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MAJOR ARTICLE

# Fluconazole in the Treatment of Cutaneous Leishmaniasis Caused by *Leishmania braziliensis*: A Randomized Controlled Trial

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***Background.*** The treatment of cutaneous leishmaniasis (CL) caused by *Leishmania braziliensis* in Brazil with pentavalent antimony ($Sb^v$) is associated with a high rate of failure, up to 45% of cases. In addition, $Sb^v$ can only administered parenterally and has important toxic effect. An effective, safe, and oral treatment for CL is required.

***Methods.*** A randomized controlled clinical trial was conducted to compare the efficacy and safety of high-dosage oral fluconazole (6.5–8.0 mg/kg/d for 28 days) versus a standard $Sb^v$ protocol (20 mg/kg/d for 20 days) for the treatment of CL in Bahia, Brazil.

***Results.*** A total of 53 subjects were included in the trial; 26 were treated with $Sb^v$, and 27 with fluconazole. Intention-to-treat analysis showed initial cure rates (2 months after treatment) of 22.2% (6 of 27) in the fluconazole and 53.8% (14 of 26) in the $Sb^v$ group ($P = .04$). Six months after treatment, the final cure rate remained the same in both groups, without any relapses. The frequencies of adverse effects in the $Sb^v$ and fluconazole groups were similar, 34.6% versus 37% respectively. One patient treated with fluconazole discontinued treatment owing to malaise, headache, and moderate dizziness (Common Terminology Criteria for Adverse Events grade 2).

***Conclusions.*** Oral fluconazole at a dosage of 6.5–8 mg/kg/d for 28 days should not be considered an effective treatment for CL caused by *L. braziliensis*.

**Clinical Trials Registration.** NCT01953744.

**Keywords.** cutaneous leishmaniasis; fluconazole; *Leishmania (V.) braziliensis*; pentavalent antimony.

Cutaneous leishmaniasis (CL) is a neglected and noncontrolled infectious disease associated with physical deformities and psychological effects. The CL burden in the public health in Brazil is increased by therapeutic challenges and long healing time [1]. Corte de Pedra is one of the most important areas of *Leishmania braziliensis* transmission in Brazil, where CL accounts for >90% American Tegumentary Leishmaniasis cases [2]. Although pentavalent antimony ($Sb^v$) has remained the first-line treatment for CL for >50 years, it cannot be considered satisfactory therapy, for many reasons: (1) $Sb^v$ requires parenteral administration with daily injections for 20–30 days, increasing patient costs and absenteeism from work; (2) toxic effects in several organs contraindicate its administration in patients with heart or renal disease; (3) it cannot be used in pregnant women; (4) lesion healing with $Sb^v$ treatment takes a long time, from 2 to 3 months; and (5) increasing resistance to $Sb^v$ therapy is an emerging problem for CL control [1, 3–5]. In addition, a failure rate of up to 50% has been reported for $Sb^v$ treatment of CL caused by *L. braziliensis* [6, 7]. Alternative drugs, such as pentamidine and amphothericin B, must also be administered parenterally, and the former may require hospitalization [8, 9].

In this context, there is an urgent need to develop a more effective and less toxic treatment that can be taken orally. Although miltefosine has been demonstrated to be a safe and effective treatment for CL by *L. braziliensis*, it is not yet available for use in Brazil [10]. Other possible oral treatments for CL include azole antifungals that show in vitro [11] and in vivo activity against *Leishmania* [12–19]. Azole antifungals inhibit 14α-lanosterol demethylation, causing accumulation of 14α-methyl sterols blocking the synthesis of ergosterol, the main sterol of *Leishmania* [11].

A noncontrolled open trial in a case series of CL caused by *L. braziliensis,* reported in 2011, documented a cure rate of 75%–100% for fluconazole given at high daily doses ranging from 5 to 8 mg/kg for 4–12 weeks, with minimal adverse effects (AEs) [20]. Fluconazole has advantages over other azoles, including a longer half-life, increased concentrations in cutaneous tissues, and low toxicity [21, 22]. We therefore performed a

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randomized controlled trial to evaluate the efficacy and safety of high-dosage oral fluconazole compared with standard-dosage parenteral $Sb^v$ for the treatment of CL due to *L. braziliensis* in the endemic rural area of Corte de Pedra in Bahia, Brazil.

## PATIENTS AND METHODS

### Endemic Area and Patient Selection

Fifty-three subjects with diagnosis of CL were recruited at the health clinic of Corte de Pedra, in the state of Bahia, northeast Brazil, an endemic area of *L. braziliensis* infection, from February 2014 through April 2015 (Figure 1).

