and daptomycin. This method was validated following FDA recommendations (good selectivity, no carry-over, linearity of the calibration curves without weighting, deviations from nominal concentrations of standards samples lower than 15%, intra- and inter-assay precision and accuracy lower than 15%). Limit of quantification was 0.2 μg of daptomycin. Calibration curve was linear up to 2.4 μg of daptomycin. Final concentrations in bones (μg/g) were normalized by the weight of bone fragments used.
Discussion: To our knowledge, this is the first method described for the extraction of daptomycin from bone fragments with further HPLC/UV determination. This method enables us to carry out DAPT-OS, a pilot, phase one clinical study to assess the penetration of daptomycin in bones.

P489
Population pharmacokinetics of Phenobarbital in neonates and infants

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Introduction: Phenobarbital is widely used for treatment of neonatal seizures and prevention of neonatal hyperbilirubinaemia. A pharmacokinetic model of phenobarbital in 35 Japanese neonates and infants was described in 2005 and an update was published in 2011 by Yukawa et al. [1, 2], studying oral and rectal routes. The objective of this study is to describe a pharmacokinetic model for estimating oral bioavailability of phenobarbital in pediatrics.
Method: Thirty-nine neonates and infants (weight: 0.7–20 kg; PNA: 0–48 weeks) hospitalized in a pediatric intensive care unit, were studied. Total mean dose of 56.0 mg (12.5–200.0 mg) per day was administered by 30-min infusion or by oral route. Blood phenobarbital concentrations were determined by immunoassay method. Pharmacokinetic analysis was performed by using a non linear mixed-effect population model (NonMEM software). Data analysis included calculation of performance error (PE), median performance error (MDPE) and median absolute performance error (MDAPE). A bootstrap was used as internal evaluation.
Result: Data were modelled with an allometric pharmacokinetic model, using three-fourths scaling exponent for clearance. This one-compartment model gave the following results. The population typical mean (percent relative standard error (%RSE)) values for clearance (CL), apparent volume of distribution (V_d) and bioavailability (F) were 0.0051 L/h/kg (7.9%), 0.65 L/kg (13.7%) and 0.53 (20.8%), respectively. The interindividual variability of V_d (%RSE) and residual variability (%RSE) were 17% (64.7%), 51% (39.4%) and 39% (50.2%), respectively. The indicators of predictive performance gave the following Results:
MDPE (95%CI) was −3.2% (0.6–19.7%) and MDAPE (95% CI) was 29.9% (0.5–16.9%).
Conclusion: The pharmacokinetic parameters of phenobarbital in neonates and infants were estimated. The predictive performance was acceptable with a small bias. These intermediate results should be confirmed by the inclusion of new patients.
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Population pharmacokinetic analyses and neonates: An overview

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Background: Three decades after its introduction, the pharmacokinetic population approaches became a reference method for drug modelling in particular in paediatrics. The main practical difficulty in clinical studies in this population is the invasiveness of the procedures and the pharmacokinetic population approaches has allowed answering to this problem with studies with sparse sampling. Numerous studies devoted to this topic were published these last 25 years and thus a synthetic view is needed.
Objective: This review summarizes all the publications concerning population pharmacokinetic approaches in paediatrics from neonate to 2 years old.
Methods: A literature search was conducted from the PubMed database, from their inception through December 2010, using the following terms: *pharmacokinetic(s), population, paediatric/pediatric and neonate(s)*. Articles were excluded if they were not pertinent. References of all relevant articles were also evaluated.
Results: Ninety-eight studies were included in this review. The following information was extracted from the articles: the drug name, therapeutic class, population size, age of patients, number of samples per patients, software used for modelling and validation methods. An increasing rate of publications over the years was observed, the NonMEM software being the most used and 44 different drugs having been studied by pharmacokinetic population. The class of antibiotics was the most studied, with emphasis on two drugs: vancomycin and gentamicin. It must be noticed that few studies have been performed on antiepileptic drugs and that the anesthetics studied were not those used in clinical.
Conclusion: This review described the numerous population pharmacokinetic models developed in neonates and sheds light on drugs for which further pharmacokinetics information is required in neonates.

