INVESTIGATION

A randomized clinical trial comparing meglumine antimoniate, pentamidine and amphotericin B for the treatment of cutaneous leishmaniasis by Leishmania guyanensis *

Estudo clínico randomizado comparando antimoniato de meglumina, pentamidina e anfotericina B para o tratamento da leishmaniose cutânea ocasionada por Leishmania guyanensis

Leandro Ourives Neves[1]
Anette Chrusciak Talhari[2]
Ellen Priscilla Nunes Gadelha[3]
Roberto Moreira da Silva Júnior[4]
Jorge Augusto de Oliveira Guerra[5]
Luiz Carlos de Lima Ferreira[6]
Sinésio Talhari[7]

Abstract: Fundamentals: American tegumentary leishmaniasis (ATL) treatment remains a challenge, since most available drugs are injectable and only a small number of comparative, randomized clinical trials have been performed to support their use. Moreover, treatment outcome may depend on the causative species of *Leishmania*.

Objectives: To evaluate and compare the efficacy and tolerability of meglumine antimoniate, pentamidine isethionate, and amphotericin B in the treatment of ATL caused by *Leishmania (Viannia) guyanensis*.

Methods: 185 patients were selected according to the eligibility criteria and randomly allocated into three groups - two groups with 74 patients each, and one group with 37 patients, which underwent meglumine, pentamidine and amphotericin B treatment, respectively. Doses, mode of administration and time periods of treatment followed the current recommendations for each drug. Patients were re-examined one, two and six months after completion of treatment.

Results: No differences were observed among the therapeutic groups in relation to gender, age, number or site of lesions. Intention-to-treat (ITT) analysis showed efficacy of 58.1% for pentamidine and 55.5% for meglumine (p=0.857). The amphotericin B group was analyzed separately, since 28 patients (75.7%) in this group refused to continue participating in the study. Mild or moderate adverse effects were reported by 74 (40%) patients, especially arthralgia (20.3%) in the meglumine group, and pain (35.1%) or induration (10.8%) at the site of injection in the pentamidine group.

Conclusion: Pentamidine and meglumine show similar efficacy in the treatment of ATL caused by *L. guyanensis*. Given the low efficacy of both drugs, there is an urgent need for new therapeutical approaches.

Keywords: Amphotericin B; Leishmaniasis, Cutaneous; Meglumine; Pentamidine; Therapeutics

Resumo: Fundamentos: O tratamento da leishmaniose tegumentar americana (LTA) ainda constitui desafio, pois a maioria dos medicamentos é injetável e têm-se poucos ensaios clínicos randomizados comparando a eficácia das drogas. Além disso, é provável que as espécies de *Leishmania* tenham influência nas respostas terapêuticas.

Objetivos: Avaliar e comparar a eficácia e a segurança dos esquemas de tratamento na LTA, ocasionada por *Leishmania (Viannia) guyanensis*.

Métodos: 185 pacientes foram selecionados, conforme critérios de elegibilidade, e distribuídos, aleatoriamente, em 3 grupos – 2 com 74 enfermos e outro com 37 - que receberam, respectivamente, antimoniato de meglumina, isotionato de pentamidina e anfotericina B em doses, períodos e vias de administração padronizados. Os enfermos foram reexaminados um, dois e seis meses após o final dos tratamentos.

Resultados: Não houve diferença entre os grupos terapêuticos em relação ao sexo, idade, número ou local das lesões. A análise por intenção de tratar (ITT) mostrou eficácias de 58,1% para a pentamidina e 55,5% para o antimoniato (p=0,857). O grupo da anfotericina B foi analisado separadamente, pois 28 (75,7%) pacientes negaram-se a continuar no estudo após a randomização. Eventos adversos leves ou moderados foram relatados por 74 (40%) pacientes, principalmente artralgia (20,3%), para o grupo do antimoniato, e dor (35,1%) ou enduração (10,8%) no local das injeções para a pentamidina.

Conclusões: A pentamidina tem eficácia similar ao antimonial pentavalente para o tratamento da LTA ocasionada por *L. guyanensis*. Face aos baixos resultados de eficácia apresentados por ambas as drogas, necessita-se, com urgência, investigar novas opções terapêuticas para esta enfermidade.

Palavras-chave: Anfotericina B; Leishmaniose cutânea; Meglumina; Pentamidina; Terapêutica

Received on 11.10.2010.
Approved by the Advisory Board and accepted for publication on 03.12.10.
* Work conducted at the Dermatology Outpatient Clinic of the Tropical Medicine Foundation of Amazonas (FMTAM) - Manaus (AM), Brazil.
Conflict of interest: None / *Conflito de interesse: Nenhum*
Financial funding / *Suporte financeiro:* Financiadora de Estudos e Projetos (Research and Projects Financing) of the Ministry of Science and Technology - FINEP.

