CLINICAL TRIAL WITH LIPOSOMAL AMPHOTERICIN B (AMBISOME®) FOR AMERICAN VISCERAL LEISHMANIASIS (AVL)

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A prospective, non-randomized study was conducted on 30 patients with American Visceral Leishmaniasis (AVL) aged 3 to 10 years from São Luis, Maranhão - Brazil, who were not previously submitted to treatment. After an informed consent form, was signed by the responsible person, all patients were submitted to anamnesis and physical examination and a standard chart was filled out. The patients were randomly divided into three cohorts of 10 patients each and submitted to the following treatment regimen with liposomal amphotericin B: cohort 1) 2 mg/kg/day applied on days 1, 2, 3, 4, 5, 6 and 10, total dose of 14 mg/kg; cohort 2) 2 mg/kg/day applied on days 1, 2, 3, 4, and 10, total dose of 10 mg/kg; cohort 3) 2 mg/kg/day applied on days 1, 5, and 10, total dose of 6 mg/kg. An intra-hospital treatment regimen was used which consisted of intravenous administration of the drug over a period of 60 min. Efficacy was 70% in cohort 1, 90% cohort 2, and 80% in cohort 3. The patients were followed up for 6 months. No significant difference was observed between the three regimens in terms of toxicity, tolerability and effectiveness when evaluated by generalizations of the Gehan model and Wilcoxon test.

Key words: *Leishmania chagasi*, visceral leishmaniasis, liposomal amphotericin B, Northeastern of Brazil.

In the 1980s, the World Health Organization (WHO) recommended pentavalent antimonials (sodium stibogluconate or N-methylglucamine antimoniate) at the dose of 20mg/Sb^{+5}/kg/day for a minimum period of 28 days, with a maximum daily dose of 850 mg/Sb^{+5}, for the treatment of visceral leishmaniasis (VL). Variable responses to this therapeutic regimen have been observed in different parts of the world, with difficulties being reported particularly in Kenia[32, 33].

In the 1990s, both the WHO and the Brazilian Ministry of Health reevaluated the question of the dose for Brazil and concluded that the standard regimen should consist of a dose of 20mg/Sb^{+5}/kg/day for a minimum of 20 consecutive days, which has shown good results[19, 33].

Over the years, alternative therapy for treatment of different forms of leishmaniasis, have been sought in an attempt to find better options than Sb^{+5}. Examples: tuberculostatic agents[24], co-trimaxazole[20], ketoconazole[30], metronidazole[16], pentamidines (mesylate/isothionate), allopurinol[14], aminosidine sulfate[29], amphotericin B[29, 31]; immunotherapeutic agents (IFN-γ, GMC-SF)[1, 26], liposomes[6,7], and, more recently, miltefosine[27], or a combination of these chemotherapeutic agents[18].

Amphotericin B is a product originating from the natural fermentation of *Streptomyces nodosus*, and is the second drug of choice for some deep mycoses and also for leishmaniasis. Among the problems related to this therapy are its side effects reported by several investigators[5, 18, 22, 33]. The clinical demand for less toxic agents resulted in the development of new lipid formulations of amphotericin B. Lipid particles were developed which, compared to deoxycholate micelles, caused fewer changes in the host cell with a consequent reduction in renal toxicity. These lipid particles are removed from the circulation by mononuclear phagocytes so that the drug is specifically distributed among cells parasitized by *Leishmania*[6, 9, 22].

The two lipid formulations of liposomal amphotericin B are amphotericin B in colloidal dispersion (Amphocil®, Liposome Technology, Menlo Park, CA) which consists of cholesterol sulfate and amphotericin B, with this formulation being 4 to 15 times more effective in the treatment of VL in infected hamsters than conventional amphotericin B deoxycholate, and Ambisome® (amphotericin B compound contain phosphatidylcholine, cholesterol, distearoyl-phosphatidylglycerol, sucrose and sodium succinate hexahydrate), the first commercially available product of this formulation which is more effective and less toxic in the treatment of systemic fungal infections[4, 6, 9, 12]. In a study on AVL, Dietze *et al*.[9] administered liposomal amphotericin B (Amphocil®) intravenously at the dose of 2 mg/kg/day to 10 patients for 10 days (group 1) and to 10 patients for 7 days (group 2) and observed cure in all of them. The authors reported a decrease in fever within an average of 4 days, a 79% reduction in splenomegaly within 2 weeks, and return to normal clinical and laboratory values 6 months after the end of treatment. Liposomal amphotericin B is the only drug approved by the US Food and Drug Administration for the treatment of VL alone (i.e., caused by *Leishmania donovani*, *L. infantum* or *L. chagasi* in immunocompetent patients, with a regimen of seven infusions of 3 mg/kg given on days 1, 5, 14 and 21, for a total dose of 21mg/kg[17]. Results from a dose-decreasing study have shown that a short course of liposomal

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amphotericin B (six infusions of 3mg/kg given on days 1, 5 and 10, for a total dose of 18mg/kg) could be safe and effective in treating children with Mediterranean VL[17].

The aim of this study was to compare three therapeutic regimens of Ambisomeâ in the treatment of AVL caused by *L. chagasi* in terms of efficacy, toxicity and tolerance.

