Efficacy and Safety of Liposomal Amphotericin B for the Treatment of Mucosal Leishmaniasis from the New World: A Retrospective Study

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Abstract. The standard treatment of mucosal leishmaniasis (ML) is pentavalent antimonials, agents with serious adverse effects. Alternative agents include amphotericin B deoxycholate and liposomal amphotericin B. We performed a retrospective study including 29 patients treated with liposomal amphotericin B, most of whom had comorbidities, history of previous treatment of ML, and contraindications to the use of antimonial pentavalent or amphotericin B deoxycholate. We observed a cure rate of 93.1%. Kidney failure was the most important side effect, reported in five patients (17.2%). This study showed a good efficacy and safety profile of liposomal amphotericin B in patients with ML and contraindications to the use of other agents.

INTRODUCTION

Leishmaniasis, a sandfly-borne infection, is endemic in several countries around the world. Different clinical presentations of the disease are possible, depending on the species of the *Leishmania* genus involved and on the host-related factors. It encompasses visceral and tegumentary forms, including cutaneous and mucocutaneous forms.^{1,2}

American tegumentary leishmaniasis (ATL) is an important public health problem in Latin America, and the mucosal form is the most severe presentation. Also known as “spundia,” mucosal leishmaniasis (ML) is characterized by destructive lesions of oral, nasal, laryngeal, and pharyngeal cavities. It is most commonly associated with *Leishmania* (*Viannia*) *braziliensis*. Mucosal involvement can cause potentially destructive lesions and respiratory disturbances, with progressive destruction of the nasal septum, soft and hard palate and severe facial disfigurement.^{1,3–5}

ML cases have been reported in South America, Asia, Europe, and Africa, but Latin America is the most important endemic area, especially the Amazon region.⁶ All major complexes may be responsible for ML over the world. The highest number of ML cases in the New World is attributed to *L. (V.) braziliensis*.⁷ *Leishmania* (*Viannia*) *panamensis*, *Leishmania* (*Viannia*) *guyanensis*, and *Leishmania* (*Leishmania*) *amazonensis* have also been involved in ML. In the Amazon region, *L. (V.) guyanensis* is a significant causative agent of ML.⁸

ML can occur simultaneously with a cutaneous manifestation (mucocutaneous leishmaniasis). However, an important aspect of ATL is the possibility of exclusive mucosal lesions, which can occur months or even years after the onset of the primary cutaneous lesion. The frequency of ML depends on the geographic location. In Andean countries, ML reaches an average of 7.1%, and Bolivia has a high frequency of 20%.^{9,10} In Brazil, the incidence can range from 0.4% to 2.7%.¹

The treatment currently available for ML is systemic, and most cases are based on use of pentavalent antimonials. This agent has an excellent cure rate. However, recurrence is

higher than other treatments and it is involved in serious adverse side effects.^{3,9} A systematic review of ML treatment reported a mean cure rate of 88% with the use of pentavalent antimonial.¹⁰ In different clinical situations, other alternative agents can be used, such as pentavalent antimonials plus oral pentoxifylline, amphotericin B deoxycholate, or liposomal amphotericin B.¹¹ Amphotericin B deoxycholate is an effective antileishmanial drug, with low rates of recurrence, but kidney failure and electrolyte abnormalities limit its use in the clinical practice.¹² Lipid-based formulations, an alternative treatment of cutaneous leishmaniasis, are important options.

Despite the development of lipid-based formulations of amphotericin B, over the last years, only few studies and case reports have been conducted using these therapeutic agents, demonstrating similar efficacy and lower toxic effects in patients with mucosal involvement, comparing with the other drugs of choice.^{3,13,14} The aim of this study was to evaluate the clinical response of liposomal amphotericin B use for the treatment of American ML. We evaluated patients from the Institute of Infectious Diseases Emílio Ribas presenting with ML and contraindication to treatment using pentavalent antimonials, according to the assessment of the medical assistance team. Twenty-nine patients received liposomal amphotericin B, and we observed a cure rate of 93.1%. Our data showed that liposomal amphotericin B could be an excellent option to treat patients presenting with ML.