[1] MSc in Tropical and Infectious Diseases - Graduate Program in Tropical Medicine, Tropical Medicine Foundation of Amazonas (Fundação de Medicina Tropical do Amazonas)/University of the State of Amazonas (Universidade do Estado do Amazonas) (FMTAM - UEA) - Professor of Clinical Dermatology at the Federal University of Amazonas (Universidade Federal do Amazonas) (UFAM) - Manaus (AM), Brazil.
[2] PhD in Tropical Medicine - Dermatologist at the Tropical Medicine Foundation of Amazonas (FMTAM) - Manaus (AM), Brazil.
[3] MSc student in Tropical and Infectious Diseases - Graduate Program in Tropical Medicine, Tropical Medicine Foundation of Amazonas/University of the State of Amazonas (FMTAM - UEA) - Nurse at the Tropical Medicine Foundation of Amazonas (FMTAM - UAS) - Manaus (AM), Brazil.
[4] PhD in Tropical and Infectious Diseases - Graduate Program in Tropical Medicine, Tropical Medicine Foundation of Amazonas/University of the State of Amazonas (FMTAM - UEA) - Biologist at the Tropical Medicine Foundation of Amazonas (FMTAM) - Manaus (AM), Brazil.
[5] PhD in Tropical Medicine – Infectious disease specialist at the Tropical Medicine Foundation of Amazonas (FMTAM) - Manaus (AM), Brazil.
[6] PhD in Pathology - Pathologist at the Tropical Medicine Foundation of Amazonas (FMTAM) - Manaus (AM), Brazil.
[7] PhD in Dermatology – President of the Tropical Medicine Foundation of Amazonas (FMTAM) - Manaus (AM), Brazil.

INTRODUCTION

Leishmaniasis includes a group of chronic infectious diseases caused by various species of protozoa of the genus *Leishmania.* They occur in tropical and subtropical regions of the Old and New World and may reach the viscera (visceral leishmaniasis), skin and/or mucous membranes. It is estimated that annually 1.5 to 2 million people are affected and that 1 to 1.5 million cases correspond to the tegumentary form of the disease. [1]

American tegumentary leishmaniasis (ATL) is an important cause of morbidity in the Americas, being observed from the southern United States to northern Argentina (except Chile and Uruguay). In the last twenty years, 549,309 cases of ATL have been registered in Brazil. The mean rate of detection of the disease in the country is 16.2 cases per 100,000 population, but in the North region it is 76.8 cases per 100,000 population.[2] ATL is a primary zoonosis of wild mammals (marsupials, rodents, edentates and primates, among others) and, secondarily, of domestic animals. Transmission can occur in a wild environment, related to the disorderly exploitation of forests and deforestation, for occupational or leisure reasons. In the rural and peri-urban environment, transmission is related to the migration process, occupation of slopes and conglomerates, associated with residual or secondary forests. [3]

The most important species in Brazil are *Leishmania (Viannia) braziliensis*, *L. (V.) guyanensis* and *L. (L.) amazonensis.* In the state of Amazonas, the cutaneous form prevails, especially in the region of the city of Manaus, with few cases of mucosal involvement. In the city of Manaus, which accounts for more than half of the cases of ATL in the state, the predominant species is *L. (V.) guyanensis.* [4,5]

The incubation period of the disease in humans is, on average, sixty days, ranging from two weeks to 24 months. [3]

Cutaneous leishmaniasis (CL) can progress to spontaneous healing or progress to formation of papules, nodules, plaques and especially ulcers. Invasion of regional lymph nodes and progression to mucosal lesions (MCL) may also occur. What will determine the infection progression and the different forms of clinical presentation of ATL is basically the type of immune response that the individual will develop - Th_1 or Th_2. This inflammatory response, on the other hand, depends on aspects not yet fully known, such as host genetic factors (existence of genes that promote Tumor Necrosis Factor, effective response of NK cells against IL-12 and adequate production of interferon gamma, among others) and the species of *Leishmania* responsible for the infection. [3,6]

The aspect of the ulcers is similar regardless of the species of *Leishmania.* Apparently, infection by *L. (V.) guyanensis* induces cellular and humoral immune response less intense than *L. (V.) braziliensis,* which could explain some specific aspects of its clinical presentation, such as a greater number of parasites in the lesions and greater resistance to antimonial therapy. [7] It seems that there is higher frequency of lymph nodes adjacent to the lesions, longer treatment is necessary and that tendency to nasal metastasis is lower. [8,9] The presence of satellite papules is thought to indicate increased risk of treatment failure. [10]

For the parasitological diagnosis of CL, direct examination or culture, using material collected for biopsy or aspirate from ulcers, is performed. Microscopic identification of smears requires experienced technicians for the diagnosis. Cultures may require a long time and there might be contamination, hindering the diagnosis. Among the indirect methods, Montenegro Skin Test (MST) has the limitation of becoming positive three to six weeks after the onset of the disease, with variable sensitivity in endemic areas. In addition, there is no standardization regarding the antigens used in its production. Polymerase chain reaction (PCR) and serological tests have been used as diagnostic options. [3,5]

