Treatment of Disseminated Leishmaniasis With Liposomal Amphotericin B

Paulo R. L. Machado,[1,2] Maria Elisa A. Rosa,[1] Luís H. Guimarães,[1,2] Fernanda V. O. Prates,[1] Adriano Queiroz,[1,2] Albert Schriefer,[1,2] and Edgar M. Carvalho[1,2]

[1]Serviço de Imunologia, Hospital Universitário Prof. Edgard Santos, Universidade Federal da Bahia, and [2]Instituto Nacional de Ciência e Tecnologia de Doenças Tropicais (INCT-DT/CNPq), Salvador, Bahia, Brazil

Background. Disseminated leishmaniasis (DL) is a severe and emerging form of American tegumentary leishmaniasis, associated primarily with infection by *Leishmania brasiliensis*. DL is defined by the presence of ≥10 mixed-type lesions such as inflammatory papules and ulcers, located in ≥2 body parts. Most patients have hundreds of lesions all over the body, and mucosal involvement is detected in up to 44% of cases. DL is a difficult to cure disease and pentavalent antimony (Sb^v) is used as standard treatment, its highest dosage being 20 mg/kg/day, for 30 days. However, less than 25% of DL cases will be cured after standard therapy, and the majority of cases will require more than one course of Sb^v for a cure. In this context, new therapies are needed that offer a higher cure rate and a better safety profile, with convenience in drug administration.

Methods. We have evaluated liposomal amphotericin B in 20 patients with DL in an open clinical trial. The total dose ranged from 17 to 37 mg/kg, used in 7 to 14 days of treatment.

Results. Cure rate at 3 months after therapy was 70%. One relapse was documented 4 months after treatment, producing a final cure rate of 65%. Although liposomal amphotericin B was considered well tolerated, mild adverse events were documented in 75% of the patients.

Conclusions. Liposomal amphotericin B is an effective therapy for DL, with a higher final cure rate of 75% observed when used in a total dose above 30 mg/kg.

Clinical Trials Registration. NCT02025491.

Keywords. American tegumentary leishmaniasis; disseminated leishmaniasis; liposomal amphotericin B.

Disseminated leishmaniasis (DL) is a severe form of tegumentary leishmaniasis caused by several leishmania species and described in the New and Old World [1–4]. DL is characterized by multiple acneiform, papular and ulcerated lesions localized on the face, chest, abdomen, and extremities. The number of lesions ranges from 10 to hundreds, and mucosal disease has been documented in up to 44% of the cases [5, 6].

Although DL had been associated with decreased immunologic response to leishmania antigen by negative leishmania skin test and low production of cytokines by peripheral blood mononuclear cells [5, 7], in situ production of pro-inflammatory and inflammatory cytokines and chemokines are similar to cutaneous leishmaniasis (CL) [8]. Furthermore, whereas immunosuppressed hosts may develop multiple CL lesions [9–11], DL cases are reported in immunocompetent individuals from endemic regions in Brazil where *L. braziliensis* is the main etiologic agent [1, 2, 5, 12]. More recently, DL has been associated with distinct genotypical characteristics of *L. braziliensis* [13]. DL should be differentiated from CL with multiple lesions in the immunocompromised, as well as anergic diffuse leishmaniasis caused by *Leishmania amazonensis*. In both conditions infiltrated nodules and plaques with a high parasite load are characteristic; in contrast with the superficial or ulcerated DL lesions that show few parasites [6, 9–11], DL is an emergent infectious disease. Although it was present in only 0.2% of the leishmaniasis

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Correspondence: Paulo R. L. Machado, MD, PhD, Serviço de Imunologia, 5° andar, Hospital Universitário Prof. Edgar Santos, Rua João das Botas s/n, Canela 40110-160, Salvador, Bahia, Brazil (prlmachado@uol.com.br).