MATERIALS AND METHODS

This retrospective study was conducted using the clinical records of patients diagnosed with leishmaniasis from January 2005 to December 2013 at the Institute of Infectious Diseases Emílio Ribas, the reference center for infectious diseases, located in São Paulo, Brazil. We evaluated a total of 29 patients with respect to efficacy and total dose of liposomal amphotericin B used for the treatment and clinical cure of ML.

Inclusion and exclusion criteria. We included the following individuals in this study: confirmed cases through clinical and laboratorial criteria and treated with liposomal amphotericin B. The medical assistance team decided for the use of liposomal amphotericin B because of contraindication to other drugs or previous treatment failure. The individuals needed to present any lesion in the nasal or oral mucosa, including ulceration and perforation of the septum, with or without extension to

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pharynx and larynx. In addition to these clinical criteria, at least one of these aspects needed to be present: individuals who lived, were born or had a history of staying in ML endemic areas for ML and who had the confirmation of the parasite presence by direct or indirect exam and had a leishmanin skin test (Montenegro test) or positive serological tests, according to the *Manual for Surveillance of American Tegumentary Leishmaniasis*, Brazil Ministry of Health.⁷ Patients with HIV coinfection and those under the age of 18 were excluded.

Laboratory diagnosis. We used several laboratory diagnostic tests depending on the clinical manifestation and availability at the moment of clinical suspicion. Such tests included direct microscopic examination, in vitro culture, anatomopathological analysis, immunohistochemistry to detect *Leishmania* spp. antigens, polymerase chain reaction (PCR) to detect *Leishmania* spp. DNA, serologic methods (indirect immunofluorescence assay [IIFA] and enzyme-linked immunosorbent assay [ELISA]), and leishmanin skin test.

Direct microscopic examination and anatomopathological analysis were considered suggestive if the presence of the parasite was observed in the lesion. Regarding serologic diagnosis, titers $\geq 1:40$ and $\geq 1:20$ were considered positive for ELISA and IIFA, respectively. Leishmanin skin test was performed by the Instituto Adolfo Lutz, São Paulo, Brazil, and was considered positive when the local induration was larger than 5 mm. The PCR technique was performed using primers that amplify a 120-bp fragment of the conserved region of a *Leishmania* kinetoplast DNA (kDNA) minicircle.

Cure criteria. Cure criteria, therapeutic failure, and relapse were defined according to the recommendations of the *Guidelines for Surveillance of American Tegumentary Leishmaniasis*, Brazil Ministry of Health.⁷ The patient was considered cured if there was regression of all signs and symptoms evaluated through the clinical history and otorhinolaryngological examination, 6 months after the end of the treatment. Therapeutic failure was determined if the patient received two successive therapeutic cycles without showing a clinical response. Relapse was defined if there was recurrence of disease within 1 year after the cure, discarding the possibility of reinfection.

Data collection and analysis. We entered all data into a standard Microsoft Excel (Redmond, WA) database; however, we did not do double data entry at the time of inputting. Also, we performed descriptive analysis, regarding clinical manifestations and treatment response.

Ethics statement. This study was assessed and approved by the Research Ethics Committee (protocol no.: 05/2014) of the Institute of Infectious Diseases Emílio Ribas, São Paulo, Brazil.

RESULTS

A total of 29 patients were diagnosed and treated with liposomal amphotericin B, from January 2005 to December 2013. Median age of patients at the time of treatment was 68.3 years (range = 34–85 years), and 20 were males (69%). Twenty-two (75.9%) individuals had some associated comorbidity. Of these, eight patients had two associated comorbidities and five had three associated comorbidities. The most reported comorbidity was systemic arterial hypertension (58.6%), followed by cardiopathy (mainly cardiac failure) (34.5%) and diabetes mellitus (24.1%). Of the individuals with at least one comorbidity, 13 (59.1%) described previous leishmaniasis

treatment with other drugs. Epistaxis was the most commonly reported symptom (31%), followed by nasal congestion (27.6%). Regarding the signals observed at the otorhinolaryngological examination during the initial evaluation, the most prevalent was crust formation (55.2%), followed by hyperemia (44.8%), edema (44.8%), perforation of the septum (41.4%), vegetant lesion (34.5%), and bleeding (6.9%).