The drug of first choice for treatment of ATL is N-methylglucamine antimoniate (NMG). As second-line treatment options, pentamidine isethionate and amphotericin B are available in health care services. [3]

It is difficult to assess the cure rates with antimonials in ATL due to lack of controlled studies and to the different dosages employed. [5]

It is likely that the species of *Leishmania* influence the therapeutic response to antimonials. [11,12] According to some studies, pentamidine is the first-line drug in the state of Amazonas in the treatment of CL by *L. (V.) guyanensis*. [8,13]

Regarding ATL treatment costs, and considering only the cost of drugs, pentamidine costs almost twice as much as antimonials. However, prolonged parenteral treatment with antimonials generates indirect expenses related to hospital supplies, any need for hospitalization or work leave. In Brazil, the annual expense only with pentavalent antimonials is R$5,490,000. [3] Thus, pentamidine constitute an important therapeutic option for the treatment of cutaneous ATL, considering its cost/benefit, besides being safe for the treatment of patients with heart disease. [8,14]

Other major drugs already used in the treatment of ATL are miltefosine, azithromycin, itraconazole, ketoconazole, allopurinol, paromomycin and pentoxifylline. [5,15]

The side effects of antimonials are well known:

cardiac, hepatic, pancreatic and renal toxicities in addition to toxicity of the musculoskeletal system. Cardiotoxicity is mainly characterized by ventricular repolarization disturbances, including T-wave changes (flattening or inversion) and increased QT interval. These changes are dose and time dependent. Antimonials are contraindicated in patients taking beta-blockers (especially propranolol and sotalol) or antiarrhythmic agents, such as amiodarone and quinidine. Side effects related to the gastrointestinal tract are common and include nausea, anorexia and abdominal pain. Hepatotoxicity is also common and may occur in up to 50% of the patients treated. [16]

Antimonials are mainly eliminated via the urine, where 80% of the drug is excreted in the first six hours after parenteral administration. After 16 hours, plasma levels of the drug drop to 1% of the values of maximum plasma concentration, and any decrease in renal *clearance* enhances the toxic effects of antimonials. Renal failure secondary to the use of antimonials in usual doses is rare. The main nephrotoxic effect of antimonials is the decrease in urinary concentrating capacity. This adverse effect, which is reversible after treatment, is secondary to the competition of the drug with the antidiuretic hormone in renal collecting ducts. Given the renal excretion of antimonials and the absence of an adjustament dosage chart in renal failure, the use of antimonials in patients with any degree of renal impairment is contraindicated. In these cases, the drug of choice is amphotericin B deoxycholate or its lipid formulations. Due to the absence of conclusive studies on the teratogenicity of antimony compounds during pregnancy, amphotericin B is also the drug of choice for treatment of CL during pregnancy. [17,18]

Amphotericin B (AB), discovered in 1956 by Gold et al., is a polyene macrolide antibiotic that interacts with ergosterol in the cell membrane, forming pores that alter ionic balance and cell permeability, causing cell death. [18] It is considered the second drug of choice when there is no response to treatment with pentavalent antimonial or when it is not possible to use it. Administration of amphotericin B is contraindicated in patients with heart, liver and especially kidney problems. The most common adverse effects are fever, nausea, vomiting, hypokalemia and phlebitis at the infusion site. These effects can be mitigated or avoided by adding antipyretics, anti-emetics, potassium replacement and hydrocortisone - 50 to 100mg – to the infusion serum. Other important adverse effects are anorexia, kidney failure, anemia, leukopenia and heart problems. Electrocardiographic and laboratory monitoring of liver enzymes, renal function and serum potassium levels should be done weekly. [3]

Gastrointestinal absorption of AB is very small. Daily AB intravenous infusions for adults at doses of 0.5 mg/kg result in plasma concentrations of about 1.0 to 1.5 μg/mL at the end of administration, which drop to 0.5 to 1.0 μg/mL within 24 hours. The drug is released from its complex with deoxycholate in the blood stream; the AB that remains in the plasma is predominantly (90%) combined with proteins, especially β-lipoproteins. Approximately 2 to 5% of each dose is present in urine, when the patient receives daily doses of AB. Liver or biliary diseases have no known effect in the metabolism of the drug in humans. Given the massive tissue uptake, there is a terminal elimination half-life of approximately fifteen days. [18]

AB is effective in cutaneous or mucosal ATL. Its use is limited, since hospitalization is needed for application. [3]

Pentamidine is a synthetic derivative of amidine and is effective against many pathogenic protozoa, including species of *Leishmania*. The discovery of the antiprotozoal activity of diamidines resulted from the search for hypoglycemic drugs that could compromise the energy metabolism of parasites. [19]

Today it is commercialized for human use only in the lyophilized form of pentamidine isethionate in vials containing 300 mg of the salt. The mechanism of action of pentamidine is thought to occur by inhibition of different cellular processes, not yet fully elucidated. Recently, resistance to pentamidine has been described in trypanosomes and leishmania. [20-22]

Pentamidine isethionate is completely absorbed following parenteral administration. According to data published by the manufacturer (Pentacarinat®/Sanofi Aventis), after intramuscular administration (4 mg/kg), the drug reaches the maximum plasma concentration of approximately 0.2 μg/mL, elimination half-life is 9.4 hours (± 2.0), renal elimination of the unmodified substance is 4.1% in 24 hours, renal clearance is 15.4 l/h (± 14.9).