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patients in our endemic area in 1986, it now occurs in more than 2% of the cases of leishmaniasis caused by *L. braziliensis* [5, 6]. Moreover, DL is a hard disease to cure, and therapeutic failure with pentavalent antimony (Sb^v) has been documented in up to 76% of the cases caused by *L. braziliensis* in the endemic area of Corte de Pedra, Bahia [8]. The majority of DL patients need several courses of Sb^v or high doses of amphotericin B deoxycholate for a cure [6]. Therefore, DL patients are exposed to relevant drug toxicity with an associated morbidity due to the long-lasting nature of the disease, leading to a high socioeconomic burden. A better therapeutic approach for DL control is urgently needed. Liposomal amphotericin B has been successfully used to treat visceral leishmaniasis [14] and severe CL or mucosal leishmaniasis cases caused by *L. braziliensis* [15–17]. In this scenario, liposomal amphotericin B appears as a promising drug for DL treatment due to its lower toxicity and short time course of therapy.

PATIENTS AND METHODS

Twenty subjects with diagnosis of DL 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 January 2011 through November 2012.

Case Definition for DL

The presence of 10 or more mixed-type lesions (eg, acneiform, papular, nodular and/or ulcerated), located in 2 or more body parts (head, trunk, arms, and legs) [5].

Inclusion Criteria

Criteria were as follows: (a) clinical diagnosis of DL based on case definition; (b) illness duration of <3 months, (c) parasite identification by culture or PCR methods, (d) no previous treatment for leishmaniasis.

Exclusion Criteria

Criteria were as follows: (a) immunodeficiency or antibodies to human immunodeficiency virus (HIV), (b) pregnancy or patients unwilling or unable to use contraceptives during therapy and through the 3-month period after therapy, (c) alanine and aspartate aminotransferases (ALT, AST) ≥3× normal reference values, creatinine, and blood urea nitrogen ≥1.5× normal reference values, (d) any evidence of serious underlying disease (cardiac, renal, hepatic, or pulmonary) including serious infection other than DL.

Liposomal Amphotericin B Administration

The patients were hospitalized at Hospital Universitário Prof Edgar Santos in Salvador-Bahia, and liposomal amphotericin B (AmBisome, Gilead Sciences, San Dimas, California) was administered intravenously in a single daily dose. The total dose ranged from 17 to 37 mg/kg, used in 7 to 14 days of treatment.

Study Procedures

Complete hemogram, AST, ALT, blood urea, and creatinine were determined in all patients on before therapy and 3 times/week up to the end of therapy. HIV serology was performed using the Determine HIV-1/2 Test (Inverness, Waltham, Massachusetts). All patients were examined by an ear, nose, and throat specialist in order to verify mucosal involvement.

Patients were monitored daily for adverse effects. Patients were seen for follow-up at 1, 2, 3, 4, and 6 months post-therapy. Clinical and laboratory AE were graded according to the Common Terminology Criteria for Adverse Events (CTCAE) of the National Cancer Institute [18].

Primary End-point

Definitive cure at 6 months after the end of treatment was defined as complete epithelialization of all ulcers and complete disappearance of inflammatory infiltrations from all nonulcerated lesions.

Secondary End-points

Initial cure at 3 months after the end of treatment with the same criteria and AE as described above.

Rescue Therapy

All patients that failed after liposomal amphotericin B therapy were treated with Sb^v (meglumine antimoniate), at 20 mg/kg/day during 30 days.

Statistical Analysis

Statistical tests were performed with GraphPad Prism 6 (GraphPad Software, Inc.). Fisher exact test was used to compare the cure rate and total drug dosage. Intent to treat analysis was performed to establish the cure rate.

Ethical Aspects

Informed consent was obtained from all patients including the permission to publish photographs.

The study was approved by the Ethics Committee of the Hospital Universitário Prof Edgar Santos from the Federal University of Bahia (registration number 55/10).

