Tamoxifen and meglumine antimoniate combined therapy in cutaneous leishmaniasis patients: a randomised trial

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Abstract

OBJECTIVES   There is a clear need for new strategies of leishmaniasis treatment. This work was conducted to evaluate the efficacy of the co-administration of tamoxifen and meglumine antimoniate (Sb^V) in a phase II pilot clinical trial in localised cutaneous leishmaniasis patients.

METHODS   A randomised controlled pilot clinical trial was conducted to evaluate the efficacy and safety of oral (40 mg/day for 20 days) or topical tamoxifen (0.1% tamoxifen citrate for 20 days) combined with meglumine antimoniate (20 mg Sb^V/kg/day for 20 days) vs. a standard Sb^V protocol (20 mg/kg/day for 20 days) for the treatment of cutaneous leishmaniasis. Primary outcome was complete epithelisation of the lesion 6 months after the end of treatment. Secondary outcomes were lesion healing 2 months after the end of treatment and frequency and severity of adverse events.

RESULTS   A total of 38 subjects were included in the trial, 15 were treated with standard Sb^V and 23 with the combination of tamoxifen and Sb^V. Of the patients treated with the co-administration scheme, 12 received tamoxifen orally and 11 were treated with topical tamoxifen. Tamoxifen administered by the oral or topical routes was well tolerated. Cure rates 6 months after the end of treatment per intention to treat were 40% in the group treated with the standard Sb^V scheme, and 36.4% and 58%, respectively, for groups treated with Sb^V plus topical or oral tamoxifen.

CONCLUSIONS   In the doses and schemes used in this study, co-administration of oral tamoxifen and Sb^V resulted in higher cure rates in comparison with the standard scheme of treatment, although not to statistically significant levels.

keywords cutaneous leishmaniasis, treatment, pentavalent antimonials, tamoxifen, topical, oral

Introduction

Leishmaniasis, an insectborne disease endemic in tropical and subtropical areas of the world, affects 0.9–1.6 million people yearly [1]. The diversity of clinical presentations correlates with parasite species but also with immune status of the host. Brazil is endemic for both visceral and tegumentary leishmaniasis, and *Leishmania (Viannia) braziliensis* is the most frequent parasite found in Brazilian patients with the tegumentary forms of leishmaniasis [2, 3].

Leishmaniasis treatment is based on a few available drugs, most of which with a poor safety profile. Pentavalent antimonials (Sb^V) were the first-line class of drugs used globally until parasite resistance was detected in India [4]. Since then, WHO recommendations replaced Sb^V with amphotericin B or miltefosine as first-

line drugs [5]. Nevertheless, in some countries, such as Brazil, Sb^V remains the main stem of leishmaniasis chemotherapy [6]. However, studies have shown that the efficacy of antimonial treatment for localised cutaneous leishmaniasis (CL) can be as low as 50% in some areas [7–9].

Globally, there is a consensus that new strategies and alternatives for treating leishmaniasis are needed. We have described the antileishmanial activity of tamoxifen, a selective oestrogen modulator, and demonstrated, in animal models of visceral and cutaneous leishmaniasis, an efficacy equivalent or superior to Sb^V [10–12]. We have also shown that tamoxifen and Sb^V, when combined, presented additive properties and that tamoxifen was effective through topical administration [12]. Based on these findings, we conducted a pilot clinical trial in localised CL patients, to test whether the combination of

tamoxifen with Sb^V (meglumine antimoniate) was superior to the standard treatment with Sb^V.

Patients and methods

Ethics

All procedures involving human subjects were approved by the Human Research Ethics Committee of the Biomedical Sciences Institute of the University of São Paulo and by the Human Research Ethics Committee of Hospital Universitário Prof. Edgard Santos of the University Federal da Bahia. The trial was registered at Plataforma Brasil (http://plataformabrasil.saude.gov.br) under the certificate CAAE: 42930015.6.3001.0049. A signed term of informed consent was obtained from all subjects.

Design

A multi-arm, phase II, randomised and controlled study was conducted to compare the efficacy of the combination therapy of tamoxifen and Sb^V *vs.* the standard Sb^V protocol.

