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Evaluation of compliance to congenital Chagas disease treatment: results of a randomised trial in Bolivia

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Background: A randomised, unblinded, clinical trial comparing two benznidazole regimens for congenital Chagas disease was carried out to determine whether simplification and reduction in the length of treatment could lead to better treatment compliance.

Methods: This study was conducted in Santa Cruz, Bolivia. Serological screening was carried out in pregnant women, and parasites were sought in the blood of newborns from seropositive mothers. Infected infants were randomly assigned to two treatment groups. Recovery was assessed by parasite seeking at 1 month and 2 months as well as serological tests at 9 months. Assessment of treatment adherence was based on weekly home visits and use of electronic monitors.

Results: Benznidazole was given to 63 newborns in group A (5 mg/kg in two daily doses for 60 days) and 61 newborns in group B (7.5 mg/kg in a single daily dose for 30 days). There was no difference in compliance between the two groups. The study confirmed the efficacy and good tolerance of both benznidazole regimens in the treatment of congenital Chagas disease.

Conclusions: The short treatment should be preferred as it allows reducing the dose of benznidazole as well as the cost of treatment.

Keywords: Chagas disease, *Trypanosoma cruzi*, Clinical trial, Compliance, Benznidazole, Bolivia

Introduction

Chagas disease, caused by the flagellate protozoan parasite *Trypanosoma cruzi*, affects up to 10 million people in Latin America.[1] Parasites are mainly transmitted by *Triatoma* sp. (kissing bugs) but also by transfusion of blood from an infected person or by congenital transmission from an infected mother.[2] In most countries, vector control has resulted in a dramatic decrease in natural transmission, bringing to light the roles of blood transfusion and congenital transmission.[3]

In Bolivia, Chagas disease involves 4 million at-risk people living 300–3500 m a.s.l.[4] Although niridazole and benznidazole may be used for the treatment of Chagas disease, in Bolivia only the latter is available. Benznidazole is given to newborns for 1–2 months at a daily dose of 5–10 mg/kg administered in one or two daily intakes.[2] Recovery is assessed both by parasite seeking and immunological tests.[4,5]

Several studies conducted in Santa Cruz de la Sierra (SCS), Bolivia, showed a 30–50% prevalence of Chagas disease among women giving birth, leading to a 6.3% congenital transmission rate, causing a dramatic incidence of congenital Chagas disease.[6–9] However, because of the length of treatment, non-compliance is a recurring problem that can lead to treatment failure. A randomised, unblinded, clinical trial was carried out in SCS comparing compliance to a reduced treatment (one daily dose over 1 month) with compliance to the regular treatment (two daily doses over 2 months). The objective was to achieve better treatment compliance using a lower dosage and simplified administration in order to improve the management of congenital Chagas disease in remote rural

health centres and, significantly, to reduce the total dose of benznidazole.

Materials and methods

The clinical trial was carried out in SCS (800 000 inhabitants) located in the eastern lowlands of Bolivia. Three maternity units were selected on logistical and administrative criteria: the Percy Boland reference municipal maternity unit (8788 childbirths in 2007); the maternity unit of the national social security fund (2214 childbirths in 2007); and the maternity unit of the French Hospital that opened in 2007 (1850 childbirths during the first year of operation). The survey was conducted from November 2006 to March 2008 at Percy Boland and from March 2007 to March 2008 at the other two hospitals.

All pregnant women attending the maternity units for delivery were informed of the investigation's goals and methods. After obtaining signed informed consent, blood samples were collected from a fingerprick of the mother. A Chagas STAT-PAK test (Chembio Diagnostic Systems, Medford, NY, USA) was immediately performed on whole blood. The result was read at 15 min and was immediately given to the mother.

