DOI: 10.1111/nmo.13180

WILEY Neurogastroenterology & Motility NGM

ORAL PRESENTATIONS: DIETARY INTERVENTIONS INCLUDING PROBIOTICS, PREBIOTICS AND SYNBIOTICS

1 | Bifidobacterium Breve NCFB 2258 stimulates vagal nerve firing across an intact colonic barrier

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Objective: Mounting evidence implicates the vagus nerve in signalling between colonic bacteria and the central nervous system (CNS), in what has been termed the microbiome-gut-brain axis. However, the mechanism by which bacteria signal across an intact barrier to their eukaryotic hosts is not understood. Bifidobacterium Breve NCFB 2258 is a commensal bacterial strain which produces polyunsaturated fatty acids (PUFAs) with reported health-promoting effects. The study aim was to investigate if this bacterial strain could signal across the gut barrier to stimulate the host nervous system.

Methods: Using ex-vivo Sprague Dawley rat colonic tissue, immunofluorescent staining and calcium imaging were utilised to investigate activation of submucosal neurons in response to mucosal application of PUFA-producing probiotic secretions (supernatants). To determine if the effects were local to the enteric nervous system of if they also stimulated colonic afferents, extracellular recordings of vagal nerve activity were also undertaken.

Results: Mucosal exposure to the bacterial supernatants stimulated increased nuclear expression of cFos and peroxisome proliferator-activated receptor alpha (PPARα) in submucosal neurons. A robust increase in neuronal $[Ca^{2+}]i$ was also observed in response to mucosal application of supernatants. This response was reduced ($P<.001$) but not abolished by the PPARα antagonist, GW6471. Similarly, exposure of the colonic mucosa to supernatants ($P<.001$) stimulated increased firing in vagal afferents. The PPARα antagonist reduced ($P<.001$) but did not abolish this response.

Conclusions: These findings illustrate that PUFA secretions from Bifidobacterium breve NCFB 2258 signal across a healthy intact gut barrier to the intrinsic and extrinsic gut nerves. Supernatants induced activation of underlying submucosal neurons, which regulate absorption and secretion, but may also act as a relay for gut-to-brain signalling. Indeed, supernatants also increased vagal afferent firing, which was mediated in part by activation of PPARα. These findings begin to elucidate the molecular mechanisms underlying signalling by specific bacteria in the microbiome-gut-brain signalling axis.

Policy of full disclosure: None.

2 | Protease activity and tryptase expression is increased in a post-inflammatory rat model for visceral hypersensitivity

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Objective: Previously, we confirmed beneficial effects of the serine protease inhibitors nafamostat mesilate and the newly developed UAMC-0050 and UAMC-1162 on visceral hypersensitivity in an acute and post-colitis rat model [Ceuleers et al. Neurogastro 2015, DDW 2016, DDW 2017]. The objective of this study was to explore which serine proteases are specifically involved based on the profiles of the inhibitors used targeting tryptase, matriptase, uPA, cathepsin G and kallikrein 2, 4, 8.

Methods: As previously described, male Sprague-Dawley rats were intrarectally instilled with TNBS (colitis) or 0.9% NaCl (control). Successively, colonoscopies were performed to document the acute colitis on day 3 and the complete healing of the mucosa in the post-colitis phase (day 10-18). Colon sampling of control, acute colitis and post-colitis rats was performed to measure the mRNA expression of the serine proteases described above by qPCR, as well as to quantify mast cell tryptase by immunohistochemistry. Next, fecal samples were collected at day 0 (control), day 3 (acute colitis) and the day of sacrifice (post-colitis) to determine general protease activity using an azocasein assay.

Results (Table 1): All TNBS rats developed acute colitis on day 3, while the post-inflammatory status was confirmed on the day of sacrifice for post-colitis rats. The qPCR experiments showed a significant downregulation of matriptase in the colon of rats with acute colitis compared to controls. In post-colitis rats, tryptase was significantly upregulated,

Background: The incidence of gastro-esophageal reflux disease (GERD) is rapidly increasing in Asian countries including Korea. However, no single ideal test is available to confirm the diagnosis. GerdQ has been used in the diagnosis of GERD in Western countries. However, its Korean version has not yet been validated. Moreover, its ideal cut-off value in determining GERD in the Korean population has not been clearly defined. Therefore, we aimed to assess the diagnostic accuracy of the Korean version of GerdQ, and to its reproducibility and concurrent validity.
Methods: After repeated translations and backward translations, the Korean version of GerdQ was prepared. Patients with symptoms suggestive of GERD were asked to answer to the GerdQ at their first visit. A second GerdQ questionnaire was then administered to the patients when they visited for their subsequent appointment for upper endoscopy, which was at least 2 weeks after the first visit. The final questionnaire was provided after proton pump inhibitor (PPI) treatment. Reflux esophagitis or pathological acid exposure was used as diagnostic references for GERD. The diagnostic accuracy of the GerdQ for GERD with regard to symptom response to PPI therapy was assessed.
Results: A total of 149 patients (52 male, 97 female) with a mean age of 52.6±14.6 years were enrolled. A GerdQ cutoff of 7 was found to the best balance with regard to sensitivity (64.6% [95% CI: 55.0-73.2], and specificity (69.4% [95% CI: 51.7-83.1)]. The intraclass correlation coefficient of two subsequently measured GerdQ scores was 0.65 (95% CI 0.52-0.75). Moreover, GerdQ had a high positive predictive value (86.9% [95% CI: 77.4-93.0]), but a low negative predictive value (38.5% [95% CI: 26.9-51.4)) for GERD.
Conclusions: The Korean version of GerdQ is a useful complementary tool for the diagnosis of GERD in primary care of Korea. Moreover, the relatively lower cut-off represents milder GERD symptoms in Korean patients than those in patients in Western countries.
Policy of full disclosure: Sponsored by Astra-Zeneca.