Ten patients (34.5%) had previous cutaneous lesions. The otorhinolaryngological examination revealed that the most affected site was the nose (82.8%), followed by palate (34.5%). History of previous treatment was present in 15 individuals, and the drugs included pentavalent antimonial, amphotericin B deoxycholate, amphotericin B lipid complex, azithromycin, pentamidine isethionate, itraconazole, and pentoxifylline. The mean total cumulative dose of liposomal amphotericin B was 32.5 mg/kg (range = 18.2–55.2 mg/kg). Seven patients presented with adverse effects during the treatment: five patients (17.2%) reported kidney failure, there was one report of myalgia and one of fever, both during the infusion. Of the 29 patients, 27 (93.1%) were considered cured and two did not meet the criteria for cure; both were classified as relapse and neither of them met the therapeutic failure criteria (Table 1). All patients are currently being followed at the Institute of Infectious Diseases Emílio Ribas.

Regarding diagnosis, leishmanin skin test was positive in 22 of 24 individuals (91.7%). ELISA was positive in 12 of 15 patients (80%) in whom the test was performed whereas IIFA was positive in 12 of 20 individuals (60%). Immunohistochemistry was conducted in 16 individuals; of these, six had a positive result (37.5%). Direct microscopic examination was carried out just in 12 cases, and six had a positive result (50%). The kDNA-PCR method was performed in samples of 19 patients and *Leishmania* DNA was detected in 18 (94.7%). Culture techniques were applied to five samples; of these, three were positive (Table 2). A total of 25 patients were submitted to a biopsy resulting in 10 patients with a suggestive exam (40%).

DISCUSSION

Although the World Health Organization guidelines consider liposomal amphotericin B as an option for the treatment of ML, there are not clinical trials comparing its use with other drugs for this purpose. Few case reports and series have been designed to demonstrate its clinical efficacy and safety.¹⁵ To our knowledge, ours is the study including the highest number of patients. Amato and others published a short report including eight patients with ML treated with liposomal amphotericin, and all patients were clinically cured during the follow-up period.³ Sampaio and others¹⁶ found similar results in a case series with six patients, five of whom were clinically cured after 26–38 months of follow-up.

As in much of Latin America, in Brazil, ML is mostly caused by *L. (V.) braziliensis*. *Leishmania (V.) panamensis*, *L. (V.) guyanensis*, and *L. (L.) amazonensis* have also been associated with ML in America, but in a few case reports and series.^{8,11} In this study, no patient included was from or born in the Amazon region. However, the kDNA-PCR method was positive in 19 patients, indicating that *L. (V.) braziliensis* is the predominant species, although similar electrophoretic patterns may occur within related species from *Viannia* subgenus, as some studies have reported.^{17–19}

TABLE 1
Characteristics and outcomes of patients with ML treated with liposomal B amphotericin