All children born to seropositive mothers were checked for parasitaemia within the first 24 h of life by venipuncture of the heel. Blood was collected in a 600 μl Microtainer tube with lithium heparin and plasma separator (Becton Dickinson, Franklin Lakes, NJ, USA). Following centrifugation, the Microtainer tube was frozen at −20 °C until a second serological test for confirmation using a third-generation ELISA (Chagatest; Wiener, Rosario, Argentina) was performed in the laboratory of the Instituto Nacional de Laboratorios de Salud (La Paz, Bolivia). A second blood sample was carried out both to measure the mother's antibody level and to serve as a baseline for further immunological measurements in the child in order to evaluate the drop in antibodies after treatment (for more technical details, see Chippaux et al.[6]). Infection in neonates was diagnosed by microscopic examination according to the technique defined by Freilij et al.[10] In the case of a positive parasitological test, the newborn was immediately enrolled by the investigator for randomisation. The random number sequence was generated using STATA 9.0 statistical software (StataCorp, College Station, TX, USA) using random block sizes of four and was stratified by centre with a 1:1 allocation. Newborns were allocated to one of the two treatment groups: treatment A, 5 mg/kg/day in two daily doses of 2.5 mg/kg for 60 days; or treatment B, 7.5 mg/kg/day in one daily dose for 30 days. Because a paediatric formulation of benznidazole is not available, 100 mg tablets (Radanil; Roche, Rio de Janeiro, Brazil) were ground up and capsules were filled with 8, 10, 13 and 15 mg of powder by a chemist to treat newborns according to their weight. Preparation of capsules was performed by specialised pharmacists. The capsules were given every week within appropriate vials (eMEM bottles, see below). No placebo was given.

Clinical surveillance was carried out at home once a week during the treatment period. Families of newborns had the telephone numbers of all the investigators to contact them in case of clinical problems. During the home visit, a clinical examination was performed by a nurse, and a standardised questionnaire was administered to check the occurrence of side effects (rash, pruritus, vomiting, diarrhoea, inconsolable crying, insomnia and agitation), with the start date and duration, and to assess the intake of capsules. In severe cases, if not attributable to an acute phase of Chagas disease (weight loss, dehydration, hepatosplenomegaly and icterus) as well as in cases of opposition from the mother, treatment was discontinued and was resumed later with parental consent. The child was weighed for dose accordance and the new dosage was provided for the following week. Compliance monitoring and evaluation were performed in three ways: first, according to the mother's statements; second, by counting at every home passage the remaining capsules, allowing calculation of the intake dose; and third, by using an 'electronic medication event monitoring' device (eMEM; AARDEX Group Ltd, Sion, Switzerland) that records the date and hour of each opening of the eMEM bottle containing the capsules. In the latter case, owing to the late procurement of eMEM bottles and/or dysfunction of some of them, only 53 patients in group A (84%) and 55 in group B (90%) benefited from a reliable measurement. Accordingly, it was possible at the end of the treatment to check the total number of openings and whether their occurrence (frequency and intervals) corresponded to the prescription and treatment group to which the child was allocated. The total dose of benznidazole related to the infant's weight and treatment interruptions (total number of days lost and number of consecutive days without treatment) were measured for each child and were then averaged for all children in each of the two groups. As the total prescribed dose was different in each group (300 mg/kg for group A and 225 mg/kg for group B), the ratio of the difference between the prescribed dose and the taken dose was calculated (differential dose = prescribed dose − taken dose/prescribed dose), which was expressed as a percentage of the total prescribed dose. The frequency of treatment interruptions for three or more consecutive days (= 'drug holidays') was assessed through electronic vials and was compared between the two groups according to the duration of treatment (1 month vs 2 months).

For all these children, treatment was completed or renewed to reach the prescribed dose. Recovery was assessed by parasitological examination at 1 month and 2 months as well as serological tests performed at 8–9 months of life, as described elsewhere.[5]

It is generally assumed that compliance means a patient takes ≥80% of the prescribed treatment.[11,12] According to the national programme of Chagas control and various teams involved in large-scale treatment in infants, including ours that followed the treatment of infants in Caraparí and Yacuiba,[13,14] compliance of treatment was approximately 75% with strict surveillance. Our goal was to reach a compliance of 90% for both treatment regimens. With $\alpha = 0.05$ and $1-\beta = 0.90$ (bilateral), the groups required 68 subjects (http://marne.u707.jussieu.fr/biostatgv/). The required number of inclusions was to be achieved within 12 months and follow-up had to last 14 months after the last inclusion.

Data were entered in Microsoft Excel 2007/2010 (Microsoft Corp., Redmond, WA, USA) and were processed with Epi Info 6 (CDC, Atlanta, GA, USA) and STATA 9.0 (StataCorp, College Station, TX, USA) statistical software. Data were analysed by intention-to-treat. Statistical comparisons were carried out using Student's *t*-test, χ^2 test and non-parametric tests (Mann–Whitney and Wilcoxon signed-rank tests). The level of significance was $p = 0.05$ and the confidence interval was 95%.