156 | Pathogenic mechanisms of esophageal peristaltic dysfunction by high resolution manometry in patients with systemic sclerosis

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Backgrounds/Aims: Our hypothesis is ineffective esophageal motility (IEM) of esophageal high resolution manometry (HRM) finding in systemic sclerosis (SSc) patients may reflect neural dysfunction with/without smooth muscle (SM) atrophy and absent contractility (AC) may reflect severe SM atrophy or extensive fibrosis. Aims of our study are to evaluate the esophageal reserved function of neuronal reflexes and muscle contractility in these three groups of SSc.
Methods: Patients diagnosed with SSc who underwent HRM during recent 3 years were retrospectively included (22 female, ages 25-75). Seventeen patients of them were underwent multichannel intraluminal impedance and pH (Imp-pH) study also. We analyzed LES pressure, HRM metrics according to the Chicago classification version 3, including distal contractile integral (DCI), DCI ratio of single swallow and multiple rapid swallow (MRS) test, and reflux parameters and the post-reflux swallow-induced peristaltic wave (PSPW) index by Imp-pH test. According to the HRM findings, patients were classified as 3 groups (normal HRM, n=5; IEM, n=8, and AC, n=9). We compared SM contractility by DCI, neuronal peristaltic reserve function by MRS test using HRM and the PSPW index using Imp-pH study in normal, IEM, and AC groups. Parameters among 3 groups were analyzed by ANOVA on ranks.

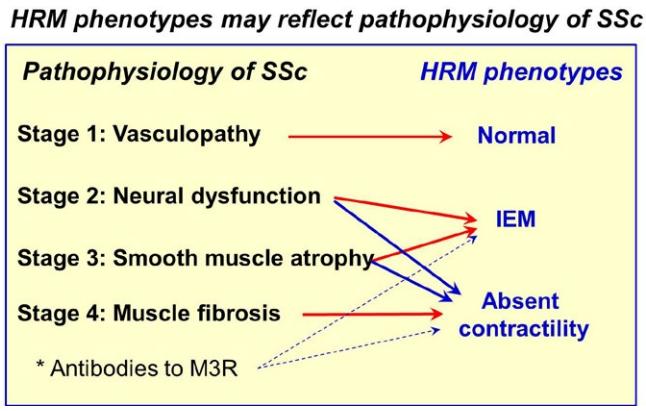

FIGURE 1

Results: DCI was significantly lower in AC and IEM group than normal group [median 0.00 (IQR 0.00-35.23), 172.80 (77.05-522.35), and 834.30 (789.85-2294.63) mmHg-s-cm respectively, *P*<.001]. DCI ratio of MRS/single swallow was lower in AC group than normal group [0.00 (0.00-0.08) vs 1.00 (0.45-1.19), *P*=.030]. Other HRM parameters including LES pressure were not different among three groups. PSPW index was lower in AC group than IEM and normal groups [0.40 (0.00-1.90), 8.95 (3.20-16.70), and 17.60 (10.0-35.40)% respectively, *P*=.023]. PSPW appearance time until 120 seconds were tended to variable in IEM group and AC group than normal group [SD 34.28 (32.29-44.89), 27.60 (16.97-32.03) and 19.60 (17.95-28.02) seconds respectively, *P*=.079]. Other reflux parameters were not different among 3 groups.
Conclusions: The AC using HRM may reflect all of SM atrophy, fibrosis and neuronal dysfunction and the IEM may reflect SM atrophy and mild to moderate neuronal dysfunction in patients with SSc (Figure 1).
Policy of full disclosure: None.

157 | The levels of pantoprazole in human breast milk and plasma: Two compartment model

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Objective: Proton pump inhibitors are the most widely used medications and their safety in the lactation is not known. Only one case report is available about PPI (omeprazole) concentrations in the human

breast milk. We aimed to evaluate the amounts of pantoprazole in human milk and plasma after oral administration to breast-feeding women and to estimate exposure of the fetus.