The main adverse reactions related to pentamidine are pain, induration and sterile abscesses at the injection site, as well as nausea, vomiting, dizziness, adynamia, myalgia, headache, hypotension, syncope, transient hyperglycemia and hypoglycemia. To avoid the effects of glucose metabolism, patients should be advised to eat before and rest for fifteen minutes after the injections. Cases of diabetes have been reported in patients who received total doses, usually greater than 2.0 g. [3]

Pentamidine is an alternative drug for cases of ATL unresponsive to pentavalent antimonials or in cases in which it is impossible to use them. Pradinaud and Talhari's studies showed good results with low doses in CL caused by *L. (V.) guyanensis* - total doses close to 1.0 g. [8,13,23]

MATERIALS AND METHODS

The research, developed at the Tropical Medicine Foundation of Amazonas (FMT-AM), is part of an open-label, controlled, randomized, multicenter study with 395 patients of both sexes from four cities: Manaus – Amazonas State, Brasilia – Federal District, Corte de Pedra – Bahia State, and Ribeirao Preto – Sao Paulo State. In Manaus, 185 patients with cutaneous leishmaniasis (CL), whose clinical diagnoses were confirmed by direct examination (scraping) and who met the eligibility criteria for the study, were evaluated. These patients randomly formed three groups, two of them with 74 and another with 37 participants. A list of random distribution was established for their allocation in the treatment groups. This list was generated by the biostatistician of the project. These patients were treated with meglumine antimoniate (NMG), pentamidine isethionate or amphotericin B (AB).

The study included patients diagnosed with the cutaneous form of ATL, who sought the FMT-AM from January 2009 to February 2010 and who agreed to participate in the investigation by signing the Informed Consent Form.

During the first appointment, medical records were filled up, with detailed examination of lesion(s), determining type, location, number and diameters (major and minor) by means of a millimeter ruler (caliper), and the ulcers were photographically recorded.

Laboratory evaluations (hematologic, hepatic, pancreatic and renal) were carried out before and after the treatment regimen, which were repeated one month later in case any changes had been detected after treatment. Samples of 20 mL of peripheral venous blood were collected from each patient for blood count, serum glucose, aminotransferases, urea, creatinine and amylase, as well as samples for urinalysis.

The patients' cardiac evaluation was complemented with electrocardiogram (ECG) at the beginning of the treatment.

Inclusion criteria were:

- Weight: greater than 8 kg;
- Gender: male or female patients;
- Clinical findings compatible with CL and positive direct examination (by smear) for *Leishmania;*
- Disease duration: between one and three months of evolution;
- Number of lesions: a maximum of six lesions (localized cutaneous leishmaniasis - LCL); presence of at least one ulcerated lesion;
- Lack of mucosal involvement and no history, confirmed or not, of cutaneous leishmanial lesion;
- Signing the Informed Consent Form (ICF).

Exclusion criteria were:

- Prior treatment with pentavalent antimonials or leishmanicidal drugs in the last six months;
- Clinical and/or laboratory evidence of cardiac abnormalities (pre-treatment ECG changes);
- Concomitant tuberculosis, leprosy, cancer, *diabetes mellitus* or other serious illness;
- Uncontrolled hypertension (HTN ≥ 160/95mmHg, verified at least three times on different days);
- Evidence of peripheral vascular involvement (presence of varicose veins in the legs or ulcerated, flat, hyperpigmented, painful lesions, even in the absence of secondary infection);
- History of alcoholism;
- Treatment with corticosteroids or other immunosuppressants;
- Pregnancy;
- AST ≥ 3 times the upper limit of normal;
- ALT ≥ 3 times the upper limit of normal;
- Serum creatinine or urea ≥ 1.5 times the upper limit of normal.

Criteria for withdrawal from the study:

- Intolerance to the treatment regimen (emergence of moderate or severe side effects that prevented continuation of the treatment regimen). Side effects were classified as mild, moderate and severe:
  - Mild side effects: presence of signs or symptoms related to the use of medication that could be tolerated by the patient, with no need for symptomatic therapy or discontinuation of the specific therapy;
  - Moderate side effects: signs or symptoms related to the treatment regimen that could be tolerated with use of symptomatic therapy. The need for temporary discontinuation of the specific treatment in such cases was considered after evaluation of the response to symptomatic therapy;
  - Severe side effects: non-manageable signs or symptoms, except with discontinuation of specific medication (changes in kidney, liver or heart function, clinically manifested).
- Non-adherence to protocol: failure in the administration of three consecutive doses, or the need for 30 days or more of treatment;
- Express request on the part of the patient to withdraw from the study.

