

A Double-blind, Randomized Trial to Evaluate Miltefosine and Topical Granulocyte Macrophage Colony-stimulating Factor in the Treatment of Cutaneous Leishmaniasis Caused by *Leishmania braziliensis* in Brazil

Paulo R. L. Machado,^{1,2} Fernanda V. O. Prates,^{1,2} Viviane Boaventura,³ Tainá Lago,¹ Luiz H. Guimarães,⁴ Albert Schriefer,^{1,2} Temis W. F. Corte,⁵ Gerson Penna,⁶ Aldina Barral,³ Manoel Barral-Netto,³ and Edgar M. Carvalho,^{1,2,3}

¹Serviço de Imunologia, Hospital Universitário Prof. Edgard Santos, Universidade Federal da Bahia, Salvador, Bahia, Brazil, ²Instituto Nacional de Ciência e Tecnologia em Doenças Tropicais, Ministério da Ciência, Tecnologia, Inovações e Comunicações, Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brasília, DF, Brazil, ³Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Bahia, Brazil, ⁴Universidade Federal do Sul da Bahia, Teixeira de Freitas, Brazil, ⁵Quatro G Pesquisa & Desenvolvimento, Sociedade de Responsabilidade Limitada, Porto Alegre, Brazil, and ⁶Núcleo de Medicina Tropical, Universidade de Brasília, Brasília-DF, Brazil

Background. The treatment of cutaneous leishmaniasis (CL) in Brazil using pentavalent antimony (Sb^v) is associated with a high rate of failure. Miltefosine has proven efficacy for CL caused by *L. braziliensis*, with a cure rate (CR) of 75%. A combined treatment with granulocyte macrophage colony-stimulating factor (GM-CSF) and miltefosine could increase CR and decrease healing time.

Methods. A randomized, double-blind clinical trial to evaluate the efficacy of miltefosine combined with topical GM-CSF (M + GM) vs miltefosine and placebo (M + P) vs Sb^v in 133 patients with CL caused by *L. braziliensis* in Bahia, Brazil.

Results. The final CR at 180 days after the initiation of treatment was 44.4% in the Sb^v group, 76.6% in the M + P group ($P = .003$ vs Sb^v), and 75.6% in the M + GM group ($P = .004$ vs Sb^v). The median healing time for cure was 102 days for the Sb^v group and 60 days for both miltefosine groups ($P = .0009$). During the 6-month follow-up period, 4 relapses were documented: 1 in the Sb^v group, 1 in the M + P group, and 2 in the M + GM group. Mild adverse events occurred in 65% of patients from the Sb^v group, 76% and 79% from the M + P and M + GM groups respectively.

Conclusions. Miltefosine is more effective than Sb^v for the treatment of CL caused by *L. braziliensis* in Brazil and accelerates the healing time. Association with GM-CSF does not improve therapeutic outcome.

Clinical Trials Registration. NCT03023111.

Keywords. cutaneous leishmaniasis; miltefosine; GM-CSF; pentavalent antimony; *Leishmania (V.) braziliensis*.

Cutaneous leishmaniasis (CL) in Brazil is an endemic, neglected, and difficult-to-treat disease. The most frequent CL causal agent in the country, *Leishmania V. braziliensis*, is associated with localized ulcerated lesions but may cause severe disease such as mucosal leishmaniasis (ML), disseminated leishmaniasis (DL), and atypical forms [1–4]. The CL burden for public health is poorly investigated but certainly heavy due to costs associated with daily transportation for at least 20 days to a site

where the injectable medication is available, absenteeism from work, a long time to heal and cure, and physical deformities with social and psychological impact [5]. In Brazil, the standard CL treatment for more than 5 decades has been pentavalent antimony (Sb^v), a parenteral and toxic medication contraindicated in patients aged >50 years and also in patients with heart disease [6, 7]. Furthermore, the frequency of therapeutic failure in up to 50% of CL in *L. braziliensis*-endemic regions where Sb^v is used is another important challenge [8, 9]. Alternative treatments are also parenteral and include toxic drugs such as pentamidine and amphotericin B [10, 11]. Therefore, oral miltefosine should be a logical alternative to the Sb^v due to its easier and domiciliary use, less toxicity, and previous data that show its effectiveness in CL caused by *L. braziliensis* and *Leishmania guyanensis* in Brazil [12, 13]. Although miltefosine is superior to Sb^v, with a cure rate of 75% in CL caused by *L. braziliensis*, there is a clear need to increase its cure rate, accelerate the healing time, and minimize side effects such as vomiting and nausea. Previous data show that topical granulocyte macrophage colony-stimulating factor (GM-CSF) associated with Sb^v therapy increases