Patient no.	Age (years) (treatment moment)	Previous cutaneous lesion	Lesion site	Comorbidities	Previous treatment	Total cumulative dose/kg	Adverse effects	Outcome
1	52	No	Nose	Hypertension	Pentavalent antimonial, amphotericin B deoxycholate	28	No	Cure
2	82	No	Nose, palate, and uvula	Hypertension, diabetes mellitus, and cardiopathy	No	44	No	Cure
3	62	No	Palate	Hypertension, cardiopathy, and dyslipidemia	No	36	Yes	Cure
4	77	No	Nose, palate	Hypertension, Chronic obstructive pulmonary disease	Pentavalent antimonial, amphotericin B deoxycholate	24	No	Cure
5	71	Yes	Nose	Hypertension, diabetes mellitus	Azithromycin	25.6	Yes	Cure
6	78	Yes	Nose	Dyslipidemia, benign prostatic hyperplasia	Pentavalent antimonial, pentamidine isethionate	28	Yes	Cure
7	64	No	Nose	Cardiopathy	No	28	No	Cure
8	77	No	Nose, pharynx	Hypertension	Pentavalent antimonial, amphotericin B deoxycholate	54	No	Cure
9	40	Yes	Palate	Hypertension, cardiopathy	Pentavalent antimonial	32	No	Cure
10	36	No	Nose	Cardiopathy	Itraconazole, amphotericin B deoxycholate	22.4	No	Cure
11	34	No	Nose	Not reported	Pentavalent antimonial, amphotericin B deoxycholate	23.1	No	Cure
12	67	Yes	Nose, palate, and uvula	Hypertension, diabetes mellitus, and cardiopathy	Amphotericin B deoxycholate	24.6	No	Cure
13	76	Yes	Palate, uvula, and jugal mucosa	Hypertension, diabetes mellitus	Itraconazole, pentoxifylline	30	No	Cure
14	78	No	Nose	Hypertension, diabetes mellitus, and cardiopathy	No	23.1	No	Relapse
15	82	Yes	Nose, palate, jugal mucosa, lip, and gum	Hypertension	Itraconazole	30	Yes	Cure
16	80	No	Nose	Hypertension, diabetes mellitus	Azithromycin, pentoxifylline	26.4	Yes	Cure
17	74	Yes	Nose	Hypertension	No	39.6	No	Cure
18	66	No	Nose	Not reported	Amphotericin B lipid complex, pentoxifylline	33	No	Cure
19	85	No	Nose	Cardiopathy, esophageal diverticulum	No	29.4	No	Cure
20	62	Yes	Nose	Not reported	No	33	No	Cure
21	54	No	Gum	Hypertension	Amphotericin B deoxycholate	21	Yes	Cure
22	71	No	Nose	Hypertension, diabetes mellitus, and cardiopathy	Pentavalent antimonial	30.4	No	Cure
23	73	No	Nose, palate, and uvula	Not reported	No	42	No	Cure
24	76	No	Nose, palate, and uvula	Not reported	No	50	No	Cure
25	74	No	Nose	Hypertension, diabetes mellitus	No	27	No	Cure
26	65	No	Nose	Not reported	No	18.2	Yes	Relapse
27	78	Yes	Nose	Cardiopathy	No	30.4	No	Cure
28	76	Yes	Palate, gum	Hypertension	No	55.2	No	Cure
29	70	No	Nose, uvula	Not reported	No	53	No	Cure
Mean	68.3	—	—	—	—	32.5	—	—

ML = mucosal leishmaniasis.

TABLE 2
Diagnosis methods performed in patients with ML treated with liposomal amphotericin B and their positivity

	Leishmanin skin test	Serology		PCR	Immunohistochemistry	Direct microscopic examination	Culture
		ELISA	IIFA				
Exam performed	24	15	20	19	16	12	8
Exam positive	22 (91.7%)	12 (80%)	12 (60%)	18 (94.7%)	6 (37.5%)	6 (50%)	3 (37.5%)

ELISA = enzyme-linked immunosorbent assay; IIFA = indirect immunofluorescence assay; ML = mucosal leishmaniasis; PCR = polymerase chain reaction.

Other species can be important in specific geographical situations, and molecular methods play a pivotal role in defining different clinical presentations, response to treatment, and choice of the best drug for each case. Guerra and others reported 46 cases of ML along the Amazon region, with 16 cases caused by *L. (V.) guyanensis*, thus showing that this species is also a significant causative agent within the region.⁸ In this scenario, the use of liposomal amphotericin B requires more caution, and studies with *L. (V.) guyanensis* are necessary to ensure the same efficacy demonstrated with *L. (V.) braziliensis*.