Methods: Twelve women who decided to stop breastfeeding were taken 40 mg pantoprazole once a day of for 7 days. Blood and milk samples were collected at day 1 and day 7 at 0-1.5-3-4.5-6-9-12th hours. A selective and rapid HPLC method was developed and validated for quantification of pantoprazole in plasma and breast milk samples using Omeprazole as internal standard. Pantoprazole was extracted from biological matrix by using Liquid-liquid extraction process. The method was validated over a linear concentration rage of 0.03-1 µg/mL and the limit of quantification (LLOQ) was 0.03 µg/mL.

Results: The plasma level was 1229.9±1160.2 (61.6-4255.6) ng/mL at day 1 and 1248.9±1448.7 (86.4-5475.8) ng/mL at day 7. The mean concentration in the milk was 61.9±36.9 (32.7-141) ng/mL for day 1 and 152.5±217.7 (32.5-762.5) ng/mL for day 7. 21 out of 97 milk samples and 47 out of 98 plasma samples were contained pantoprazole in all time points. The frequency of pantoprazole at day 1 was 20.8% in the milk and 43.8% in the plasma while they were 22.4% in the milk and 54.2% in the plasma at day 7.

Conclusions: The two compartment model was proposed to describe time profiles of pantoprazole in plasma and milk (plasma as central, milk as peripheral compartment). Pantoprazole's level of milk compartment was far less than plasma compartment. Since, the uncoated pantoprazole is unstable in acidic pH, the systemic dose received by the infant from the breast milk might be even lower. Our limited data implicate that women might not stop breastfeeding when taking pantoprazole.

Policy of full disclosure: None.

158 | Anti-inflammatory mechanisms of action in FD and IBS: The example of STW 5

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Introduction: Inflammation is involved in the etiology of Functional Dyspspsia (FD) and Irritable Bowel Syndrome (IBS) as a potential cause. The question is how it can be addressed by therapy, as eg, the NSAIDs, used as anti-inflammatory drugs, can cause inflammations in the GI tract. As an example for an anti-inflammatory drug not causing GI side effects, data for a drug used in FD and IBS, STW 5 were analyzed. Methods: Data from in vitro studies were revealed and analyzed for elucidating mechanisms of action underlying anti-inflammatory effects.

Results: STW 5 activated COX-1, but not COX-2 mRNA expression, which was in contrast to the control substances, like ASS and diclofenac, which inhibited COX-1 and COX-2 mRNA Expression (1). STW 5 inhibited the increased gene expression and reduced significantly the release of TNF-alpha by activation of adenosine A2A receptors in LPS (100 ng/mL)-stimulated human monocytes, while having no effect in untreated cells (2). Radioligand binding assays confirmed the affinity of STW 5 to adenosine A2A receptors.

Conclusion: The mechanism of action of STW 5 is anti-inflammatory, despite not involving COX-1 or COX-2 inhibitory properties. This is a base for the very good tolerability of this medicinal product.

Policy of full disclosure: O. Kelber is employee of Innovation and Development, Phytomedicines Supply and Development Center, Bayer Consumer Health, Darmstadt, Germany. K. Nieber has received honoraries and travel grants from Innovation and Development, Phytomedicines Supply and Development Center, Bayer Consumer Health, Darmstadt, Germany.

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159 | Melanin-concentrating hormone receptor 1 expression in colon: A multiplex immunohistochemical study with colon from "normal" donors and patients with Inflammatory Bowel Disease (IBD)

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The neuropeptide melanin-concentrating hormone (MCH) regulates appetite but there is increasing evidence that MCH may contribute to the inflammatory pathology associated with inflammatory bowel disease (IBD) via activation of the G-protein coupled receptor, MCHR1; thus mRNA levels for both MCH and MCHR1 are elevated in human inflamed colonic mucosa. To investigate this further, the present study used multiplex immunohistochemistry to investigate the expression of MCHR1 at the protein level in resected colon samples from patients with IBD in comparison to 'normal' colon (resected colon tissue at least 15 cm away from the borders of a tumour) along with phenotypic markers to identify expressing cells. Specific MCHR1 immunoreactivity (rabbit polyclonal primary anti-MCHR1 affinity purified vs isotope control) was evident in colon from either 'control' donors or patients with IBD. MCHR1 immunoreactivity was evident in the outer regions of cells consistent with a cell membrane expression. Infiltration by immune cell subsets was evident in colon samples from either 'control' donors or patients with IBD although immune cell infiltration was particularly evident in the latter samples when using antibodies to phenotypic markers such as CD3 (T cells), CD11c (eg, dendritic cells, monocytes, macrophages) and CD14 (monocytes, macrophages). Multiplex immunohistochemistry with the phenotypic markers allowed demonstration of MCHR1 immunoreactivity co-localisation to often be evident. MCHR1 immunoreactivity was also evident in epithelia identified by their location and morphology. The present study has demonstrated the expression of MCHR1 immunoreactivity in the colon from 'control' donors