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Correspondence: P. R. L. Machado, Hospital Universitário Prof. Edgard Santos. Serviço de Imunologia, 5º andar, Rua João das Botas, s/n - Canela 40110-160 Salvador-Bahia, Brazil (prlmachado@hotmail.com).

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cure rate and accelerates the healing time of CL [14, 15]. In addition to these advantages, combined therapy, if successful, could prevent resistance development and provide a lower daily dosage of miltefosine with reduced costs and fewer side effects.

Our aim in this randomized, placebo-controlled, double-blind clinical trial was to compare the efficacy of 3 arms of treatment for CL, that is, standard Sb^v, miltefosine combined with topical GM-CSF, and miltefosine combined with topical placebo, in a highly endemic region of CL caused by *L. braziliensis*. Moreover, we determined if miltefosine alone or in association with GM-CSF was able to decrease the healing time of CL.

METHODS

Endemic Area and Case Definition of CL

The patients were recruited at the Health Clinic of Corte de Pedra (85% of cases) and Health Post of Jequiricá (15%) in Bahia, northeast Brazil, an endemic area of *L. braziliensis* infection. CL was diagnosed by the presence of 1 or more ulcerative lesion(s) at a skin site, with laboratory confirmation by detection of *L. braziliensis* DNA using polymerase chain reaction (PCR) or by histopathology showing amastigotes in the tissue. Women of childbearing age were included only after a negative beta Human chorionic gonadotropin (HCG) test to exclude pregnancy and used a parenteral contraceptive during 3 months.

Patient Selection

Inclusion criteria were age between 18 and 65 years, 1 to 3 ulcers at any location of the body, lesion between 10 and 50 mm in size in a single dimension, and a period of between 30 and 90 days from the onset of the skin lesion. Patients with previous CL treatment; patients with evidence of ML, DL or CL; pregnant or breastfeeding mothers; and patients living with human immunodeficiency virus or any chronic disease were not included.

Sample Size, Randomization, and Group Assignment

The total sample size of 120 patients was obtained considering a variation of 30% of the cure rate in the control group compared with the intervention groups, with an alpha of 0.05 (2-tailed) and 85% of power. Randomization was done according to a computer list obtained at www.randomization.com and allocated at a rate of 1:1 into 3 groups: Sb^v (control), miltefosine combined with topical GM-CSF (M + GM), and miltefosine and placebo (M + P). Two blinded clinicians for the assignment group performed the physical examination and determined the therapeutic outcome. Due to the impossibility of blinded patients who were in the control group and used parenteral Sb^v, patients and clinicians were instructed to not exchange any information regarding the treatment.

Histopathology and PCR

All patients were biopsied from the edge of the ulcer, and 2 skin fragments were obtained for histopathological

analysis and PCR. DNA isolation, purification, and amplification were performed as described elsewhere [12]. Detection of the subgenus *Viannia* applied the primers GGGGTTGGTGAATATAGTGG and CTAATTGTGCACG. The *Leishmania*-specific band consists of 120 base pairs and that for *Viannia* of 750 base pairs.

Drug Administration

Miltefosine (Impavido, Paesel + Lorel GmbH & Co) was administered in capsules that contained 50 mg at a dosage of 2.5 mg/kg of body weight (maximum daily dose, 150 mg) for 28 consecutive days. The daily dose was divided into 2 or 3 intakes, always given with meals. Patients were asked to return the blisters for verification of regular use and adherence. The control group was treated by intravenous route with Sb^v (Glucantime, Aventis) at a dosage of 20 mg/kg/day for 20 days. Healthcare providers registered the Sb^v dosage and date administered at health posts near the patient's home.