RESULTS

The epidemiological and clinical features of the 20 DL patients and therapeutic outcome are shown in Table 1 and Supplementary Table. DL was more common in the male gender (18 of 20 cases), and the number of lesions ranged from 5 to 207, with a mixed pattern, including papules, superficial nodules, and ulceration. Nasal mucosal involvement was found in 53% of the cases, with the majority of patients presenting a superficial degree of inflammation without ulceration being classified

Table 1. Demographic, Clinical, Laboratorial and Therapeutic Findings of 20 Patients With Disseminated Leishmaniasis Treated With Liposomal Amphotericin B

Characteristics	Findings
Age (years) (mean ± SD)	38.6 ± 15.6
Gender M:F (n)	18:2
No. of lesions – median (range)	58 (14–207)
Regional lymphadenopathy (%)	10/19 (52.6)
Nasal mucosal involvement (%)	9/17 (53)
Positive leishmania skin test (%)	12/18 (66.6)
Positive leishmania culture (%)	11/20 (55)
Positive PCR (%)	14/20 (70)
Liposomal Amphotericin total dosage (mean ± SD)	28 ± 6.3/kg
Cure rate at 3 months after therapy (%)	14/20 (70)
LAB <21 mg/kg	0/2
LAB 21–30 mg/kg	7/10 (70)
LAB > 30 mg/kg	7/8 (87.5)
Final cure rate (6 months after therapy) (%)	13/20 (65)
LAB < 21 mg/kg	0/2
LAB 21–30 mg/kg	7/10 (70)
LAB > 30 mg/kg	6/8 (75)

Abbreviations: LAB, liposomal amphotericin B; PCR, polymerase chain reaction; SD, standard deviation.

Dose stratification was chosen to compare the lowest cumulative dosage (eg, <21 mg/kg) with 2 schedules using an intermediate cumulative dose (21–30 mg/kg) and the highest cumulative dosage (>30 mg/kg).

according to Lessa et al (2012) as grade I or II, with one patient presenting grade III (infiltration and ulceration in nasal septum) [19]. The parasite isolated and identified by culture and polymerase chain reaction (PCR) in all DL cases was *L. braziliensis*.

The mean hospitalization period was 9.8 days ranging from 7 to 14 days. Liposomal amphotericin B dosage ranged from 17 to 37 mg/kg (28 ± 6.3). Complete epithelialization of all ulcers and disappearance of inflammatory infiltrations from all cutaneous and mucosal lesions at 3 months after the end of treatment (Figure 1) was documented in 14 of 20 DL cases, with an initial cure rate of 70%.

In one case, a relapse occurred 4 months after therapy, making the overall final cure rate 65% at 6 months. A follow-up at 6 months post-therapy showed no other relapses. All therapeutic failures presented a major improvement, with a >80% regression in the number of the lesions associated with a reduction in size and clinical activity. Patients who were cured were treated with Sb^v at 20 mg/kg/day for 30 days, except in one case, where a cure was achieved only after the second Sb^v course.

Correlation between clinical cure and the total amount of drug administered showed a higher final cure rate of 75% for patients treated with a dose >30 mg/kg vs 58% of cure compared to doses ≤ 30 mg/kg. A total dose lower than 21 mg/kg was used

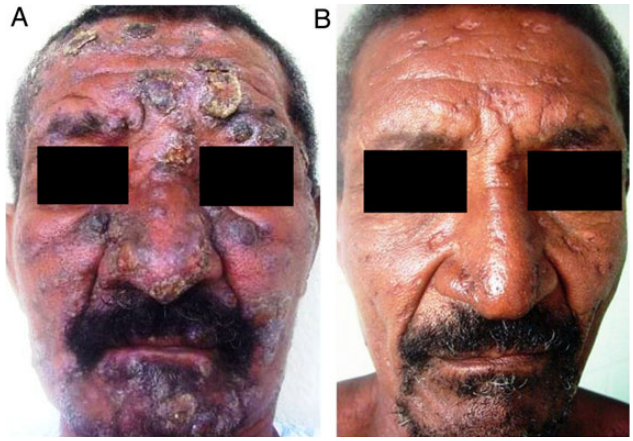

Figure 1. Therapeutic outcome in a disseminated leishmaniasis patient treated with liposomal amphotericin B. *A*, Before treatment. *B*, Three months after treatment.

in 2 subjects, and therapeutic failure was documented in both (Table 1).

AE were documented in 75% of the patients during treatment (Table 2). The most common adverse effects were fever (35%), nausea (35%), phlebitis (35%), dorsal pain (25%), vomiting, and headache (15%). The majority of these adverse effects occurred during or after the infusion and was controlled by symptomatic medication. Interestingly, there was no correlation between the incidence or intensity of AE and total drug dosage. Mild transient creatinine elevation was detected in 6 patients (30%), and in 3 cases it was 2 times the previous normal reference values. Hipokalemia was detected in 2 patients. Raised creatinine levels associated with a higher total dosage (>30 mg/kg) was observed in 2 patients.