Laboratory diagnosis:

- Direct examination - scraping of the border of up to three lesions was performed, followed by staining with Giemsa or Panotico.
- Biopsy - two fragments were collected for histopathology and species identification by PCR.
- Identification of strains - PCR-RFLP *(restriction fragment length polymorphism)* was performed, with *primers* amplifying a region of the hsp70 gene and of the *mini-exon,* according to studies by Garcia et al. and Marfurt et al., respectively. [24,25]

Administration of treatments:

After registration, diagnostic procedures, heart, kidney and liver evaluation and randomization, the patients were allocated to the study. The main objective was to assess response to therapy in patients with LC, caused by *L. (V.) guyanensis* by registering clinical cure or treatment failure.

Antimonial - the drug is presented in 5ml ampoules containing 81 mg of pentavalent antimonial (Sb^{+5}) per mL. The dose was 15 mg/kg/day for 20 days, administered intravenously (IV) or intramuscularly (IM). It was recommended that the IV application be slow, for about five minutes, and dilution was not necessary; rest was recommended after application. The maximum dose was 15 mL/day. The patient received the medication, which was applied in a heath center.

Pentamidine - three doses of 4mg/kg were administered every 72 hours via deep intramuscular injection with the patient in a supine position. The contents of a vial was dissolved in 6ml of sterile water for injection. This application was done at the outpatient clinic of dermatology, after administration of a carbohydrate-rich meal to reduce its hypoglycemic effect. The maximum dose was 300 mg/dose.

Amphotericin B – 1mg/kg/day was administered IV for 20 days. On the first two days, the maximum low dose was (0.5 mg/kg/day) for evaluation of the patient's clinical and laboratory tolerance to the medication. These first two doses were not considered in the calculation of the twenty days of treatment. Venous infusion was performed at Hospital-Dia at the FMT-AM, following all the recommended procedures for administration of the drug.

Follow-up – clinical follow-up was conducted 30, 60 and 180 days after the end of the treatment. Laboratory evaluations were repeated after 30 days. The other evaluations were clinical: vital signs, weight and photographic documentation of lesions (Figures 1 to 4).

Criteria for healing:

Apparent cure - complete epithelization of all ulcers and absence of any signs of inflammatory reaction up to 60 days after completion of treatment.

Permanent cure - complete healing of all ulcers and absence of any signs of inflammatory reaction 180 days after completion of treatment.

Therapeutic failure - appearance of any new lesion or 50% increase in lesions previously documented sixty days after completion of treatment.

Rescue treatment: performed in cases of clinical aggravation attributed to leishmaniasis, despite treatment. The drug of choice was pentamidine isethionate, with the same dosage used in the initial treatment.

RESULTS

During 13 months (January 2009 to February 2010), 313 patients with CL and positive direct examination were evaluated for the study. Of these patients, 128 were excluded, for they lived far from Manaus

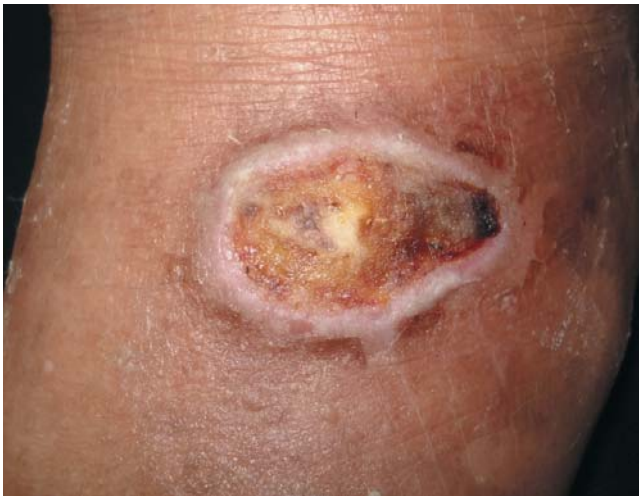

Figure 1: Patient 1: ulcerated lesion in the dorsal region of the left foot, before treatment with pentamidine

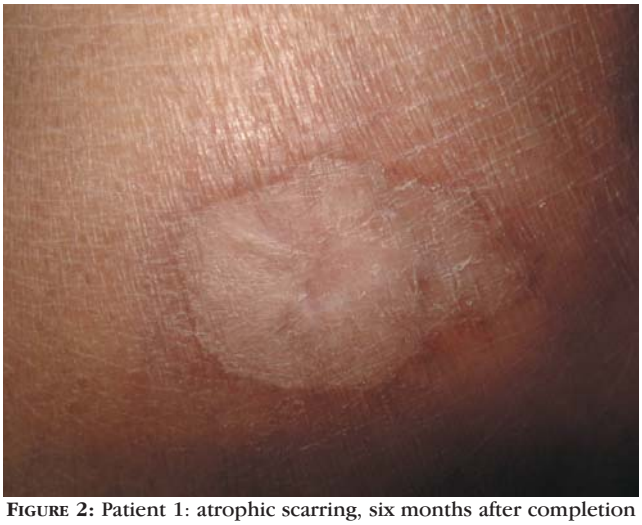

Figure 2: Patient 1: atrophic scarring, six months after completion of treatment with pentamidine