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Evaluation of therapeutic drug monitoring (TDM) of cyclosporine (CsA) and tacrolimus (TAC) on the Architect® Abbott system

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Introduction: CsA and TAC are immunosuppressant indicated in graft rejection prevention and autoimmune diseases. Their narrow therapeutic range and large pharmacokinetic variability, require TDM to prevent transplant rejection and minimize toxicity.
CMIA (Architect®) whole blood levels of immunosuppressant were compared with FPIA (AxSYM®) levels for CsA or MEIA (IMX®) levels for TAC.
Materials and methods: Patients' samples and external quality controls were tested: 131 for CsA and 199 for TAC. Each assay was performed following the manufacturer's recommendations. Statistical analysis was performed using the Passing-Bablock regression, CUSUM test and Bland-Altman plots (MedCal®).
Results: A positive bias of 0.24 μg/L was observed between MEAI and CMIA for TAC. Passing- Bablock regression [$y = 0.01505 + 0.9785x$] and CUSUM test ($P > 0.1$) showed no significant deviation from linearity between the two methods. Bland-Altman plots showed a bias of −1.5 μg/L, with a standard deviation of 65.5 μg/L, between FPIA and CMIA for CsA. Passing-Bablock regression ($y = -13.8500 + 1.0597x$) and CUSUM test ($P < 0.05$) confirmed this deviation.
Discussion: FPIA whole blood CsA levels exhibit higher results than after CMIA. Previous studies have shown same differences depending on the immunological technic used[1], in relation with a variable cross-reactivity rate of CsA metabolites (AM1 and AM9). Oellerich team evaluate cross-reactivity rate on AxSYM® at 14.5% for AM9 and 8.6%for AM1 [1]. On Architect® the rates are below 1%[2]. Because the CsA's metabolites are less active, the CMIA results are a better estimate for clinical use. For the TAC, we obtain similar results with both assays. The lower limit of quantification on Architect® technic (2 μg/L) improves TDM performance for patients treated with low doses, as recommended by the European conference consensus on TDM TAC[3].
Conclusion: The analytical performances of CMIA and the clinically acceptable differences between the results of two assays for each molecule, allow immunosuppressant TDM on Architect® system.
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P492
Comparison of four Bayesian methods for the estimation of past and the prediction of future concentrations of amikacin in elderly patients

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The Bayesian approach is considered as the most efficient method for dose adjustment of aminoglycosides [1]. The Maximum A Posteriori (MAP) estimation is the classical method used in such Bayesian framework. Jelliffe and colleagues have developed three other Bayesian methods, the Multiple Model (MM), Interacting Multiple Model (IMM), and Hybrid Multiple Model (HMM) (www.lapk.org). The objective of this study was to compare the performance of these four methods.
Amikacin concentrations from geriatric patients collected from 2001 to 2010 in our institution were analysed in two ways. First, the entire data set of each patient was fitted with the four methods. Then, the data were analyzed sequentially, for three occasions of therapeutic drug monitoring (TDM). On each occasion, the four methods were used to estimate the past concentrations available at this time and to predict the subsequent concentrations to be observed on the next occasion. A nonparametric population model of amikacin implemented in the MM-USC*Pack software was used to analyze all data. Mean error and mean squared error of prediction precision were used to assess bias and precision of each method.
Four-hundred and six amikacin concentrations from 96 patients were available. In the analysis of the entire past therapy, bias and precision of the four methods were significantly different ($P < 0.001$). IMM was much more precise (2.3 mg^2/L^2) than the MAP, HMM and MM methods (10.2, 10.9, and 25.5 mg^2/L^2, respectively). In the sequential analysis, IMM also best fitted the past concentrations on each occasion. In the prediction of future concentrations, biases of the four methods were significantly different. The MM method better predicted future concentrations estimated from sparse data with bias values of 0.001, 0.35, 1.00, and 1.10 mg/L for the MM, IMM, HMM, and MAP methods, respectively.

In this study, the Bayesian IMM method better fitted past concentrations of amikacin, while the MM method better predicted future concentrations. The classical MAP showed no advantage over the new Bayesian methods. This study also questions the paradigm 'the better the estimation, the better the prediction' in clinical pharmacokinetics.

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Therapeutic drug monitoring of azathioprine in myositis

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Introduction: Azathioprine is an immunosuppressive drug prescribed in second line treatment for myositis. Because of its pharmacokinetic inter individual variability and its haematopoietic and liver toxicity, therapeutic drug monitoring is important. Interindividual differences in therapeutic efficiency might be explained by the polymorphism of thiopurine S-methyltransferase (TPMT), the enzyme involved in the metabolism of azathioprine.

Objective: The aim of our work was to study the relation between azathioprine blood level, patient TPMT genotype and tolerance.

Patients, materials and methods: 6 thioguanine (6TG) and 6 methylmercaptopurine (6MMP) were quantified in erythrocytes from patients treated with azathioprine and corticoids for myositis. Both metabolites were quantified using reversed-phase high-performance chromatography (HPLC) with UV detection at two different wavelengths: 303 nm and 342 nm. Biological parameters (liver enzymes and blood count) were used for hemato and hepatotoxicity assessment. Patients were genotyped for TPMT*3A, TPMT*3B, TPMT*3C and TPMT*2 wich are the most frequently reported genetic variants.