Participants

Patients were spontaneously seeking medical attention at the health centre of Corte de Pedra, in the state of Bahia, Brazil, an endemic area of *L. braziliensis* transmission. Inclusion criteria were untreated CL with 1–3 months of active disease, with diagnostic confirmation through positive identification of amastigotes in histopathological examination or positive culture or positive polymerase chain reaction for *L. braziliensis*, performed as described in Ref. [7]. Recruitment required individuals to be 18–65 years of age, with a number of lesions ranging from one to five, with the presence of ulcerated lesions with sizes varying between 1 and 5 cm in diameter. Exclusion criteria were as follows: pregnant or breastfeeding women, childbearing-age women unwilling to adhere to contraceptive measures during treatment and until 2 months after the end of treatment; previous history of leishmaniasis treatment; malnutrition; concomitant diseases such as cardiac, pulmonary, hepatic, cancer, tuberculosis, malaria, AIDS, any other infectious disease; laboratory evidence of liver or kidney disease.

Intervention

All groups were treated with the standard regimen of Sb^V (meglumine antimoniate—Glucantime®) 20 mg Sb^V/kg/day, intravenously, daily for 20 days. The group treated with the association with oral tamoxifen received 20 mg/day tamoxifen citrate every 12 h for 20 consecutive days. The group treated with the association with topical tamoxifen was given a cream formulated in oil-free vehicle at 0.1% tamoxifen citrate [13]. Patients were instructed to apply enough cream to cover the lesions twice a day for 20 days.

Outcomes

Primary endpoint was complete epithelisation of the lesion(s) 6 months after the end of treatment. Secondary endpoints were initial cure at 2 months after the end of treatment, frequency and severity of adverse events (AEs).

Randomisation

Patients were randomised by www.randomization.com and allocated at a rate of 1:1:1 into three groups: oral tamoxifen plus Sb^V, topical tamoxifen plus Sb^V and Sb^V monotherapy. Randomisation codes were generated by MEFD in a single block (block size = 38). Sequentially coded numbers associated with intervention arm and allocation were kept under the responsibility of MEFD, and kept in opaque and sealed envelopes. Enrolment was performed by PRLM and CSR, who were also in charge of patient care. Enrolled participants were assigned to interventions by MEFD. Outcome assessment was performed based on physical examination and without collecting any information regarding use of medications or side effects by PRLM and by EMC (blinded). Participants and care providers were not blinded because interventions were not similar.

Clinical and laboratory investigations

All patients were evaluated at days 0, 15, 30, 60, 90 and 210 after recruitment into the study. Lesions were measured (two measures across the ulcer in its larger diameters) with a calliper and evaluated as to their epithelisation status. Blood tests (haematological parameters, hepatic aminotransferases, urea, creatinine, sodium and potassium) and urinalysis were performed on day 0, 15, 60 and 90. All patients were also monitored for treatment adherence and adverse events (AEs). Patients were asked to return the blister packs or cream tubes of tamoxifen/placebo to verify compliance. Clinical and laboratory AEs were graded according to the Common Terminology Criteria for Adverse Events of the National Cancer Institute, ranging from 0 to 5 [14]. The frequency of occurrence of each AE was evaluated per treatment group.

Statistical analysis

Statistical analyses were performed using GraphPad Prism software, version 5.0, for Mac (GraphPad Prism Inc., San Diego, CA) and SPSS®, version 20, for MAC®. Values of $P < 0.05$ were considered significant. Intention-to-treat analysis was performed to establish the cure rates. For quantitative variables, difference between three groups was determined by the Kruskal–Wallis test and between two groups by the Mann–Whitney test. For categorical variables, the comparison between three groups was calculated using the chi-squared test and two groups using Fisher's exact test.

Results

Thirty-eight patients attending the health centre of Corte de Pedra, Bahia state, northeast Brazil, and diagnosed with CL from November 2015 through November 2016 were enrolled in the study (Figure 1). Patients were 19–53 years of age, and 60% were male. Most subjects presented with only one lesion (74%, 28/38) with mean duration of disease of at least one month. Only two patients had a negative PCR test, but the diagnosis was confirmed by histopathology or positive culture. The frequency of lymphadenopathy in association with the cutaneous lesion was different between groups, being detected in 93% of patients treated only with Sb^V and observed in 64% and 33% of patients treated with the topical or oral association, respectively. Other characteristics did not vary significantly between groups (Table 1).