Table 2. Liposomal Amphotericin B Toxicity and CTCAE Grade in DL Patients

Symptom	Frequency (%)	CTCAE Grade[a]
Fever	35	1: 6 cases; 2: 1 case
Nausea	35	1: 4 cases; 2: 1 case; 3: 2 cases
Phlebitis	35	1: 7 cases
Dorsal pain	25	1: 2 cases; 2: 2 cases; 3: 1 case
Vomiting	15	1: 2 cases; 2: 1 case
Headache	15	1: 1 case; 2: 2 cases
Chills	10	1: 1 case; 2: 1 case
Palpitations	5	1: 1 case

Abbreviations: AE, adverse events; CTCAE, Common Terminology Criteria for Adverse Events; DL, disseminated leishmaniasis.

[a] CTCAE grade: 1. Minor AE; asymptomatic, interventions or medications are generally not indicated. 2. Moderate AE; usually symptomatic and local treatment or medications may be indicated. 3. Severe and undesirable AE; disruptive symptoms, more serious interventions may be indicated.

DISCUSSION

DL is considered one of the most severe forms of CL not only due to the great number of cutaneous lesions but also given a high rate of mucosal involvement [1, 2, 5, 6]. Indeed, DL is associated with a high rate of therapeutic failure (76%) even after a prolonged treatment with Sb^v in its highest dosage [8]. Therefore, the majority of DL patients need several Sb^v courses or prolonged use with a high dosage of amphotericin B deoxycholate to cure, being exposed to an increased morbidity associated with drug toxicity, and a long-lasting disease [6].

Liposomal amphotericin B has been advocated as less toxic and faster to administer than amphotericin B deoxycholate and has a similar therapeutic response for infectious diseases including visceral leishmaniasis [14] and cutaneous or mucosal leishmaniasis [15–17, 20, 21]. The optimal schedule for liposomal amphotericin in CL is yet to be determined. Previous data from case reports or small trials show a wide range in the total dose of liposomal amphotericin (7.5–30 mg/kg) for cutaneous or mucocutaneous leishmaniasis [15–17, 20, 21]. As this is the first time that liposomal amphotericin B was used for DL, we decided to treat patients with a minimal total dose of 21 mg/kg. However, 2 patients had to stop the treatment before it ended due to adverse effects.

In this study, liposomal amphotericin B had a higher cure rate (65%) than the cure rate observed for Sb^v (24%) in patients with DL caused by *L. braziliensis* from the same endemic area [8]. Moreover, we have found that a higher cure rate was associated with a higher total dosage but not necessarily with an increase in AE. A similar correlation between higher dosages and therapeutic outcome in CL was also observed by others [15–17, 20, 21].

Although no serious AE were recorded, the use of liposomal amphotericin B may be associated with several adverse effects, as well as elevation of creatinine and urea. Nevertheless, the advantages of liposomal over amphotericin B deoxycholate include milder AE and a shorter time of therapy (7–15 days compared with 1–2 months).

Despite our previous experience with a high rate of Sb^v failure in DL, we decided to use Sb^v in the patients who failed to respond to liposomal amphotericin B for 2 reasons: First, because of the availability of the drug, it is provided by the government as the standard therapy for DL. Second, because despite the fact that a complete cure was not observed in 35% of patients treated with liposomal amphotericin B, there was a great reduction of the number and in the inflammation of lesions after liposomal amphotericin B.

Our study has limitations. It was not a formal dose finding study, and liposomal amphotericin B dosage varied according to adverse effects and clinical outcome, based on clinical decision. Patients were not included by randomization, and this may be associated with a selection bias.

The data from our study suggest that liposomal amphotericin B should be considered as an important drug in the therapy of DL and should be used at a total dosage ≥30 mg/kg. The higher cure rate and the short therapy time with the use of liposomal amphotericin are essential to overcoming the important socioeconomic burden of DL.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. All authors: No potential conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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