Results: Five patients (four female and one male) with myositis and long term antimetabolite therapy were included in the study. Blood sampling was performed between 4 months and 4 years after treatment initiation. Patients (aged from 36 to 78 years, mean 59 ± 12.5) were treated with 75–150 mg by oral route. Double TPMT variants were detected in one subject (TPMT*3C et B). Mean trough metabolite concentrations in erythrocytes ranged from 131 to 365 (mean 241) pmol/3.10^8RBC and from 63 to 5623 (mean 3211) pmol/3.10^8RBC for 6TG and 6MMP respectively. Moreover, the full blood count and the hepatic function tests were within normal ranges.

Discussion and conclusion: These preliminary results show no haematopoietic and liver toxicity in our patients. In addition, no major toxicity was detected in patient with the double TPMT variations. However, 6TG concentrations are interpreted with therapeutic range used in IBD. Therefore, future studies will be set up in order to establish a therapeutic range for azathioprine metabolites in the treatment of myositis.

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Evaluation of the QMS® Teicoplanin (Microgenics) reagent for serum quantification of teicoplanin on the CDx90® automated analyser, in view of replacing the TDx/FLx

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Aims: To evaluate the performance of QMS® Teicoplanin (Microgenics) reagent for serum quantification of teicoplanin on the CDx90® automated analyser, in view of replacing the TDx/FLx.

Materials and methods: The QMS® Teicoplanin method used on the CDx90 automated analyser is an immunoturbidimetry technique by kinetic inhibition of agglutination of the particles in solution. The performance checks of this method were performed in our laboratory in compliance with the requirements of the standard ISO 15 189 and Cofrac (French Accreditation Committee) document SH GTA 04. The expected performance criteria have been fixed in accordance with the vancomycin state-of-the-art requirements. The QMS® Teicoplanin technique has also been compared to our routine.

Method: Teicoplanin® (LABfx/Eurobio) reagent on the TDx/FLx (Abbott), using the fluorescence polarization immunoassay (FPIA) principle. Finally, the HPLC-UV method was used for the final analysis in cases where discordance was seen (a difference of $> \pm 2\sqrt{2} \times CV$ is acceptable, i.e. 28%). One hundred and thirty-six samples were tested: 54 internal quality control samples, to test the intermediate precision (three concentration levels, analysed in 18 separate runs); 82 clinical samples from our routine. These samples were used for repeatability testing (two samples repeated 15 times), contamination testing, linearity testing and for the comparison of the two methods (82 samples). 47/82 samples were controled using HPLC-UV.

Results: The repeatability (≤3.2%) and the intermediate precision (≤3.9%) are excellent, there is no inter-sample contamination and the linearity is verified. However, numerous conflicting results are seen between the CDx90 and the TDx (59/82 i.e. 72%), with lower results reported on the CDx90 (on average −31.9%) than on the TDx/FLx. By HPLC-UV, the results obtained on the CDx90 are confirmed more often (82%) than those obtained on the TDx/FLx.

Conclusion: The QMS® Teicoplanin technique on the automated analyser CDx90 gives a good analytical performance and could be considered as a replacement for the TDx/FLx for therapeutic teicoplanin follow-up testing on plasma samples. However, the measured levels in plasma differ depending on the method used. Each laboratory should therefore check the reference values proposed for result interpretation. The HPLC-UV technique can also be an alternative to immunoassays.

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Stability tests of 4 immunosuppressive drugs in whole blood according to EN 15189 COFRAC standards

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Introduction-objectives: The method for determination of four immunosuppressants in whole blood (cyclosporin, tacrolimus, sirolimus and everolimus) was recently switched in our laboratory from an immunological technique to a chromatographic technique LC-MS-MS. The accreditation of this analysis according to the EN 15189 COFRAC standard prompted us to think about the pre-analytical phase and particularly on the stability of the received samples. Maximum delay between two analytical series of immunosuppressant drugs in our laboratory is 3 days. Are the samples put in refrigerator or freezer stable for at least 3 days?

Methods: For each molecule, 10 blood samples on EDTA at different concentration levels were selected. The samples were aliquoted and stored in refrigerator at +4°C and in freezer at −20°C for 1–3 days. These samples were analyzed by LC-MS-MS (3200 QTRAP AbSciex®). The stability storage was assessed by comparing freshly whole blood samples with stored samples.

Results-discussion: The whole blood samples stored at −20°C for 1, 2 or 3 days showed acceptable analytical recovery and imprecision compared to freshly prepared samples. The observed stability of the four immunosuppressive drugs in whole blood was consistent with those documented in literature, although in most cases, the studies were conducted on spiked samples.