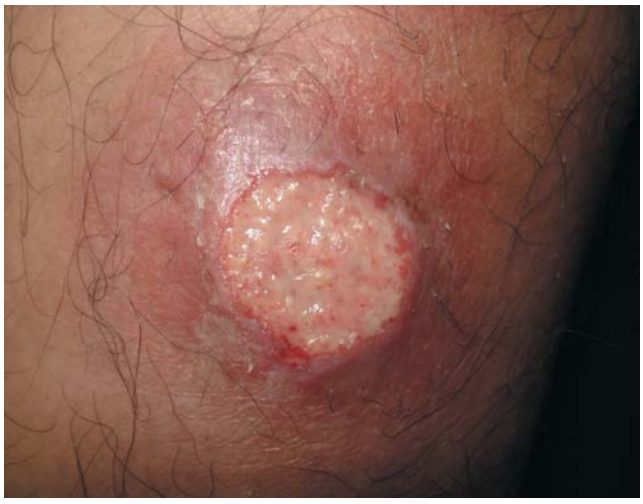

Figure 3: Patient 2: ulcer in the posterior region of the left leg before treatment with meglumine

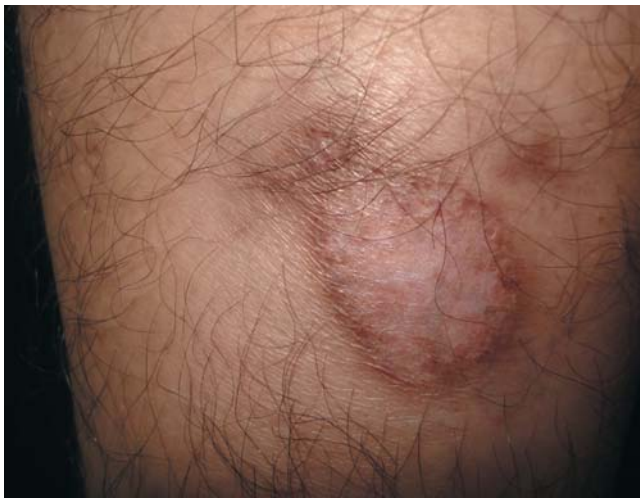

Figure 4: Patient 2: Scar, six months after completion of treatment with meglumine

(n=57), were unavailable for appointments (n=10), refused to participate (n=10), had more than six lesions (n=9), had no ulcerated lesions (n=14), had lesions larger than 5 cm (n=2), had undergone previous treatment (n=11), were pregnant (n=6), presented associated diseases (n=5), were in transit (n=4).

Overall, 185 patients met the eligibility criteria and were enrolled in the study: 74 for the antimonial, 74 for pentamidine and 37 for amphotericin B. All patients acquired the disease in endemic areas on the outskirts of Manaus. The patients enrolled were of both sexes: 44 women and 141 men, with ages ranging from 5 to 65 years. There was no statistically significant difference between the treatment groups regarding sex, age, number or location of lesions (Tables 1 and 2). In 163 (88.1%) patients, the species found was *L. Guyanensis;* nine (4.9%) had *L. brasiliensis* and thirteen (7.0%) did not have the species identified (Table 3). To calculate efficacy, only patients with *L. guyanensis* were evaluated, with eleven patients being excluded from the antimonial group and twelve from the pentamidine group; the amphotericin B group was analyzed separately, for 28 (75.7%) of its patients refused to continue participating in the study after randomization. For the analysis of efficacy, 58 patients in the antimonial group and 58 in the pentamidine group were considered. Five patients from the antimonial group and four from the pentamidine group were lost during follow-up. Two patients in the antimonial group and one in the pentamidine group withdrew from the study after randomization, for they prefered other medications (Table 4).

Toxicity and tolerability: The drugs used in the study were well tolerated. No serious adverse events occurred. Two patients in the AB group had to discontinue treatment, one due to hypokalemia and the other due to nausea during infusion. Among the patients in the antimonial group, 20.3% had arthralgia and 9.5% had headache. Among the patients who used pentamidine, the most common side effects were pain (35.1%) or induration (10.8%) at the injection site (Table 5).

Efficacy: The cure rates after six months (permanent cure) were 55.5% and 58.1% (p=0.857) for the antimonial and pentamidine, respectively, considering only patients with *L. guyanensis.* If we analyze only treatment, regardless of the species of Leishmania involved, the antimonial obtained 56.9% efficacy and pentamidine obtained 60.3% efficacy (p = 0.737) in 145 patients (Table 6).