The trial report complies with CONSORT statements.[15]

Results

The incidence of congenital Chagas disease was lower than expected, requiring extension of the clinical trial. However, the number of included children reached almost the expected number and the statistical power of the trial was reduced to 0.85.

Following diagnosis of infection with *T. cruzi*, infants were assigned to each group and were followed until negativation of *T. cruzi* antibodies (Figure 1). The two groups were comparable at the moment of randomisation (Table 1). Undesirable events observed during monitoring are detailed in Table 2. There was no significant difference in the prevalence of undesirable events between the two treatment groups, especially if considering that the duration of treatment A was twice that of treatment B. Most adverse events were immediately followed by a brief interruption of treatment (<3 days), either on the initiative of the mother or upon the recommendation of team staff. Most of them were explained by other causes than the treatment with benznidazole. In particular, rashes all appeared >2 weeks after the beginning of treatment and disappeared very quickly with a topical or antihistamine treatment in the case of suspicion

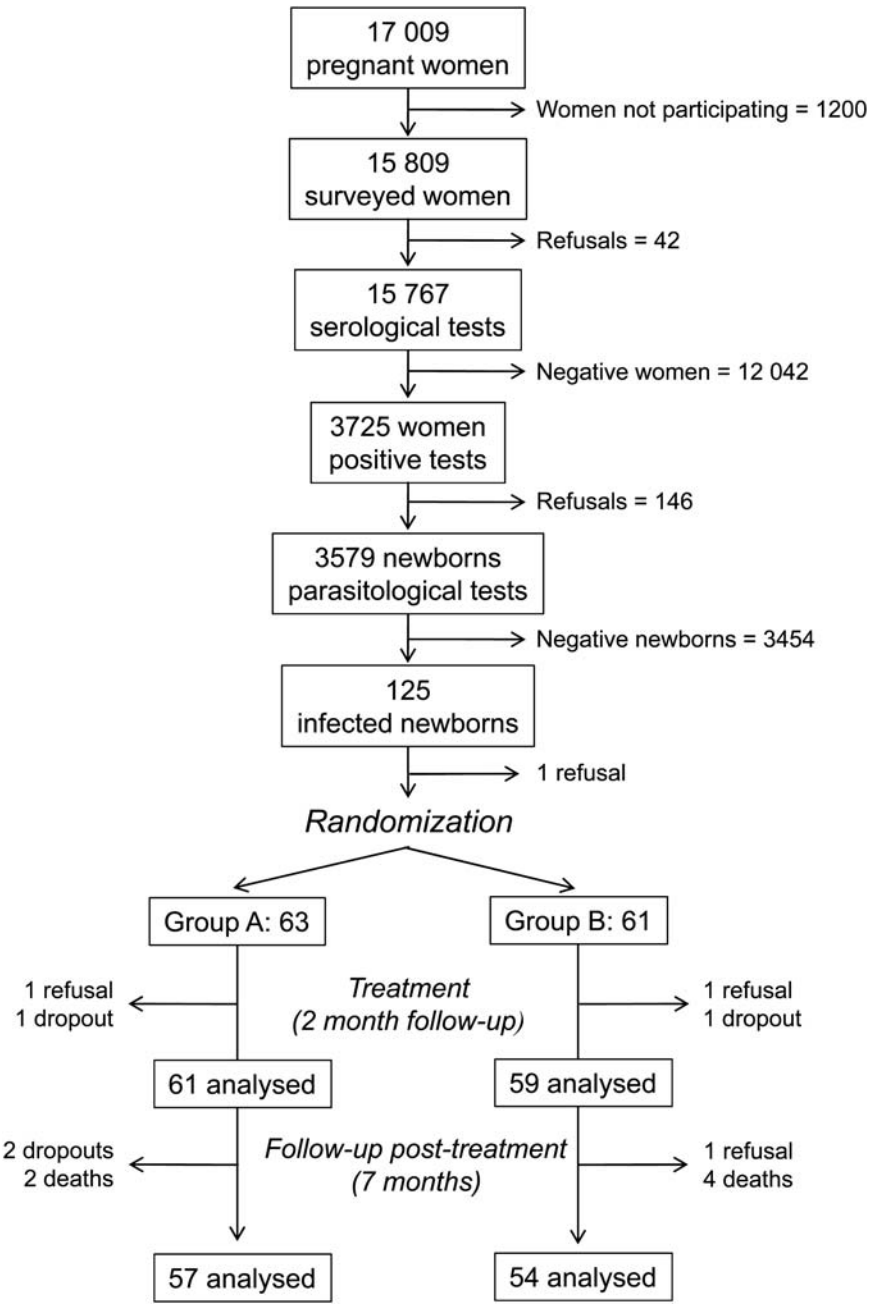

Figure 1. Flow of participants through the clinical trial.

Table 1. Comparison of parameters related to mothers and newborns between the two treatment groups

Parameter	Group A[a]	Group B[a]	p-value[b]
Mothers			
No.	63	61	
Address in Santa Cruz (n)	30	31	0.45[c]
Rural origin (n)	23	23	0.84
Level of education (n)	56	53	
Nil	3	3	0.79[d]
Primary	28	29	
Secondary	22	20	
University	3	1	
Age (years) (mean ± SD)	26.29 ± 1.84	26.61 ± 1.68	0.80
Gravidity (mean ± SD)	1.68 ± 0.59	1.95 ± 0.48	0.49
Parity (mean ± SD)	1.25 ± 0.47	1.72 ± 0.46	0.05
Living children (mean ± SD)	1.25 ± 0.47	1.67 ± 0.45	0.21
Amenorrhea (weeks) (mean ± SD)	37.67 ± 0.63	37.86 ± 0.56	0.66
Newborns			
No.	63	61	
Normal delivery (n)	33	40	0.27