Conclusion: The quality of the result depends on the quality of the sample... The stability of the samples in pre-analytical and post-analytical phases has to be known in order to optimize the storage conditions (temperature, light exposition...). The accreditation of our laboratories according to the EN 15189 COFRAC standard prompted us to reassess processes too often based on habits and not on tests in situ or on the bibliography.

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Adalimumab pharmacokinetics and concentration-effect relationship in Crohn's disease

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Objectives: Adalimumab, an anti-TNF-α antibody, is effective in active Crohn's disease (CD). Subcutaneous injections of adalimumab lead to highly variable concentrations between patients and this pharmacokinetic (PK) variability partly explains the variability of clinical effect. However, adalimumab PK after subcutaneous injection has never been reported. The goal of this study is to build a simplified PK model for therapeutic drug monitoring (TDM) of adalimumab in CD patients and to describe the concentration-effect relationship of adalimumab in these patients using PK-PD modeling.

Methods: Adalimumab PK data were taken from a multicentric observational study in which 168 CD patients were included. Trough adalimumab concentrations, CRP levels and antibodies toward adalimumab (ATA) were measured. The PK of adalimumab was described using a one-compartment model with first order elimination. Because adalimumab absorption is slow after SC injection, it was approximated using a zero-order infusion rate. The relationship between adalimumab concentrations and CRP levels was described using an indirect response model with inhibition of CRP input. A population approach was used and models were run simultaneously. Sex, age, body weight, smoking status and the presence of ATA were tested as covariates on each pharmacokinetic and PK-PD parameter.

Results: A total of 341 adalimumab trough concentrations and 317 CRP concentrations were available in the 65 eligible patients. Adalimumab PK and the relationship between adalimumab concentration and CRP data were satisfactorily described by the models. The following PK and PK-PD parameters were estimated (interindividual coefficient of variation): apparent volume of distribution (Vd*) = 17 L (13%), apparent clearance (CL*) = 0.41 L/day (83%), zero-order CRP input rate (k_{in}) = 143 mg/L/day (116%), first-order CRP output rate (k_{out}) = 9.2/day and adalimumab concentration leading to 50% decrease of k_{in} (C_{50}) = 5.9 mg/L (127%). Presence of ATA was found to increase CL* seven times.

Discussion: This simplified PK model, for which the absorption of adalimumab is approximated by a zero-order infusion rate, provides a satisfactory description of adalimumab concentrations in CD patients. This model allows TDM of adalimumab in CD patients and the analysis of its concentration-effect relationship.

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High performance liquid chromatographic quantification of rifampicin in human plasma: method for therapeutic drug monitoring

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Rifampicin is a major antituberculosis drug used also in other infection such as staphylococcal infection. Therapeutic drug monitoring (TDM) of this drug is necessary in children, in patients with, mycobacterium tuberculosis positif culture after 2 months treatment, AIDS or gastro-intestinal diseases causing absorption problems of this drug and in patients with renal or hepatic insufficiency. TDM of rifampicin has been shown useful clinically by various teams to enhance efficacy and avoid toxicity of this drug.

The present study was undertaken for developing and validating a simple and rapid High Performance Liquid Chromatography (HPLC) method with Ultraviolet (UV) detection, which could be useful for TDM of rifampicin in routine setting.

The method is based on the precipitation of proteins in human plasma with methanol and direct injection into HPLC system. A C18 column and a simple mobile phase consisting of 0.05 M dipotassic hydrogen phosphate buffer and acétonitrile (53/47, V/V) and 0.086% diethylamin, pH = 4.6 was used. The flow rate was 1 mL/min and the effluent was monitored at 340 nm. The assay method is linear in the concentration range 1–20 μg/mL ($r^2 > 0.99$). The limit of quantifi-

cation and limit of detection of rifampicin were respectively 0.632 μg/mL and 0.208 μg/mL. Intra and inter day coefficient of variation and bias were below 10% for all samples, suggesting good precision and accuracy of the method. Recoveries were >90% in plasma samples volume of 100 μL. The method is being successfully applied to therapeutic drug monitoring of rifampicin in plasma samples of patients with tuberculosis and staphylococcal infections.

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Monitoring of imatinib plasma levels by liquid chromatography with ultraviolet detection

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Imatinib is a selective and specific tyrosine kinase inhibitor which has demonstrated significant clinical efficacy in some type of cancer such as Chronic Myelogenous Leukemia (CML) and Gastro-Intestinal Stromal Tumor (GIST). Imatinib is administrated as oral drug. Its bio-availability presents high variability and several studies have shown no correlation between doses and plasma concentrations of this drug but the presence of correlation between clinical response and its plasma concentrations. This drug presents also several dose dependent side effects. So therapeutic drug monitoring (TDM) of imatinib has been shown useful clinically by various teams to enhance efficacy and avoid toxicity of this drug.