DISCUSSION

Although the ATL treatment recommended by the Ministry of Health (MOH) is only one for the whole country, regardless of the *Leishmania* species causing the disease, there are few clinical trials showing the real efficacy of these drugs. It is known that the response to treatment with antimonial (first-line drug) may vary according to the parasite strain, the patient's immune status and clinical form. Molecular studies on the parasite have shown its wide diversity, including subspecies of *L. (V.) brasiliensis.* [26] Genetic variations of a species may give rise to features such as antigenic diversity, virulence, pathogenicity and drug resistance. Genetic and racial differences may play a role in disease resolution. It is believed that the severity of the disease may interfere with therapeutic response, and some species of parasites are innately

Table 1: Analysis of homogeneity between the treatment groups with respect to age and gender

	Meglumine(%)	Pentamidine(%)	Amphotericin B(%)	Total
Age groups				
Under 18	14 (48.3)	15 (51.7)	9 (24.3)	38
18 |-- 36	43 (51.8)	40 (48.2)	19 (51.4)	102
36 |-- 54	16 (50.0)	16 (50.0)	7 (18.9)	39
54 and over	1 (25.0)	3 (75.0)	2 (5.4)	6
Mean ± SD	28.5 ± 11.4	29.3 ± 12.7	28.8 ± 13.6	
	p value = 0.94**	0.89 *		
Sex				
Female	18 (48.6)	19 (51.4)	7 (18.9)	44
Male	56 (50.5)	55 (49.5)	30 (81.1)	141
	p value = 0.726*			

* Fisher's Exact Test / ** Kruskal-Wallis
SD = Standard Deviation

Table 2: Analysis of homogeneity between the treatment groups regarding location of the lesions

Location	Meglumine	Pentamidine	Amphotericin B	*p*value*
Head				
no	72	68	32	0.099
yes	2	6	5	
UL				
no	45	49	23	0.783
yes	29	25	14	
LL				
no	35	34	17	0.984
yes	39	40	20	
TR				
no	60	60	29	0.933
yes	14	24	8	

* Chi-square Test
UL = Upper limbs
LL = lower limbs
TR = Trunk

more susceptible to antimonials. [27]

L. (V.) guyanensis is the second most prevalent species in Brazil and one of the most common in the Amazon region. Only two clinical efficacy trials identifying species have been conducted in the last 10 years. In 2001, Romero et al. obtained a cure rate of 26.3% for pentavalent antimonial. More recently, in 2009, pentavalent antimonial was compared to miltefosine, and the cure rates were 53.3% and 70.7% ($p = 0.05$), respectively.[5,11] This difference in terms of efficacy could be explained by the likely genetic variability among strains of *L. (V.) guyanensis,* resulting in different susceptibilities to treatment. A study conducted in French Guiana, using *ribosomal fingerprinting analysis*, identified two distinct populations of *L. (V.) guyanensis.* [28]

Pentamidine, the second treatment option for ATL in Brazil, [3] is the first-line drug in Suriname and French Guiana, where *L. Guyanensis* predominates. [29] In French Guiana, mesylate and pentamidine isethionate at a dose of 4mg/kg/day in two applications with an interval of 48 hours led to cure of 100% of the patients with CL, without significant adverse effects. [10] In Suriname, a retrospective study on patients treated between 1979 and 2000 with pentamidine mesylate (120mg/day/IM for seven days) or with pentamidine isethionate (300mg/week/IM for three to five weeks) found cure rates of approximately 90%. Bitter taste in the mouth, nausea, pain at the injection site and cardiorespiratory symptoms (nasal congestion, dyspnea, hyperpnea and palpitations) were the adverse effects reported. [30]

In Brazil, a study comparing the efficacy of three applications of pentamidine isethionate at a dose of 4mg/kg/day, intramuscularly, on alternate days and NMG at a dose of 20 mg Sb^{+5}/kg/day, intravenous-

Table 3: Results of identification by PCR-RFLP

Identification by PCR-RFLP	Meglumine	%	Pentamidine	%	Amphotericin B	%
L. braziliensis	4	5,4	5	6,8	0	0,0
L. guyanensis	65	87,8	63	85,1	35	94,6
Negativo em biópsia	5	6,8	6	8,1	2	5,4

Table 4: Comparison of therapeutic efficacy between meglumine and pentamidine, only for CL by *L. guyanensis*

	Meglumine	%	Pentamidine	%
Cura definitiva	35	53.8	36	57.1
Clinical Failure	23	35.4	22	34.9
Loss	5	7.7	4	6.3
Excluded	2	3.1	1	1.6
Total		65		63
	$p = 0.99$ *			
ITT	35/63	55.5	36/62	58.1
CI 95%		(42.5 - 68.1)		(44.8 - 70.5)
	$p = 0.857$*			
PP	35/58	60.3	36/58	62.1
CI 95%		(46.7 - 72.9)		(48.4 - 74.5)
	$p = 0.99$*			