Gender (boys/girls) (n)	32/31	36/25	0.17[d]
Birth weight (g) (mean ± SD)	3030 ± 154.5	2927 ± 173.2	0.39
Apgar 1 (mean ± SD)[e]	7.79 ± 0.16	7.93 ± 0.14	0.20
Apgar 5 (mean ± SD)[e]	8.94 ± 0.12	9.03 ± 0.12	0.26
Head perimeter (cm) (mean ± SD)	33.77 ± 0.47	33.57 ± 0.88	0.70
Length (cm) (mean ± SD)	50.25 ± 0.81	49.49 ± 0.88	0.21

[a] Group A, 5 mg/kg in two daily doses for 60 days; group B, 7.5 mg/kg in a single daily dose for 30 days.
[b] Student's *t*-test (unless otherwise stated).
[c] Mann–Whitney test.
[d] χ^2 test.
[e] Apgar score evaluates the newborn's condition at 1 min (Apgar 1) and 5 min (Apgar 5).

Table 2. Adverse events according to treatment regimen

Symptom	Group A (n = 63) n (%)	Group B (n = 61) n (%)	Total (n = 124) n (%)
Overall condition[a]	6 (10)	6 (10)	12 (10)
Digestive disorders	6 (10)	3 (5)	9 (7)
Local spots	3 (5)	3 (5)	6 (5)
Systemic rash	6 (10)	7 (11)	13 (10)
Other cutaneous symptoms	3 (5)	0	3 (2)
Total infants with adverse events[b]	24 (38)	19 (31)	43 (35)

No significant difference between the treatment groups (χ^2 test).
[a] Anaemia, respiratory, heart or neurological disorders, accidents, etc.
[b] Any infant may have suffered several adverse events.

of allergy. However, no rash recurred when the treatment was resumed after the interruption.

All parasitaemias were negative at the end of the first and second months. Serological data were detailed in a previous article[6] intended to show the persistence of maternal antibodies in infants and to evaluate the recovery time in order to give recommendations regarding serological criteria of recovery. Briefly, all infants but one were serologically negative at 9 months. However, the child retained highly positive antibody titres until 15 months, compatible with the persistence of infection attributed to lack of drug intake. As a consequence, although the clinical condition and weight development were normal, the case management responsibility was transferred to the national programme of Chagas control.