The present study was undertaken for developing and validating a simple and rapid HPLC method with UV detection, which could be useful for TDM of imatinib in routine setting.

Samples were prepared in a simple and single step by precipitation of plasma protein with methanol. Imatinib was separated using a Lichocart cartridge column (250 × 4 mm) filled with Lichrospher 1000 RP-8, having a 5 μm practical size maintained at 25 °C. The samples were eluted in a mobile phase consisting of 0.02 M dipotassic hydrogen phosphate buffer and acétonitrile (73:27, V:V) at a flow rate of 1 mL/min. The mobile phase was filtered through a 0.22 μm filter and degassed under vacuum prior to use. The detector wavelength was set at 265 nm. Calibration plots in spiked plasma were linear in a concentration range of 500–4000 ng/mL. Inter and intra-day coefficient of variation (precision) and bias (accuracy) were < 10%. Limit of detection (LOD) and limit of quantification (LOQ) of imatinib were 125.41 and 380 ng/mL, respectively. Mean recovery of imatinib ranged from 93.84% to 109.68 ng/mL. The cross validation showed that the number of samples within a 20% difference from reference value was 19 out of 24 samples. Method developed is flexible and may be easily useful for analyzing clinical samples containing imatinib.

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Quality management in pharmacological and toxicological bioanalytical procedures for the chromatographic quantitative determination of drugs: new guidelines from the European Medicines Agency

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Introduction: Quality is a major outcome in bioanalytical procedures, even more since the French legislation made the accreditation procedure mandatory for medical analysis laboratories. Most of drug measurements for pharmacokinetic/pharmacodynamic analysis in clinical trials or in therapeutic drug monitoring or toxicological analysis require chromatography-based methods. New guidelines from the EMEA for the validation of such methods will come into effect in February 2012. The aim of our work was to set-up these guidelines for the routine validation of the methods developed in a pharmacology-toxicology laboratory.

Methods: We performed a critical analysis of the previously mentioned guidelines (EMEA/CHMP/EWP/192217/2009), together with the EMEA reflection paper on guidance for laboratories that perform the analysis or evaluation of clinical trial samples, the international standards ISO15189 and ISO22870 and the COFRAC guide (SH-GTA-04). We then compared the key elements with the commonly used FDA guidelines (Bioanalytical Method Validation, 2001) and set-up a practical methodology to apply these recommendations.

Results: The EMEA guidelines repeat most of the current FDA requirements in terms of analytical performances (accuracy, precision, selectivity, stability…), but their practical evaluation is better defined. New items have appeared such as the evaluation of the matrix effect in mass spectrometry, interferences with drug metabolites, or analysis of samples from patients with renal or hepatic impairment. Despite their scientific justification, some of these items may be quite hard to assess, due to the difficulty to obtain the drugs of interest (metabolites of the parent drug…) or sufficient amounts of the requested biological samples. The guidelines also focus on the continuous process of the validation, which should be carried on during all the method lifetime, on the traceability of the reagents used, and on the redaction of validation reports. Particularly for clinical trials, quality management has to be set-up in the laboratory, but the items are close to those necessary for the accreditation procedure.

Conclusion: These new guidelines which are being applied in our laboratory describe analytical features that should be used for the setting-up of chromatographic methods reliable for the determination of drug concentrations in biological matrixes, during either medicines development stages or in routine analysis.

P500

Development of a LC-MS/MS method for TDM of antibiotics (Isoniazid, Rifampicin, Clarithromycin, Moxifloxacin, Ciprofloxacin, Ofloxacin and Cefepime)

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Objective: TDM of antibiotics is currently performed in the laboratory of Pharmacology and Toxicology University Hospital of Amiens by HPLC-DAD. In order to increase sensibility and specificity of TDM and to reduce sample volume required of Isoniazid, Rifampicin, Clarithromycin, Moxifloxacin, Ciprofloxacin, Ofloxacin and Cefepime assays, we implemented a LC-MS/MS technique for simultaneous determination of these antibiotics in serum.

Material and methods: The volume of sample required is only 100 μL. Chromatographic separation (Prominence, Shimadzu®) was performed with a reverse phase column (Restek PFP Propyl Column-50 × 2.1 mm) using reserpin as internal standard. Detection was performed with a triple-quadrupole mass spectrometer (3200 QTRAP, ABSciex®) that monitored two specific transitions per compound in electrospray, positive-ion selected reaction monitoring mode. Calibration samples and internal controls have been prepared and injected to define the linearity of the method. We then tested pre -analytical treatment by deproteinization. Analytical validation of the method has been performed, in accordance to EN 15189 COFRAC standards, before its clinical use. We then compared determination of concentrations in patients with the two methods.