Intention-to-treat analysis showed initial cure rates at day 90 (approximately 60 days after the end of treatment) of 53% in the Sb^V group, confirming previous studies in the same area [7]. Groups treated with topical and oral tamoxifen had 45% and 67% cure rates, respectively. At the primary endpoint, 6 months after the end of treatment, two relapses were identified in the group treated with Sb^V, leading to an efficacy of 40% in this group at this point. The group treated with Sb^V associated with topical tamoxifen did not show any improvement in cure rates as compared to the single drug scheme. Efficacy in the group treated with oral tamoxifen and Sb^V was higher than in the control group but not significantly so (Table 2).

Four patients did not complete the study: one from the Sb^V, one from the Sb^V plus topical tamoxifen group and two patients from the oral tamoxifen group. Two of these were lost to follow-up, and two had severe AEs with irregular use of medication.

On a per-protocol analysis, cure rates did not significantly differ between groups, although there was a trend towards an improved outcome in the group treated with the association with oral tamoxifen, with 70% cure rate at the primary endpoint (Table 3).

AEs were documented in 87% of subjects, with similar frequency in the three groups (87%, 82% and 92% for Sb^V, Sb^V plus topical and Sb^V plus oral tamoxifen) (Table 4). AEs were generally mild, and the most common complaints were arthralgia and myalgia, which is commonly associated with Sb^V use. One patient dropped out of Sb^V plus oral tamoxifen treatment due to grade 3 headache and palpitation on the second day of treatment. One patient from the Sb^V plus topical tamoxifen group stopped therapy due to angio-oedema after the second Sb^V application.

Biochemistry and blood analysis did not show differences between groups on tests performed at days 0, 15, 60 and 90 (Table 5 and data not shown).

Discussion

This is the first report of the use of tamoxifen in the treatment of human leishmaniasis. Previous work in animal models had shown that 20 mg/kg tamoxifen orally for 15–20 days in mice and hamsters was effective in controlling or reducing lesions and parasite load in animal models of cutaneous and visceral leishmaniasis [10–12, 15]. Topical administration of tamoxifen in a CL experimental model showed very promising results [12], with efficacy superior to high doses of Sb^V upon infection with *Leishmania* (*L.*) *amazonensis*. Those data encouraged us to design the trial with one arm employing topical administration of tamoxifen.

Half-maximal inhibitory concentration (IC_{50}) of tamoxifen *in vitro* against *L.* (*V.*) *braziliensis* intracellular amastigotes is 1.9 ± 0.2 µM [11]. In mice, administration of a single 20 mg/kg dose of tamoxifen results in maximal plasma concentrations of 40.8 ng/ml or 0.11 µM. In humans and rats, tamoxifen has been shown to accumulate in tissues with concentrations that can be 10-fold to 60-fold higher than in serum [16].

The conversion of effective doses in mice (20 mg/kg/day) for VL or CL by allometric scaling into a 'human equivalent dose' provides an estimate of 1.53 or 1.62 mg/kg/day, respectively, using the dose factor or correction factor [17]. These doses would translate into the administration of 90–100 mg tamoxifen/day to patients.