There were two deaths in group A and four deaths in group B during the post-treatment follow-up, i.e. between 2 months and 1 year. Three of them occurred in very premature infants whose weight remained below normal during the follow-up. One death was associated with pneumonia treated by an overdose of codeine; another was due to a domestic accident and the last from infectious disease of undetermined cause. However, absence of parasitaemia at 1 month and 2 months and the

Table 3. Comparison of child weights during follow-up between the two treatment groups

	Child weight (g) (mean ± SD) [no. of children]		
	Group A	Group B	p-value[a]
Birth weight	3031 ± 154 [63]	2927 ± 173 [61]	0.39
Weight at month 1 ± 3 days	3439 ± 184 [60]	3325 ± 206 [59]	0.43
Weight at month 2 ± 5 days	5014 ± 244 [59]	4743 ± 339 [50]	0.20
Weight at month 9 ± 1 month	8683 ± 393 [43]	8722 ± 505 [44]	0.91

[a] Student's *t*-test.

decrease in antibody titres suggested that these children were no longer infected with *T. cruzi*. The trend of the weight curve in both groups was not significantly different between birth and 9 months (Table 3).

According to questionnaires, 57/61 children (93%) in group A and 55/59 children (93%) in group B received ≥80% of the prescribed dose ($p = 0.75$). Electronic monitors showed 50/53 (94%) and 45/55 (82%) receiving ≥80% of the prescribed dose in groups A and B, respectively ($p = 0.16$). The differential dose was 24 ± 10.1% for infants in group A and 20 ± 8.7% for infants in group B ($p = 0.89$).

The percentage of days without treatment was 8.9 ± 3.3% in group A and 7.6 ± 4.4% in group B ($p = 0.64$), representing, respectively, 5.3 days and 2.3 days on average. Adherence to treatment was constant throughout the duration of treatment in group A: there was no significant difference ($p = 0.8$) between the number of days without treatment during the first month (8.9 ± 2.8%) and during the second month (8.4 ± 4.5%). Similarly, there was no significant difference when comparing the number of days without treatment in group B and the number of days without treatment during either the first month ($p = 0.61$) or the second month ($p = 0.79$) of treatment in group A.

Although treatment compliance did not differ between groups A and B, regardless of the estimation method, the study identified some inconsistencies between the different methods or expression of measurements. The discrepancy between the intake dose and the frequency of electronic monitor openings was significantly different ($p = 0.007$) between group A (4/53; 7.5%) and group B (13/55; 23.6%), meaning that in the latter group the frequency of bottle openings was inconsistent with the number of remaining capsules (or intake doses) in 25% of the newborns. All other parameters were similar comparing groups A and B.

Discussion

To our knowledge, there are no randomised clinical trials regarding treatment of newborns using benznidazole to assess both its efficacy and tolerance. The rationale for the treatment protocol used here was based on previous studies, often involving treatment of malignant diseases, the initial indication of benznidazole, and treatment of chronic Chagas disease. The required concentration to stop parasite growth in culture is 0.4–0.8 μg/ml and the dose necessary to kill the parasite is 3–6 μg/ml.[16] According to the authors, the half-life of the molecule is 14 h on average. According to Workman et al.,[17] administration of 2–4 mg/kg/day would achieve the therapeutic dose. We failed to find any rationale for dosage and treatment duration (1, 2 or 3 months, according to authors) in the treatment of congenital Chagas disease. Russomando et al.[18] successfully treated six infants using 7 mg/kg/day divided into two daily doses for 60 days. Benznidazole administered at a dose of 10 mg/kg/day in two daily takings for 1 month showed excellent tolerability and efficacy in newborns.[19] Blanco et al.[20] treated three infected newborns with 5 mg/kg/day for 30 days taken orally and divided into two or three fractions after meals. Schijman et al.[21] obtained satisfactory results in infants treated with a daily administration of 5–8 mg/kg in two doses for 60 days.

It was not possible to obtain laboratory confirmation with ELISA for Chagas infection in mothers before parasitological diagnosis in the newborn. Indeed, the first diagnostic test was performed most often at the time of delivery and we had to wait several weeks for the result of the second test. All neonates from mothers serologically positive by the Chagas STAT-PAK tests have been enrolled to avoid no-shows in further convocations. We had calculated that the number of false positives, including newborns from non-infected mothers, was about 5% of newborns,[22] which was accepted by the ethics committee.