Results: The technique we have developed shows analytical performances comparable to those currently published. Linearity for each molecule is compatible with a clinical use (from 0.5 to 100 μg/mL). Intra- and interday reproducibility shows variation below 20%. Contamination between samples is negligible.

Discussion: As liquid/liquid extraction requires the use of toxic solvants and a high sample volume, we preferred deproteinization despite a lower extraction rate. Correlation of the two methods led us to define corrective factors for clinicians. Indeed, LC-MS/MS concentrations were inferior to those measured by HPLC/DAD, because of a higher sensitivity and thus lower interferences.

Conclusion: The method we have developed shows good analytical performances and allows rapid response to clinicians. It has allowed us to improve sensibility and specificity of the TDM of seven antibiotics. Moreover, the simultaneous determination saves time and sample volume.

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Therapeutic drug monitoring of posaconazole in adult patients with haematological malignancies under posaconazole prophylaxis

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Objective: Posaconazole (PCZ) is given at 200 mg three times daily as a fungal prophylaxis in patients at high risk of developing invasive fungal infections (IFIs). Low PCZ plasma concentrations have been associated with an increased risk of clinical failure in the prophylactic indication. The objective of this study was to analyze the PCZ plasma concentration in patients with haematological malignancies under PCZ prophylaxis.

Methods and patients: We determined PCZ plasma levels between January 2010 and October 2011 in 33 patients with acute myeloid leukaemia or myelodysplastic syndrome (eight females/25 males) with a mean ± SD age of 57.2 ± 15.3 years. PCZ plasma levels were determined by a validated high performance liquid chromatography method with ultra-violet detection (LOQ: 0.1 μg/mL).

Results and discussion: Forty-one blood samples were studied. The median PCZ plasma concentration was 0.33 μg/mL (range <0.1–1.5 μg/mL). Overall, 78% of PCZ plasma concentrations were under the recommended threshold of 0.50 μg/mL and 83% were under a cut-off of 0.7 μg/mL recommended by FDA based on available exposure-response relationships. Treatment duration of PCZ was in mean of 18 days and PCZ plasma levels were measured after a mean of 9 days of PCZ prophylaxis. Three patients developed 'possible' or 'probable' IFIs. A low exposure of PCZ was observed in these patients: 0.23, 0.36 and 0.36 μg/mL and a treatment with voriconazole and/or amphotericin B was initiated. PCZ was well tolerated. Treatment was switched to caspofungin or voriconazole in three patients for intolerance: gastrointestinal adverse effect ($n = 2$) and toxidermitis ($n = 1$). These results enhance the high prevalence of PCZ suboptimal concentrations under prophylaxis. Then, PCZ therapeutic drug monitoring (TDM) is essential in order to early detect patients with low concentrations and identified the etiology of such results. However, given the low number of patients developing probable or proven IFIs, PCZ cut-off under prophylaxis should be reevaluate.

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Serum determination of teicoplanin with HPLC as an alternate to immunoanalysis

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Introduction: Teicoplanin is is a glycopeptide antibiotic used in the prophylaxis and treatment of serious infections caused by Gram-positive bacteria. Monitoring of teicoplanin levels is recommended in severe or deep-seated infections or in prolonged courses. The Abbott TDx fluorescence polarization immunoassay (FPIA) which has been largely used in France will be soon unavailable. HPLC methods exist as possible replacements for serum teicoplanin measurement. We present a simple and accurate HPLC method we retained and adapted (McCann et al, 2002), easily usable by the analysts of the Therapeutic Drug Monitoring group.

Method: After de-proteinisation with acetonitrile followed by an extraction with chloroform, an aliquot of the aqueous supernatant phase was injected in a reverse-phase C18 column, at 210 nm. The validation included repeatability, reproducibility, and accuracy tests. A series of patient samples was compared with the two methods at disposal.

Results: The inter-assay coefficients of variation were 8.1%, 7.1% and 6.1% at 12.4, 24, 50.6 mg/L, respectively. The intra-assay coefficients of variation were 1.5%, 2.1% and 3.1% at 13.4, 24, 55 mg/L, respectively. The HPLC method is linear over the range 10–100 mg/L. The limit of detection is 5 mg/L. The mean error calculated ($n = 28$) on patient samples determined in duplicate was 4.5%. The liquid extraction coupled with HPLC correlated well with FPIA: HPLC = 0.981 TDx – 2.532, $R^2 = 0.906$ ($n = 54$).

Conclusion: The HPLC method described here is simple, robust, highly reproducible and suited to a clinical laboratory with the appropriate equipment. The absence of divergence between results obtained with the two methods did not require any change in the recommended concentrations.