In this study, the cure rate was 93.1%, and just two patients were not considered cured. Of these, the first used a total dose of 23.1 mg/kg whereas the other, a total dose of 18.2 mg/kg (the lowest dose used for all patients). Both individuals were retreated with liposomal amphotericin (60 and 37 mg/kg, respectively) and had a better outcome after the second treatment, reaching the cure criteria. Factors such as dose, secondary bacterial infections, and host immunity may have been involved in the worst outcomes. To date, the ideal dose of liposomal amphotericin B for ML treatment has not yet been established. For the treatment of the localized form of American cutaneous leishmaniasis, a study comparing low-dose liposomal amphotericin B (total dose of 7.5 mg/kg) with *N*-methyl glucamine showed that only 50% of patients achieved a clinical cure in the liposomal amphotericin B group, in comparison with a 100% clinical cure in the *N*-methyl glucamine group.²⁰ For the ML treatment, the World Health Organization guidelines recommend a total dose of 40–60 mg/kg, but we think that an intermediate dose can be used, according to our findings and the results reported in other studies.¹⁵ We used a mean total dose of 32.5 mg/kg (range = 18.2–55.2 mg/kg) with a good cure rate.

Situations for the use of liposomal amphotericin B have not been well established, partly because there are not studies comparing liposomal amphotericin B to other drugs. Because of this, in Brazil, liposomal amphotericin B is indicated for the treatment of American cutaneous leishmaniasis just in cases of failure with all other options recommended or if there is an absolute contraindication to use other drugs. *N*-methyl glucamine antimoniate, a pentavalent antimonial, is the parenteral drug most commonly used in Brazil and the first choice according to the *Manual for Surveillance of American Tegumentary Leishmaniasis*.⁷ Despite their potential toxicity, due to the therapeutic failure rate of antimonials and the frequent relapses, in some situations, it is necessary to repeat the treatment.²¹ The side effects include liver failure, pancreatitis, constitutional symptoms, and abnormal electrocardiographic findings (T wave inversion; QTc interval prolonged), which can affect 10–30% of individuals treated.^{21–24} These effects limit the use of antimonials in patients with previous comorbidities and advanced age. Amphotericin B deoxycholate, liposomal amphotericin, azole

drugs, miltefosine, and pentamidine are recommended as a second-line therapeutic option and in cases of therapeutic failure with antimonials, according to the Pan American Health Organization guidelines.²⁵

Liposomal amphotericin yields better results than amphotericin B deoxycholate as described in literature, even in those cases in which therapy with antimonials had previously failed.²¹ Furthermore, the use of amphotericin B deoxycholate is accompanied by dose-limiting toxicities, most importantly, infusion-related reactions and nephrotoxicity, and lipid-associated amphotericin B formulations provide a safer alternative than conventional amphotericin B with significantly less nephrotoxicity.²⁶ In this study, the mean age at the moment of treatment was 68.3 years, and 23 (75.9%) individuals had some comorbidity. In addition, 15 individuals (51.7%) had undergone previous treatment, with a relapse. These were the main criteria used by the medical assistance team to indicate the use of liposomal amphotericin. It is possible that a regimen containing lipid-associated amphotericin B formulations should be preferable in case of advanced age, important comorbidities, and clinical failure to other treatments. However, comparative and prospective studies are required to determine if this is the best alternative in these cases. Still, we consider using liposomal amphotericin B as the first choice to treat patients that present absolute contraindication to other drugs or some medical condition that may worsen during the treatment thus calling for discontinuation of therapy.

It is also important to observe that liposomal amphotericin B use is not free of collateral effects. Seven patients in the study population had adverse effects; five of them (17.2%) had renal failure. Considering the high median age and comorbidities reported, it is possible that, besides the drug effect, some patients had a previous unknown renal impairment. However, it is important to highlight that none required discontinuation of treatment. Amato and others published a retrospective study including 30 patients treated with amphotericin B deoxycholate. Of these, 17 interrupted the treatment because of acute kidney failure.⁹ Our study suggests that liposomal amphotericin can be a safer drug. As a result, it can increase the probability of the patients completing the treatment and influence the outcome and cure rate.

This study showed good safety and efficacy of liposomal amphotericin B in patients with ML. It can be used in the context of advanced age and comorbidities, as an off-label use. Because this is a retrospective study with many limitations, we consider its data useful, taking into account the lack of data on this subject. Although prospective studies are necessary to establish the best dose and to compare the clinical outcomes regarding other drugs of choice, the data presented indicate that total doses of about 30 mg/kg can be safe and effective.

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