### Group Assignment

Patients were randomized by a computer list obtained in www.randomization.com and allocated at a rate of 1:1 into 2 groups: fluconazole (intervention) and $Sb^v$ (control). Clinicians blinded to the assignment group performed the physical examination to evaluate cure. Both clinicians and subjects were instructed to not exchange any information regarding the treatment. A single-blind randomized controlled clinical trial was performed.

### Case Definition of CL

CL was defined as the presence of an ulcerative lesion at a skin site, with laboratory confirmation by means of histopathology or polymerase chain reaction (PCR) results positive for *Leishmania* [23].

### Inclusion and Exclusion Criteria

Inclusion criteria were as follows: (1) diagnosis of CL based on case definition, (2) illness duration >1 month and <3 months, (3) age 18–65 years, (4) 1–3 ulcerated lesions, and (5) major ulcer diameter ranging from 10 to 50 mm. Patients with CL duration <1 month were not included because most of them present in a preulcerative phase of the disease with a surprisingly poor therapeutic outcome [24].

Exclusion criteria were as follows: (1) pregnancy or breastfeeding among women, (2) any uncontrolled active infectious or severe disease, and (3) allergy to fluconazole or $Sb^v$.

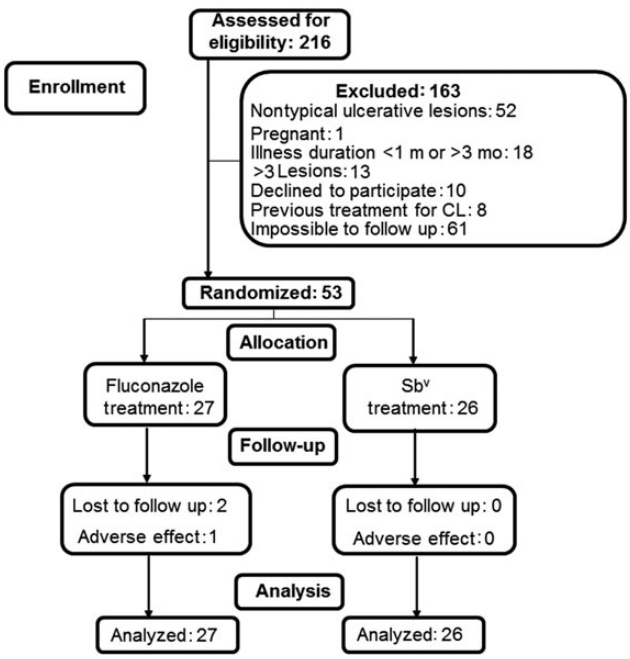


**Figure 1.** Trial flowchart. Abbreviations: CL, cutaneous leishmaniasis; $Sb^v$, pentavalent antimony.

### Drug Administration

Fluconazole was administered orally in capsules containing 150 mg of the drug at a dosage of 6.5–8 mg/kg/d for 28 days. The control group was treated with $Sb^v$ (Glucantime), administered intravenously at a dosage of 20 mg/kg/d for 20 days.

### Study Procedures

Complete blood cell count and aminotransferase (aspartate and alanine aminotransferase), urea, creatinine, and glucose levels were determined in all patients before and 15 days after therapy. Those with abnormal parameters were followed up until normalization. All patients were submitted to rapid human immunodeficiency virus test and female patients also underwent a beta human chorionic gonadotropin test before inclusion. Patients were followed up at 2 weeks and 1, 2, and 6 months after therapy. If a patient did not return for follow-up at the specified time, visits were conducted in the patient's home on the same day or within 7 days of the missed appointment. Patients' ulcers were measured bidirectionally with standardized calipers at the initial visit and at each follow-up visit. The involved area was calculated as the product of the 2 measurements. Standardized digital photographs of patients' were obtained lesions at the same time points.

Patients were monitored for AEs and treatment adherence. Patients returned the blister packs of fluconazole (used and unused) to verify compliance. Clinical and laboratory AEs were graded according to the Common Terminology Criteria for Adverse Events of the National Cancer Institute [25]. Interim analysis to access efficacy and safety was performed 3 times by the data safety and monitoring board, 8 months after the beginning of the study and then at 2-month intervals.

### Primary and Secondary End Points

The primary end point was definitive cure 6 months after the end of treatment, defined as complete epithelialization of all lesions without raised borders, infiltrations, or crusts. Secondary end points included initial cure 2 months after the end of treatment (with the same criteria listed above), healing time for ulcerative lesions, and clinical and laboratory AEs.

### Rescue Therapy

All patients who needed rescue therapy after treatment failure at 2 months were given $Sb^v$, 20 mg/kg/d for 20 days in fluconazole group and for 30 days in the $Sb^v$ group.