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P506

Pharmacokinetic and pharmacodynamic variability of fluindione in octogenarians

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Context: Fluindione is an oral antivitamin K (AVK) agent comprising about 80% of oral anticoagulant prescriptions in France. AVK are characterised by a narrow therapeutic window, with bleeding being the major adverse event, leading to a high number of hospital admissions [1]. Elderly patients, in whom the prevalence of diseases with an indication for anticoagulant treatment, such as atrial fibrillation, is increased, often receive concurrent medications with potential interactions and may suffer from other morbid conditions such as denutrition, and these reasons have been proposed to explain the increased risk of bleeding in that population [2]. The PREPA observational study was designed to investigate the factors influencing pharmacokinetic and pharmacodynamic variability in the response to fluindione in a general population of octogenarians inpatients.

Methods: Measurements of fluindione concentrations and INR (International Normalised Ratio) were obtained from 131 inpatients initiating fluindione treatment. Treatment was adjusted according to routine clinical practice. The data were analysed using non-linear mixed effect models, and the parameters were estimated using the MONOLIX software.

Results: The pharmacokinetics of fluindione was monocompartmental, while the evolution of INR was modelled according to a turnover model (inhibition of vitamin K recycling). Interindividual variability was very large. Clearance decreased with age and with prior administration of cordarone. Patients who underwent surgery before the study had lower IC50, leading to an increased sensitivity to fluindione. The interindividual variability was large.

Conclusion: AVK administration is challenging because of the long equilibration half-time, and could benefit from modelling-based approaches to better anticipate the fluctuations of INR. Pharmacokinetic exposure is substantially increased in elderly patients, warranting a lower dose of fluindione.

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Tacrolimus-Telaprevir interaction monitoring: case report in a liver transplant patient with recurrence of hepatitis C virus (HCV)

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Introduction: Telaprevir, a new hepatitis C virus protease inhibitor, is a substrat and inhibitor of the enzyme cytochrome P450 3A4 (P4503A4) and has the potential to saturate or inhibit P-gp in the gut. Tacrolimus is a substrat of both P450 3A4 and P-glycoprotein (P-gp), a transmembrane transporter. Therefore, coadministration with telaprevir may increase the systemic exposure to tacrolimus. In the literature, there are no guidelines for dose adjustments of these drugs in transplant patients because telaprevir has not been studied in pre- or post-transplant patients. Garg and al., in a healthy volunteer study, found that steady-state telaprevir increased the blood concentrations of 1 mg of tacrolimus significantly (dose-normalized (DN) exposure (DN_AUC) approximatively by 70-fold and the terminal elimination half life by 5-fold).

Observation: We report the case of a 60-years-old double liver transplant male recipient treated by once-daily prolonged release tacrolimus, who presented recurrence of HCV infection. He started ribavirin and interferon but, because of a severe anemia leading to decrease dosage, telaprevir was introduced.

In order to manage interaction between tacrolimus and telaprevir, the first step was the conversion from Advagraf (5 mg) to Prograf (0.5 mg), a twice daily tacrolimus formulation. The effect of telaprevir (750 mg q8h) on tacrolimus pharmacokinetics was studied 6 h after a single dose of tacrolimus. Then, it was decided to administrate empirically tacrolimus twice a week and to develop a therapeutic drug monitoring (target under 5 µg/L) every day at 6 a.m. until telaprevir steady state at 7 days. Forty-five days after, no adverse reactions causing by tacrolimus overdose was noted.

Discussion/Conclusion: The degree of the interaction between telaprevir and tacrolimus is not predictable. In our case, we found a discrepancy with the study by Gard et al. maybe because this study was conducted in healthy volunteers and because a single dose 0.5-mg of tacrolimus was administrated. A 35 fold diminution dose could be considered as a valuable protocol, but an inpatient drug monitoring is necessary. Further studies are urgently required and clinicians should be aware that coadministration of telaprevir and tacrolimus could lead to serious of life-threatening events.

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Comparison between a chromatographic and three coagulation assays for dabigatran and rivaroxaban quantification

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Objectives: New anticoagulants such as dabigatran or rivaroxaban supposedly don't require any monitoring thanks to their more predictable pharmacological profile, but specific clinical conditions might benefit from coagulation testing. Numerous dedicated tests are appearing but their relation to drug exposure is unclear. We developed and validated a simple liquid chromatographic method with tandem mass spectrometer detection and applied it to samples of patients undergoing total knee or hip replacement for comparison with functional assays.