Doses of tamoxifen used in the treatment of breast cancer are generally 20–40 mg QD for 5–10 years. After 28 days of treatment with 20 mg oral tamoxifen citrate, patients were shown to present 83.6 (8.7–134.4) ng/ml serum concentration and 866.5 (413.4–1466) ng/g in
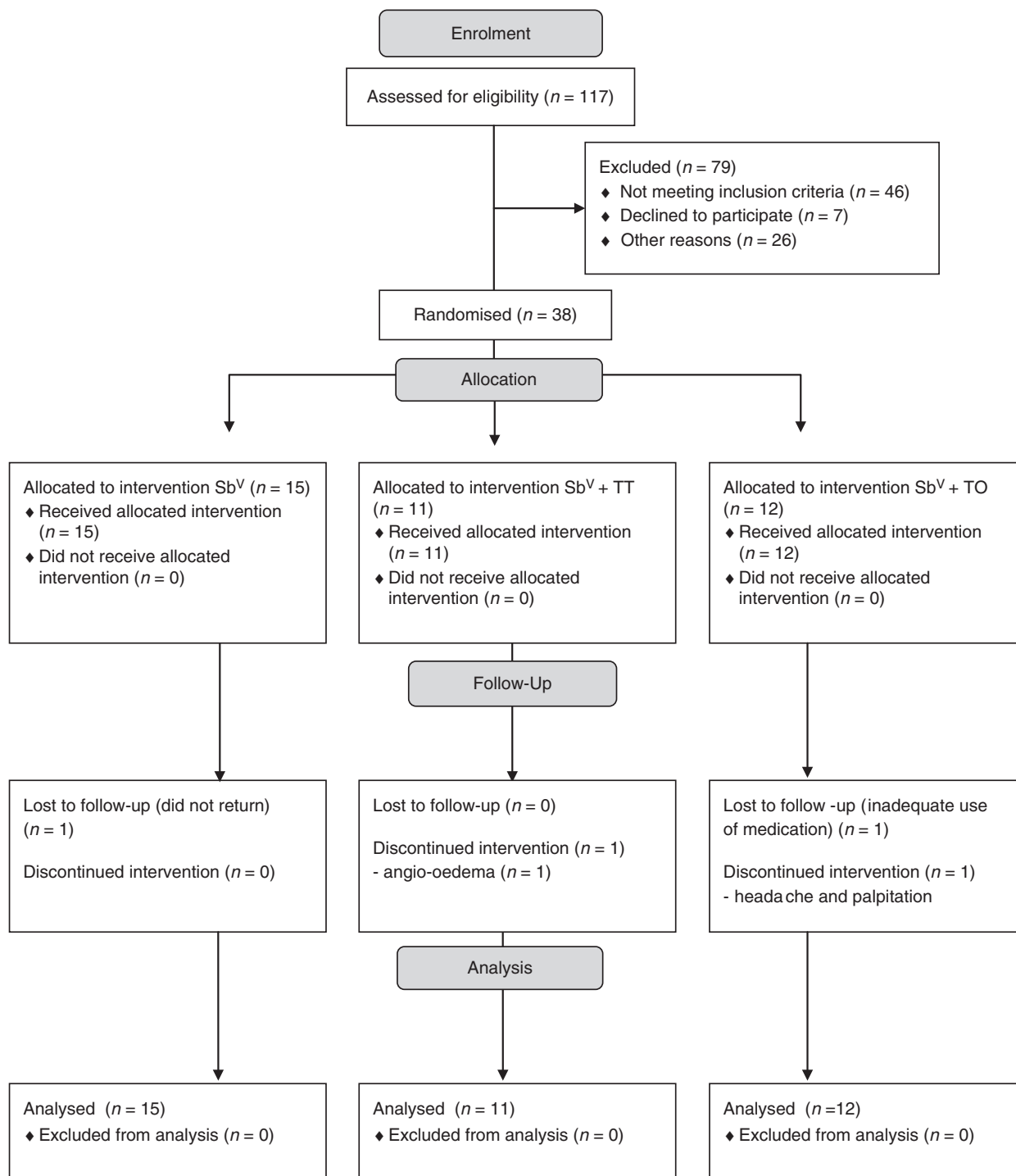
Fig. 1 CONSORT 2010 flow diagram allocation.

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Table 1 Baseline characteristics of the 38 CL patients in the study by treatment groups Sb^v, Sb^{v}plus topical tamoxifen (TT) and Sb^v plus oral tamoxifen (TO) (intention-to-treat analysis)

	Sb^v (*n* = 15/39%)	Sb^v +TT (*n* = 11/29%)	Sb^v +TO (*n* = 12/32%)	*P*-value
Age (years)*	29 (19–44)	43 (32–53)	35 (24–47)	0.12†
Male‡	12 (80%)	5 (46%)	6 (50%)	0.14§
Weight (kilograms)*	69 (20)	68 (13)	64 (16)	0.29†
Number of lesions‡				
Single	11 (73%)	8 (73%)	9 (75%)	0.99§
>1	4 (27%)	3 (27%)	3 (25%)	
Area of the lesion (mm^2)*	165 (345)	144 (245)	208 (538)	0.81†
Lymphadenomegaly‡	14 (93%)	7 (64%)	4 (33%)	0.01§
Area (mm^2)§	225 (393)	150 (285)	162.5 (684)	0.78†
Time of illness (days)*	32 (10)	32 (10)	41.5 (19)	0.47†
Site of the body‡				
Lower limbs	8 (53%)	5 (46%)	10 (83%)	0.14§
Others sites	7 (47%)	6 (55%)	2 (17%)	
IDRM area (mm^2)*	300 (169–400)	225 (144–289)	195 (105–284)	0.20†
Negative PCR‡	1 (7%)	1 (10%)	0 (0%)	0.62§
Side effects‡	13 (87%)	9 (82%)	11 (92%)	0.78§

*Median (interquartile difference).
†Kruskal–Wallis test.
‡Absolute frequency (relative frequency).
§Pearson's chi-squared test.