Topical GM-CSF 0.01% and placebo of gel creams were produced by 4G Company (Porto Alegre, Brazil). The rhGM-CSF was purified according to Schwanke et al 2009 [16]. Ointments were prepared with 1.5% (w/w) aqueous polycarbophil gel that contained 10 µg/g of rhGM-CSF. Placebo was prepared in the same way, without the rhGM-CSF. Patients were oriented to cover the lesions with topical placebo or GM-CSF twice daily during the period of miltefosine use (28 days).

Study Procedures

A complete hemogram, aminotransferases (aspartate aminotransferase [AST], alanine aminotransferase [ALT]), urea, creatinine, and blood sugar levels were determined at the start of treatment and on day 15 of therapy. Patients were seen for follow-up every 2 weeks in the first month, every month up to day 90, and 6 months post-therapy. Patients who did not return for follow-up were asked to return or were visited at home within 7 days of the missed appointment.

The ulcers were measured using a standardized caliper and photographed at the initial visit and at each follow-up visit. Clinical and laboratory adverse events (AEs) were graded according to the Common Terminology Criteria for Adverse Events [17].

Clinical Endpoint Criteria

The primary endpoint was final cure at 180 days after initiation of therapy. The secondary endpoints were initial cure at 90 days after the initiation of therapy, healing time in days, and clinical and laboratory AEs. Cure was defined by complete reepithelialization without raised borders, infiltrations, or crusts of all lesions. Failure was defined as the presence of active ulcer or a healed lesion but with raised borders. All patients who failed at day 90 received Sb^v at 20 mg/kg/ day for 30 days or amphotericin B (0.5 to 1 mg/kg total dose).

Statistical Analyses

The data are presented as proportions, 95% confidence intervals, means, and standard deviations. The normally distributed variables were compared using the *t* test. The proportions were compared with the unpaired *t* test or Fisher exact test for categorical variables. The survival curve was analyzed using the log-rank test for trend. The intention-to-treat analysis was used to calculate the cure rates. All statistical analyses were performed with GraphPad Prism 7.02 software for Windows. A *P* value of < .05 was considered statistically significant.

Ethics

Prior to enrollment in the study, a written informed consent was obtained from all patients. The Medical School from Federal University of Bahia, Brazil, Ethics Committee approved the study.

RESULTS

We included 133 CL patients from May 2016 and August 2019; 127 (95.5%) had a positive PCR for *L. braziliensis*. All patients with negative PCR had the diagnosis confirmed by the presence of amastigotes on histopathology. Among the 133 patients, age ranged from 18 to 61 years (34 ± 12.1), with a predominance of males (69%) and no difference between the 3 groups (Table 1). A single ulcer was presented by 76% of patients, 2 ulcers by 18%, and the remaining presented 3 lesions. The main lesion was considered the one with the biggest diameter and was located on the lower limbs in 73% of the cases. The demographic, clinical, and PCR data for the 3 groups are provided in Table 1.

Efficacy

By intention-to-treat analysis, the initial cure rate at 90 days in the M + G group was 80.5% compared with 78.7% in the M + P group and 46.6% the control group (Sb^v). The final cure rates at 6 months were 75.6% in the M + G group, 76.6% in the M + P group, and 44.4% in the Sb^v group. The healing time was longer in the Sb^v group (112 ± 69.7 days) compared with the M + P (72 ± 42.8 days) and M + GM groups (70 ± 37.8 days; *P* = .0013 and *P* = .001, respectively; Table 2).

At day 90 after initiation of therapy, 51% of patients in the Sb^v group remained with active ulcers compared with 20% in the M + P group and 18% in the M + GM group (*P* = .0009, log-rank test for trend; Figure 1).

Relapses due to reactivation of the ulcers and/or infiltration of borders during the 6-month follow-up period were documented in 4 patients: 1 from the Sb^v group, 1 from the M + P group, and 2 from the M + GM group. Six patients did not complete treatment due to irregular use or because they abandoned the study. One patient was in the Sb^v group, 2 in the M + GM group, and 3 in the M + GM group (Table 2).