Materials and methods/patients: Dabigatran and rivaroxaban were quantified in a single 6 min run after solid phase extraction, separation on an Atlantis C18 5µ column (Waters®) and detection in positive electrospray mode using isotopic internal standards ([$^{13}C_6$]-Rivaroxaban and [$^{13}C_6$]-Dabigatran, Alsachim®) for quantification. Coagulation assays were derived from the diluted thrombin time and two antiXa methods for dabigatran and rivaroxaban quantification respectively. Plasma samples from patients treated with rivaroxaban (n = 42) or dabigatran (n = 35) were collected at peak concentration several times after surgery (rivaroxaban: n = 136, dabigatran: n = 92) and were tested using chromatographic and coagulation methods.

Results: Calibration curves were linear between 0.5 and 500 ng/mL (30–400 ng/mL for coagulation assays). Intra- and inter-assay variability ranged from 2 to 8.4% for dabigatran and from 3.2 to 7% for rivaroxaban. Matrix effect and extraction recoveries were within 80–120%. Patients concentrations reproduced previously described results (107 ± 75, 92 ± 78 ng/mL for rivaroxaban and dabigatran respectively, mean ± SD) with all methods, with a high inter- and intra-patient variability. Discrepancies >20% were found in 20% and 29% of samples for rivaroxaban and 30% for dabigatran assays. Discordant values might be explained by icteric and blurred plasma samples that might disturb the optic measurement by coagulation tests. Deming regressions provided correlation coefficient > 0.9 and slopes of 1.1 for all comparisons.

Discussion: Biases between methods were acceptable. We observed a high concentration variability, which confirms the need of validated monitoring methods. Functional coagulation tests showed a reliable correlation to drug concentrations and are more suitable for routine practice as they are available on automated systems often present in 24H emergency units.

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Determination of iohexol and its application to glomerular filtration rate estimation: validation of a new UPLC-MS/MS method with ICP-MS comparable performances

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Aim: Repeated iohexol measurements and calculation of its clearance are commonly used to estimate GFR in patients with renal insufficiency. Our laboratory had developed a method using Inductively-Coupled Plasma- Mass Spectrometry (ICP-MS), with a very low limit of quantification (0.0001 mg/L). Good Laboratory Practice recommendations insist on the necessity whenever possible for back-up methods. As other described techniques (1–4) would not be sensitive enough, we decided to validate a new UPLC-MS/MS method that would satisfy our requirements.

Methods: The method was set up on a Waters Xevo-TQ UPLC-MS/MS device. After a Water/Methanol/$ZnSO_4$ defecation with ioversol as an internal standard, iohexol was eluted from a C_{18}-BEH column within 1.5 min with a gradient acetonitrile/water mobile phase. Analytical validation was realized according to the recent French Society of Analytical Toxicology recommendations. Correlation to the former ICP-MS method was realized comparing the results for nine patients for whom a kinetic iohexol elimination study had been realized, with blood samples drawn at 0, 60, 120, 180, 240, 480 and 1440 min after iohexol administration.

Results: Extraction efficiency was satisfying, with a variation coefficient of <20% for the three levels of control, respectively 0.78, 12.5 and 50 mg/L. Limits of detection and quantification were determined at 0.0159 and 0.0396 mg/L respectively. Linearity was demonstrated between 0.78 and 100 mg/L (R^2 = 0.99959). Precision was established, with variation coefficients for repeatability and reproducibility of <10% for the three levels of control. Accuracy was statistically demonstrated and uncertainty calculated at 0.51, 2.29 and 1.58 mg/L respectively for the three levels of control. No matrix effect was detected. Good correlation was established with ICP-MS method, for each sample (R2 = 0.9888) and for the corresponding calculated clearances.

Conclusion: This new UPLC-MS/MS method for iohexol determination has proved highly performing, more sensitive that the existing UPLC method (4). Even if ICP-MS stays more sensitive, both methods are comparable in terms of exactness and precision.

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NC-IUPHAR : from nomenclature to a worldwide pharmacology resource-Michael Spedding,

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How can we manage knowledge about all the receptors and drug binding sites in the human genome?

The nomenclature committee of NC-IUPHAR meets twice yearly in Paris to manage its database (www: iuphar-db.org) and 60 subcommittees, uniting the efforts of ~600 scientists freely giving their time. The database, managed by Tony Harmar in Edinburgh, has two full time curators and aims to give authoritative background knowledge of protein structure, functionally relevant polymorphisms, the key pharmacological principles involved in ligand/protein interactions and ''gold-standard'' ligands. There are tight interactions with IUPAC for chemistry and HGNC for gene nomenclature. Key initiatives are alliances with ASPET (nomenclature published in Pharmacological Reviews), Discoverx for orphan GPCRs and tyrosine kinase receptors, and with the BPS (links with GRAC, more general pharmacology reviews and ''guidetopharmacology'', a web-based initiative which will eventually be a general knowledge base). Classifications of epigenetic targets, MiRs, transporters etc. are ongoing. NC-IUPHAR is a community-based activity and all contributions are welcome.