Assessment of compliance is important because it impacts the efficiency of the treatment and risk of resistance. Reducing the duration of treatment may have beneficial consequences (lower cost, lower total dose intake, fewer side effects) but also negative effects. Indeed, the total dose may be dangerously too low to be effective and may jeopardise the treatment outcome. In this study, recovery was confirmed after treatment in all infants, with negative parasitaemia and a significant decrease in antibody titre by the ninth month. Evolution of the weight curves was similar in the two groups and showed normal trends. It seems that both doses were effective.

Inflammatory reactions and skin manifestations are the most common side effects of benznidazole.[23–26] Paraesthesia and polyneuritis of peripheral nerves are also reported as serious toxic effects induced by benznidazole when treatment achieves the total dose of approximately 18 g in adults. This dose-dependent phenomenon is logically less frequent in children than in adults.[26,27] Parents did not report adverse effects, which can mean either the absence of side effects or that a few mild symptoms were not considered serious enough to mention. However, the apparent absence of side effects in this study is likely to substantiate the good tolerance of benznidazole in newborns.

This study was designed to address treatment compliance in order to reduce constraints in public health strategies, especially during long and costly treatments. There is no gold standard for measuring compliance. During treatment for several weeks, the risk of permanent or temporary interruption of treatment is high, especially in ambulatory patients.

Three independent methods were used to assess compliance, each of them presenting advantages and flaws that have been reviewed by Costagliola and Barberousse.[28] Whilst they are not

consistent because they measure different parameters, they should be correlated with the patient outcome, which is the best criterion.[12,29] The questionnaire, provided it covers a period <7 days,[28] gives acceptable results but overestimated adherence[30,31] compared with the count of capsules.[11,32] However, none of the methods estimates the number of 'drug holidays'. In the treatment of infectious diseases, this parameter is critical because it can lead to poor outcome of the patient, emergence of drug resistance and depletion of the anti-infectious armamentarium.[12] Although use of an electronic monitor may seem more reliable because each opening records the date and time, opening the monitor is not synonymous with drug intake. This could explain discrepancies observed between the amount of intake drug and the number of openings of the electronic vials. On the other hand, the technique is considered intrusive, resulting in unnatural behavior.[28] This defect may impact our study, overestimating treatment compliance in the patients and giving an unrealistic view of natural compliance.

The discrepancies between questionnaires and monitors may be due either to reporting errors from mothers or data collection by the investigation team.[33] However, when the 'drug holiday' was too high, replacement therapy was prescribed leading to a resumption of the treatment, which also explains some differences between the intake doses and number of days with treatment.

Conclusions

Treatment of congenital Chagas disease by benznidazole was effective and well tolerated. The overall results of this randomised trial showed that treatment compliance was not significantly improved by its simplification or reduction in length. In addition, the short treatment could be penalised by a lower adherence, suggesting that treatment compliance could improve with the experience that would be gained from the longer treatment. However, the latter suffers from longer and more expensive monitoring and a higher dosage.

Whatever the method of assessment, treatment adherence was acceptable and close to the aim (i.e. 90%). The efficacy of treatment in all children, even those who took a dose significantly lower than the prescribed dose (<20%) or those showing irregular intake of drug, suggested that the dosage is sufficient and covers a correct margin of efficiency. Finally, treatment simplification would help both to reduce the dose and maintain the same efficiency. In addition to reducing the administered dose of benznidazole by 25%, we must add the 50% reduction in the time of treatment monitoring by health personnel, which reduces by at least one-third the total cost of treatment and its monitoring. The greater simplicity of the short-term treatment and the dose reduction suggest that it could be widely used in Bolivia to treat newborns congenitally infected with *T. cruzi*.

Authors' contributions: J-PC, ANS-C and LB conceived the study; J-PC, ANS-C, JRP and LB designed the study; DS designed the immunological aspects of the study; JAS performed the ELISA serological analysis and quality control of STAT-PAK in Santa Cruz; DS and JAS analysed and interpreted the immunological data; J-PC, ANS-C, JRP and LB analysed the data; ANS-C and LB interpreted the data; ANS-C drafted the manuscript; JP-C, JRP, DS, JAS and LB critically revised the manuscript for intellectual content. All authors read and approved the final manuscript. J-PC is guarantor of the paper.

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