### Sample Size and Statistical Analysis

The sample size of 70 patients was obtained considering a variation of 30% of the cure rate (55% in the control vs 85% in the study group), with an α value of 0.10% and 80% power. The normally distributed variables were compared using the Student *t* test. Proportions were compared with the $\chi^2$ test or Fisher exact test for categorical variables. Intention-to-treat analysis was performed to establish the cure rates. All statistical analyses were performed with SPSS 21.0 software for Windows. Differences were considered statistically significant at $P < .05$.

### Ethical Aspects

This trial was conducted according to the Declaration of Helsinki. Before enrollment in the study, informed consent was obtained from all patients. The study was approved by the Ethics Committee of the Federal University of Bahia, Brazil (registration 296.392/2013).

## RESULTS

From the total of 216 eligible patients, 53 were included according to our inclusion and exclusion criteria (Figure 1). A positive PCR result for *L. braziliensis* was found in 94.3% (50 of 53). The 3 patients with a negative PCR result had a CL diagnosis confirmed by pathology and a positive *Leishmania* skin test result.

Of the 53 patients, 35 were male and 18 female, and their ages ranged from 18 to 53 years. Comparison between groups showed a lower mean age in the fluconazole group ($P = .01$). The groups also differed on the location of ulcerated lesions, with 26 of 27 patients (96.3%) in the fluconazole arm presenting with ulcers on the lower limbs, compared with 17 of 26 (65.4%) in the $Sb^v$ arm ($P = .005$). The other clinical and demographic characteristics evaluated were similar between groups (Table 1). Most patients (60.3%; 32 of 53) had regional lymphadenopathy in association with the ulcerated skin lesion(s). Forty-two patients (79%) had a single lesion, 9 (16.9%) had 2, and 2 (3.8%) had 3.

**Table 1. Demographic and Clinical Characteristics and Laboratory Results in Patients With Cutaneous Leishmaniasis Treated With Pentavalent Antimony or Fluconazole**

| Characteristic or Result | $Sb^v$ Treatment (n = 26) | Fluconazole Treatment (n = 27) | *P* Value |
|---|---|---|---|
| Age, mean (SD), y | 35 (10.7) | 27.9 (9.2) | .01[a] |
| Male sex, % | 20/26 (76.9) | 15/27 (55.6) | .18[b] |
| Weight, mean (SD), kg | 68 (14.8) | 64.5 (15.2) | .41[a] |
| Lesions, mean (SD), No. | 1.3 (0.6) | 1.2 (0.4) | .74[a] |
| Major lesion in lower limbs, % | 17/26 (65.4) | 26/27 (96.3) | .005[c] |
| Ulcer area, mean (SD), $mm^2$ | 393 (337.9) | 270.6 (247.6) | .14[a] |
| Lymphadenopathy, % | 13/26 (50) | 19/27 (70.4) | .22[b] |
| LST area, mean (SD), $mm^2$ | 237.2 (160.5) | 290.1 (154.5) | .23[a] |
| Positive PCR result, % | 24/26 (92.3) | 26/27 (96.2) | .61[c] |

Abbreviations: LST, *Leishmania* skin test; PCR, polymerase chain reaction; $Sb^v$, pentavalent antimony; SD, standard deviation.

[a] Student *t* test.

[b] $\chi^2$ Test.

[c] Fisher exact test.

**Table 2. Therapeutic Outcome in Patients With Cutaneous Leishmaniasis Treated With Pentavalent Antimony or Fluconazole**

| Outcome | $Sb^v$ Treatment (n = 26) | Fluconazole Treatment (n = 27) | *P* Value |
|---|---|---|---|
| Cure rate, No. % | | | |
| Initial (2 mo) | 14/26 (53.8) | 6/27 (22.2) | .04[a] |
| Final (6 mo) | 14/26 (53.8) | 6/27 (22.2) | .04[a] |
| Healing time for ulcerative lesions, mean (SD), d | 117.2 (52.5) | 157.6 (55.7) | .01[b] |
| Patients who needed rescue therapy, No. (%)[c] | 7/26 (26.9) | 16/27 (59.3) | .04[a] |

Abbreviations: $Sb^v$, pentavalent antimony; SD, standard deviation.

[a] $\chi^2$ Test.

[b] Student *t* test.

[c] To avoid unnecessary treatment and to protect subjects from $Sb^v$ toxicity, we asked all patients with failure at 2 months and little clinical activity (epithelization, >80%–90%) to come back after 15 days to receive a prescription for rescue therapy if epithelization was still not complete. Therefore, the rescue therapy numbers are different from those for patients whose cutaneous leishmaniasis was not cured. Cure was achieved in all subjects in both groups treated with rescue therapy.