Table 2 Therapeutic outcome 3 months – D90 – and 7 months – D210 – from the beginning of the treatment by treatment group (intention-to-treat analysis)

	Sb^V† (*n* = 15/39%)	Sb^V+TT (*n* = 11/29%)	Sb^V +TO (*n* = 12/32%)	*P*-value
Cure rate at D90	8 (53%)	5 (45%)	8 (67%)	0.58*
Cure rate at D210	6 (40%)	4 (36.4%)	7 (58%)	0.82*
Relapse at D210	2 (25%)	1 (20%)	1 (12%)	0.81*

*Pearson's chi-squared test.
†Treatment groups: meglumine antimoniate alone (Sb^v), Sb^{v}plus topical tamoxifen (Sb^v +TT) and Sb^v plus oral tamoxifen (Sb^v +TO).

Table 3 Therapeutic outcome 3 months – D90 – and 7 months – D210 – from the beginning of the treatment by treatment group (per-protocol analysis)

	Sb^V† (*n* = 14)	Sb^V +TT (*n* = 10)	Sb^V +TO (*n* = 10)	*P*-value
Cure rate at D90	8 (57%)	5 (50%)	8 (80%)	0.35*
Cure rate at D210	6 (43%)	4 (40%)	7 (70%)	0.63*
Relapse at D210	2 (25%)	1 (20%)	1 (12%)	0.81*

*Pearson's chi-squared test.
†Treatment groups: meglumine antimoniate alone (Sb^v), Sb^{v}plus topical tamoxifen (Sb^v +TT) and Sb^v plus oral tamoxifen (Sb^v +TO).

normal breast tissue [18]. Given the results observed in this pilot study, the use of higher doses of tamoxifen in CL patients could be an option. In the treatment of bipolar disease, tamoxifen has been shown to be effective in doses of up to 80 mg/day for 21 days, without serious AEs [19–21].

In *L. (V.) braziliensis* infections, isolated topical treatment is not an option given the possibility of complications such as mucosal or disseminated leishmaniasis. Therefore, topical tamoxifen was co-administered with the standard drug. The tamoxifen cream was given to the patients, who were instructed to apply it over the lesion twice a day for 20 days. As this application was not supervised, it is possible that the use was irregular or inadequate. Additionally, CL ulcers may present secretion and secondary infection, which may inhibit tamoxifen penetration and activity.

Oral tamoxifen use is probably easier to adhere to and was somewhat controlled by the return of empty blisters. Although not significant, cure rates in the group receiving oral tamoxifen were higher than in the other two groups, a finding that may be clinically relevant and deserves future assessment. It is possible that our small sample size was inadequate to show a higher

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Table 4 Frequency of occurrence of each side effect per treatment group and respective CTCEA grade

	Sb^v (*n* = 15)	CTC Grade*	Sb^v + TT (*n* = 11)	CTC Grade*	Sb^v + TO (*n* = 12)	CTC Grade*	*P*-value
Fever	4 (26%)	1 (*n* = 2) 2 (*n* = 2)	1 (9%)	2 (*n* = 1)	5 (42%)	1 (*n* = 3) 2 (*n* = 2)	0.21^a
Chills	1 (7%)	2 (*n* = 1)	1 (9%)	2 (*n* = 1)	1 (8%)	2 (*n* = 1)	0.97^a
Arthralgia	7 (47%)	1 (*n* = 4) 2 (*n* = 3)	8 (73%)	1 (*n* = 3) 2 (*n* = 5)	7 (58%)	1 (*n* = 5) 2 (*n* = 2)	0.41^a
Myalgia	7 (47%)	1 (*n* = 2) 2 (*n* = 5)	6 (55%)	1 (*n* = 3) 2 (*n* = 3)	6 (50%)	1 (*n* = 4) 2 (*n* = 2)	0.92^a
Insomnia	1 (7%)	1 (*n* = 1)	2 (18%)	1 (*n* = 1) 2 (*n* = 1)	5 (42%)	1 (*n* = 3) 2 (*n* = 2)	0.08^a
Headache	0	0	2 (18%)	2 (*n* = 2)	1 (8%)	3 (*n* = 1)	0.24^a
Angio-oedema	0	0	1 (9%)	3 (*n* = 1)	0	0	0.28^a
Palpitation	0	0	1 (9%)	1 (*n* = 1)	1 (8%)	3 (*n* = 1)	

*CTCEA grade (absolute frequency).
^aPearson's chi-squared test.