Tolerability and Toxicity

AEs were documented in the majority of patients irrespective of the treatment group: 65% in the Sb^v group vs 76% in the M + P group vs 79% in the M + GM group. The AEs that were more frequent in the 2 miltefosine groups were nausea (43%), vomiting (29.5%), and headache (8%). In the Sb^v group, arthralgia and/or myalgia (31%), fever (17.7%), and headache (13.3%) were more frequent. Two patients who used miltefosine stopped treatment due to intense vomiting, despite the use of ondansetron 4 mg (Vonau, Biolab Sanus). One patient in the Sb^v group also discontinued treatment due to intense arthralgia and myalgia. The others patients in the 3 groups had mild and transient symptoms.

DISCUSSION

Even today, treatment of CL remains a challenge due to growing ineffectiveness, long time to heal, and toxicity associated with available drugs [5–7, 9]. These negative outcomes may be associated with several factors. One of the most important is an ancient, parenteral, and toxic drug that has been used in monotherapy for decades due to the paucity of studies to test new drugs and combination schemes in order to improve cure rates and accelerate healing time. A successful combined therapy could also be tested with lower dosages of drugs and therefore provide less toxicity and lower costs.

Table 1. Demographic, Clinical, and Laboratory Characteristics of 133 Patients With Cutaneous Leishmaniasis Included in the Trial

Characteristic	Pentavalent Antimony (n = 45)	Miltefosine + Placebo (n = 47)	Miltefosine + Granulocyte Macrophage Colony-stimulating Factor (n = 41)	<i>P</i> Value
Age, mean ± SD, y	33 ± 13.1	36 ± 11.8	33 ± 12.1	ns ^a
Male-to-female ratio	35/10	34/13	25/16	
Number of lesions, mean ± SD	1.4 ± 0.65	1.3 ± 0.79	1.5 ± 0.71	ns ^a
Biggest ulcer diameter, mean ± SD, mm ²	22 ± 8.9	21 ± 7.8	18 ± 9.0	ns ^a
Positive polymerase chain reaction for <i>Leishmania braziliensis</i>	43/45 (95%)	44/47 (94%)	40/41 (98%)	ns ^b

Abbreviations: SD, standard deviation; ns, non-significant.

^aUnpaired *t* test.

^bFisher exact test.

Table 2. Therapeutic Outcome for Cutaneous Leishmaniasis Patients in the 3 Arms of the Trial

Therapeutic Outcome	Pentavalent Antimony (n = 45)	Miltefosine + Placebo (n = 47)	Miltefosine + Granulocyte Macrophage Colony-stimulating Factor (n = 41)	PValue
Initial cure rate (90 d) (%)	21/45 (46.6)	37/47 (78.7)	33/41 (80.5)	.0023 ^a ; .0017 ^b
Final cure rate (6 m) (%)	20/45 (44.4)	36/47 (76.6)	31/41 (75.6)	.004 ^c
Healing time (d) range (mean ± standard deviation)	17–313 (112.3 ± 69.7)	15–198 (72.1 ± 42.8)	30–180 (70.1 ± 37.8)	.009 ^d
Relapse (%)	1/21 (4.7)	1/37 (2.7)	2/33 (6)	ns ^e
Irregular use (%)	1/45 (2.2)	3/47 (6.4)	2/41 (4.9)	ns ^e

^aUnpaired t test, pentavalent antimony (Sb^v) vs miltefosine (M) + placebo (P).

^bUnpaired t test, Sb^v vs M + granulocyte macrophage colony-stimulating factor (GM).

^cUnpaired t test, Sb^v vs M + P; unpaired t test, Sb^v vs M + GM.

^dLog-rank test for trend.

^eFisher exact test.

Abbreviations: ns, non-significant.

Parenteral Sb^v remains the standard CL treatment in Brazil [6, 12]. We showed that GM-CSF compared with Sb^v in CL was able to not only increase the cure rate but also shorten the healing time [14, 15]. More recently, miltefosine treatment of CL showed superiority over Sb^v in disease caused by *L. braziliensis* and *L. guyanensis* in Brazil due to easier administration by oral route and higher cure rates [12, 13, 18]. However, about 20% of patients remained with active disease and needed another treatment in order to be cured. Although evidence is growing that indicates that miltefosine is a better therapeutic approach than Sb^v for CL and should be provided as the first choice of standard treatment [18], some concerns related to its use should be kept in mind. Side effects, such as nausea and vomiting, occur in at least 40% of patients and may interfere with adherence and regular use by auto-administration [12, 13]. Monotherapy after years of use may decrease its cure rate, as has been observed with Sb^v in CL as well as with miltefosine in visceral

leishmaniasis [19]. Therefore, an increase in miltefosine actual cure rate of about 80% in CL caused by *L. braziliensis* is highly desirable due not only to the aggressiveness of this parasite but also to shorter morbidity associated with long time to heal and additional treatment courses.