### Efficacy

Intention-to-treat analysis by groups showed initial cure rates (2 months after treatment) of 22.2% in the fluconazole and 53.8% in $Sb^v$ group ($P = .04$). Six months after the end of therapy, the cure rates in both groups remained the same, with no relapses (22.2% vs 53.8%, respectively). The healing time was longer in the fluconazole group (157.6 days vs 117.2 days the $Sb^v$ group; $P = .01$) (Table 2). All patients in both groups treated with

**Table 3. Frequency of Adverse Effects and Common Terminology Criteria for Adverse Events Grade of Patients With Cutaneous Leishmaniasis Treated With Pentavalent Antimony or Fluconazole**

| | Fluconazole Treatment (n = 27) | | $Sb^v$ Treatment (n = 26) | |
|---|---|---|---|---|
| Adverse Effect | Frequency, % | CTCEA Grade | Frequency, % | CTCEA Grade |
| Dizziness | 22.2 | 1 (5 patients); 2 (1 patient) | 0 | 0 |
| Nausea | 11.1 | 1 (3 patients) | 0 | 0 |
| Headache | 7.4 | 2 (1 patient); 1 (1 patient) | 7.7 | 1 (2 patients) |
| Vomiting | 3.7 | 1 (1 patient) | 0 | 0 |
| Myalgia | 3.7 | 1 (1 patient) | 23 | 1 (6 patients) |
| Malaise | 3.7 | 2 (1 patient) | 0 | 0 |
| Fever | 0 | 0 | 7.7 | 1 (2 patients) |
| Arthralgia | 0 | 0 | 11.5 | 1 (3 patients) |
| Anorexia | 0 | 0 | 3.8 | 1 (1 patient) |
| Weakness | 0 | 0 | 3.8 | 1 (1 patient) |
| Pain at lesion site | 0 | 0 | 3.8 | 1 (1 patient) |

Abbreviations: CTCEA, Common Terminology Criteria for Adverse Events; $Sb^v$, pentavalent antimony.

rescue therapy had their CL cured (Table 2). Owing to the low cure rate in the fluconazole group after interim analysis, the data safety and monitoring board decided to interrupt the recruitment of new patients.

### Tolerability and Toxicity

The overall incidence of AEs was 35.8% and similar in both groups (34.6% in the $Sb^v$ vs 37% in the fluconazole group). Only 1 patient in the fluconazole group discontinued treatment because of malaise, headache, and moderate dizziness (Common Terminology Criteria for Adverse Events grade 2); this patient was referred for rescue therapy with $Sb^v$ for 20 days. The remaining patients had mild and transient AEs (Table 3).

## DISCUSSION

The treatment of CL with $Sb^v$ has toxic effects and suboptimal efficacy [4, 26–29], leading to studies to evaluate oral treatment with several drugs, including triazole antifungal agents [14, 16, 30]. Previous data on the use of fluconazole to treat Old World CL caused by *Leishmania major* for 6 weeks showed high cure rates with daily doses of 200 mg (79%) [14] or 400 mg (81%) [30]. Similarly, an open and noncontrolled study in Brazil, including 28 patients with New World CL caused by *L. braziliensis*, evaluated the use of fluconazole in high daily doses of 5–8 mg/kg until complete healing of skin lesions was achieved, with cure rates ranging from 75% to 100% [20]. The different fluconazole cure rate found in our study compared with other trials of the same drug in CL caused by *L. major,* may be explained by heterogeneous sensitivity to fluconazole among *Leishmania* species, as already described with other drugs [3, 5, 31, 32]. *L. braziliensis* is responsible for the most severe form of CL, with rapid evolution of skin lesions, a tendency to slower healing of ulcerative lesions, and lower cure rates after $Sb^v$ treatment, when compared with other species [2, 33].

It is likely that the different therapeutic outcome described by our study, compared with the data from Sousa et al [20], may have at least 2 causes. First, it is known that *L. braziliensis* has a high genetic polymorphism [34], which influences clinical presentation and therapeutic response [35, 36]. *L. braziliensis* from different geographic regions also differ genetically [37] and may present a heterogeneous response to drugs [38] Second, the earlier study was an open and noncontrolled study in a case series of CL caused by *L. braziliensis*. Fluconazole was used in escalating doses from 5 to 8 mg/kg/d to treat CL for 4–12 weeks, and cure rates differed according to dosage. Our present study was a randomized controlled trial with a fixed high dosage, and the treatment duration was previously established. A course of 28 days was chosen because it resembles the 20–30-day treatment course used for $Sb^v$ or miltefosine [10].

Our data show that fluconazole administered orally at a dose of 6.5–8 mg/kg/d for 28 days is not an effective therapy for CL in a high-transmission area for *L. braziliensis.* In addition, the final low cure rate in $Sb^v$ group (53.8%) alerts us as to how much CL caused by *L. braziliensis* can still be considered a therapeutic challenge. Our data highlight the importance of encouraging research for new effective therapies with oral drugs for CL treatment, preferably in randomized controlled clinical trials, stratified by geographic region of study and by *Leishmania* species.

## Notes

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