* Fisher's Exact Test
CI = confidence interval
ITT = intention to treat analysis
PP = protocol analysis

ly, for twenty days showed similar efficacy - 71% and 73.2%, respectively. Most species, identified by monoclonal antibody technique in 21 isolates, were *L. (V.) brasiliensis* (57.14%). We observed a reduction in treatment time and less cardiac toxicity in the pentamidine group. The main adverse events were dizziness, near syncope and pain at the injection site. [14] In Manaus, Amazonas State, the results obtained with pentamidine are similar to those observed by Pradinaud[23] and Talhari et al.[8,13]

Amphotericin B is effective in the cutaneous and mucosal forms of ATL; however, its use is limited, given the need for hospitalization for its application. In this trial, 28 (75.7%) of the patients randomized to this group refused to continue in the study when they learned that they would need to come to the hospital for at least 20 days. Given the small number of patients treated with amphotericin B, this drug has not been considered in the analysis of this clinical trial.

The data from the statistical analysis, with 58.1% efficacy for pentamidine and 55.5% for the antimonial, showed that both drugs are similar. However, when evaluating effectiveness, it is observed that treatment with pentamidine is more convenient for patients, since it takes only three injections at intervals of two days. On the other hand, treatment with antimonial is longer - a total of 20 injections, intravenously. A major drawback to pentamidine is that it requires deep intramuscular injections in an outpatient basis, given the possibility of immediate side effects such as hypoglycemia and emergence of reactions at the site of application, if administration is superficial.

The cost of pentamidine is greater than that of the antimonial. However, from an operational point of view, expenses with the antimonial become larger, since it takes several daily applications, long trips, interferes with work and often requires payment for the applications.

CONCLUSION

The authors conclude that the antimonial and pentamidine have similar efficacy in the treatment of ATL caused by *L. (V.) guyanensis.* Pentamidine has the advantage of being used for a shorter period of time and of having fewer adverse effects. For most patients,

Tabele 5: Comparison between adverse effects associated with treatment with meglumine and pentamidine

Adverse effects	Meglumine	%	Pentamidine	%
Arthralgia*	15	20.3	3	4.1
Asthenia	4	5.4	2	2.7
Headache	7	9.5	2	2.7
Visual disorders	1	1.4	1	1.4
Pain at injection site*	0	0.0	26	35.1
Emaciation	1	1.4	0	0.0
Induration*	0	0.0	8	10.8
Fever	2	2.7	3	4.1
Arterial hypotension	0	0.0	1	1.4
Loss of appetite	2	2.7	0	0.0
Myalgia	3	4.1	1	1.4
Nausea or vomiting	2	2.7	1	1.4
Palpitations/Tachycardia	0	0.0	1	1.4
Fullness	1	1.4	0	0.0
Skin Reactions	6	8.2	3	4.1
Flu-like symptoms	1	1.4	0	0.0
Sleepiness	1	1.4	1	1.4
Dizziness	2	2.7	9	12.2

* Significant for Fisher's Exact Test

Table 6: Comparison of therapeutic efficacy between meglumine and pentamidine, regardless of the species

	Meglumine	%	Pentamidine	%
Cura definitiva	41	55.4	44	59.5
Clinical Failure	25	33.8	25	33.8
Loss	6	8.1	4	5.4
Excluded	2	2.7	1	1.4
Total	74		74	
	$p = 0.834$ *			
ITT	41/72	56.9	44/73	60.3
CI 95%		(4.7 - 68.6)		(48.1 - 71.5)
	$p = 0.737$*			
PP	41/66	60.3	44/69	62.1
CI 95%		(49.3 - 73.8)		(51.3 - 75.0)
	$p = 0.86$*			

* Fisher's Exact Test
CI = confidence interval
ITT = intention to treat analysis
PP = Protocol analysis

it is more difficult to use the antimonial, for they live on roads far from health facilities and it is often difficult for them to find qualified people for the application of intravenous medication.

The low efficacy results for the antimonial and pentamidine, as well as the operational difficulties related to amphotericin B, show the urgent need for new therapeutic options in the treatment of ATL. ❑

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MAILING ADDRESS / ENDEREÇO PARA CORRESPONDÊNCIA:
Leandro Ourives Neves
Av. Pedro Teixeira, 25, Dom Pedro I
69040 000 - Manaus, AM, Brazil
E-mail: lourives@ig.com.br

How to cite this article/*Como citar este artigo*: Ourives-Neves L, Chrusciak-Talhari A,, Gadelha EPN, da Silva Júnior RM, Guerra JAO, Ferreira LCL, Talhari S. A randomized clinical trial comparing meglumine antimoniate, pentamidine and amphotericin B for the treatment of cutaneous leishmaniasis by Leishmania guyanensis. An Bras Dermatol. 2011;86(6):1092-101.