A decrease in the healing time of CL is necessary. Treatment with Sb^v and miltefosine lasts 20 and 28 days, respectively, but cure or failure is only determined up to 90 days after initiation of therapy. The rationale for choosing GM-CSF to evaluate its association with an anti-Leishmania agent in CL therapy was based on several findings: GM-CSF increases parasite killing by direct activation of macrophages [20–22] and enhances tissue healing and scar formation as shown in chronic venous leg ulcers [23]. In addition, previous studies have shown the increased efficacy of GM-CSF compared with antimony therapy in CL. For instance, intralesional GM-CSF achieved 70% cure 40 days after therapy onset compared with 10% in the placebo group (Sb^v and intralesional saline) and a final cure rate of 80% vs 50% respectively [14]. We also showed that topical GM-CSF diluted in saline plus Sb^v cured 100% of CL patients compared with 50% of patients in the control group [15]. Moreover, in 5 CL patients refractory to Sb^v (failure in at least 2 previous courses), cure was achieved with topical GM-CSF combined with another course of Sb^v [24].

The data from this clinical trial did not show any benefit in combining topical GM-CSF with miltefosine to increase the cure rate or accelerate the healing time of CL. This may be due to several factors. The medium used for GM-CSF may have had a lower final GM-CSF concentration than intralesional applications or dressings diluted in saline as reported elsewhere [14, 15]; patients may have incorrectly used the topical GM-CSF; or since miltefosine monotherapy achieves nearly 80% cure, the benefit of combining it with GM-CSF to improve this cure rate may require higher concentrations of GM-CSF in the topical preparation. We chose topical use by auto-application due to its practical approach in an endemic rural area where patients have difficulty returning to the health post for dressing changes, which occurs 3 times a week for 3 weeks [15].

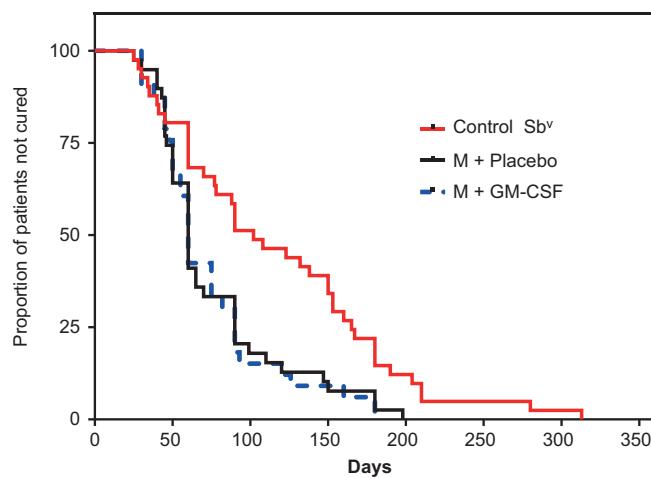


Figure 1. Kaplan-Meier curve comparing time to cure in the 3 arms of cutaneous leishmaniasis treatment. Time to cure is the number of days required for complete healing of the ulcers without any sign of clinical activity such as inflammation or raised borders. $P = .0009$; log-rank test for trend. Abbreviations: GM-CSF, granulocyte macrophage colony-stimulating factor; M, miltefosine; Sb^v, pentavalent antimony.

The results of this trial confirm the superiority of miltefosine compared with Sb^v in the treatment of CL caused by *L. braziliensis* in Brazil. Additionally, we showed that miltefosine accelerates the healing time of CL ulcers. We conclude that miltefosine should be implemented as the first therapeutic choice for CL and that efforts should be made to evaluate combined therapies in order to increase cure rates and shorten the healing time of CL, preventing morbidities and development of drug resistance.

Notes

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