Table 5 Haematological and biochemical parameters from CL patients at treatment day 15

Parameter	Unit	Sb^V	Sb^V + TT	Sb^V + TO	*P*-value	Post-test result
Hb	g/dL	1.12 (1.08–1.15)	1.11 (1.07–1.13)	1.13 (1.1–1.15)	0.7003	n.s.
RBC	10^3/L	0.66 (0.63–0.69)	0.66 (0.62–0.68)	0.68 (0.64–0.69)	0.6915	n.s.
Platelets	10^3/L	2.39 (2.31–2.48)	2.45 (2.3–2.47)	2.49 (2.41–2.54)	0.1475	n.s.
WBC	10^9/L	0.77 (0.69–0.87)	0.92 (0.83–0.99)	0.99 (0.81–1.04)	0.1091	n.s.
Neutrophils	10^9/L	0.42 (0.34–0.56)	0.61 (0.49–0.69)	0.69 (0.54–0.81)	0.0833	n.s.
Monocytes	10^9/L	2.73 (2.60–2.79)	2.7 (2.83–2.78)	2.76 (2.62–2.99)	0.4224	n.s.
Lymphocytes	10^9/L	0.45 (0.41–0.51)	0.59 (0.4–0.66)	0.41 (0.34–0.56)	0.3589	n.s.
Albumin	g/dL	0.58 (0.53–0.65)	0.58 (0.55–0.65)	0.66 (0.57–0.72)	0.2035	n.s.
Globulin	g/dL	0.61 (0.55–0.66)	0.7 (0.63–0.72)	0.68 (0.57–0.7)	0.0732	n.s.
Total protein	g/dL	0.91 (0.88–0.93)	0.95 (0.93–0.95)	0.95 (0.94–0.97)	0.0018	*,#
TGO	g/dL	1.33 (1.12–1.51)	1.30 (1.04–1.47)	1.41 (1.26–1.47)	0.7353	n.s.
TGP	U/ml	1.25 (0.98–1.45)	1.25 (1–1.50)	1.32 (1.25–1.39)	0.6051	n.s.
ALP	U/ml	1.82 (1.73–2.01)	1.77 (1.65–1.82)	1.80 (1.71–1.95)	0.4134	n.s.
Sodium	mEg/L	2.14 (1.14–2.15)	2.14 (2.14–2.15)	2.14 (2.14–2.15)	0.9266	n.s.
Potassium	mEg/L	0.82 (0.69–0.86)	0.78 (0.62–0.84)	0.75 (0.59–0.9)	0.6613	n.s.

Values represent median and interquartile ranges. Log$_{10}$-transformed data (which presented Gaussian distribution) were analysed using one-way ANOVA with Tukey's multiple comparisons post-test. Column with *P*-values represents the one-way ANOVA comparisons. *P*-values for post-tests are represented by: *$P < 0.05$ for Sb^V vs. Sb^V + topic tamoxifen; #$P < 0.05$ for Sb^V vs. oral tamoxifen; n.s. non-significant. Abbreviations: ALP, alkaline phosphatase; GGT, gamma-glutamyl transpeptidase; Hb, haemoglobin; RBC, red blood cell count; WBC, white blood cell count. Treatment groups: meglumine antimoniate alone (Sb^v), Sb^v plus topical tamoxifen (Sb^v +TT) and Sb^v plus oral tamoxifen (Sb^v +TO).

efficacy of this association. A high-powered trial with a larger number of subjects from our and other endemic areas will be necessary to better define this point. If used in different areas, possible differences in response according to *Leishmania* species must be taken into account. However, *in vitro* studies demonstrated equal susceptibility to tamoxifen in all *Leishmania* species tested [22]. Additionally, oral tamoxifen could be tested in higher dosages due to its well-known low toxicity.

In summary, in this pilot clinical trial, the co-administration of topical or oral tamoxifen citrate in doses used regularly for the treatment of breast cancer with Sb^V standard schedule was not superior to Sb^V alone in the treatment of localised CL.

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