Material and Methods

Study design

A comparative, prospective, non-randomized study evaluating the efficacy of liposomal amphotericin B (Ambisome®) was conducted on 30 children with VL. The subjects were hospitalized on the ward of Infectious Diseases, Mother-Infant Hospital, Federal University of Maranhão - UFMA, São Luis, Brazil, during the use of the therapy. The study was approved for the Ethics Committee of research involving humans of the University Hospital of UFMA.

Patients

Included in the study were 30 children aged 3 to 10 years from the Ilha de São Luis, Maranhão, with a diagnosis of VL without previous treatment. After an informed consent form, was signed by the responsible person, all patients were submitted to anamnesis and physical examination and a standard chart was filled out. The patients were randomly divided into three cohorts of 10 subjects each and submitted to the following treatment regimen: cohort 1) 2 mg/kg/day applied on days 1, 2, 3, 4, 5, 6 and 10, total dose of 14 mg/kg; cohort 2) 2 mg/kg/days on days 1, 2, 3, 4 and 10, total dose of 10 mg/kg; cohort 3) 2 mg/kg/day on days 1, 5 and 10, total dose of 6 mg/kg.

Excluded were patients younger than 3 years, patients with severe concomitant disease, subjects with a history of allergy to Sb^{+5} and/or amphotericin B, and patients with AVL during the last 6 months or with hemoglobin levels <5g/dL, total leukocytes <2,000/mm^3, platelets ≤40,000/mm^3, aminotransferase (AST/ALT) levels four times above normal, and urea and creatinine levels two times above normal.

Parasitological diagnosis

The parasitological diagnosis of AVL was made for all patients based on visualization of *Leishmania* amastigotes in Giemsa-stained bone marrow or splenic aspirates by microscopy and/or culture.

Study procedures

Signs and symptoms compatible with AVL (fever ≥38.0ºC), hemoglobin concentration, white blood cell count, platelet count, and levels of aspartate aminotransferase (AST), blood urea nitrogen, creatinine, potassium, albumin and globulin were determined for all patients. We considered important aspects in the evaluation of these parameters: anemia (hemoglobin <9.0mg/dL, leukopenia <4,000 white blood cells/μL, thrombocytopenia <150,000 platelets/μL) and laboratory parameters (serum *Leishmania* antibodies by immunofluorescent antibody test at a titer ≥1:40). Blood for laboratory determinations was drawn on days 1, 2, 3, 4, 5, 6 and 10 for cohort 1, on days 1, 2, 3, 4 and 10 for cohort 2, and on days 1, 5 and 10 for cohort 3.

Nutritional indicators were also determined for all children based on the calculation of Z scores or standard deviation (SD) for height/age and weight/height according to the North American curves of the National Center for Health Statistics (NCHS) adopted by the WHO as an international reference standard. Malnutrition was considered to be mild when the values were 1 to 1.9 SD below the median weight or height, moderate in the case of 2 to 2.9 SD, and severe in the case of 3 SD or more[15].

Determination of spleen size

Spleen size was assigned a value according to the following scale, with the organ palpable beyond the costal margin being given a value equivalent to size in centimeters: 0 = organ palpable at the costal margin at rest, 2 = organ palpable on deep inspiration, and 3 = organ undetectable on deep inspiration (i.e., the normal finding for individuals without a history of splenic pathology). All spleens were measured from the costal margin to the tip of the organ. Each spleen size measurement was performed by two clinicians (GMV and JMLC). Percent regression in spleen size refers to the percentage that the size regressed toward a value of -3.

Drug administration

Ambisome® was supplied by NeXstar Pharmaceuticals Inc. ready for clinical use according to manufacturers instructions, and was infused over a period of at least 2hs in 5% dextrose solution into a peripheral vein.

Follow-up evaluation

The patients remained hospitalized for 10 days under daily clinical follow-up. Each day on which the drug was administered the patients were monitored for fever, chills, tachycardia, somnolence, phlebitis, bone pain, anorexia, abdominal pain, and diarrhea. In cohort 1, vital signs (blood pressure, pulse rate, respiratory frequency, and temperature) were measured at 30-min intervals during the 2-h for drug infusion.

Laboratory exams (hemoglobin concentration, white blood cell count, platelet count, and levels of AST, blood urea nitrogen, creatinine, potassium, albumin and globulin) were repeated for all patients 24, 60 and 180 days after discharge from the hospital.

Definitions of cure, failure and relapse

Cure was defined as the regression of signs and symptoms and normalization of laboratory parameters during the 6-month follow-up; improvement was defined as the partial regression of signs and symptoms with a tendency toward normalization of laboratory parameters during follow-up; failure was defined as the persistence or reactivation of signs and symptoms after

treatment, with a positive myelogram or splenic aspirate; relapse was defined as the reappearance of parasites after their initial disappearance.

Statistical analysis

The results were compared between groups by analysis of variance and by the nonparametric Mann-Whitney and Wilcoxon tests[10, 11]. The efficacy of treatment was compared between groups based on survival analysis using generalizations of the Gehan model and the Wilcoxon test, with the level of significance set at 0.05.

Results

In the sample studied, 63.3% of the patients were males, with an equal proportion of whites and non-whites, and 66% ranged in age from 3 to 5 years. With respect to origin, 53% of the patients were from the municipality of São Luis, capital of the State of Maranhão - Brazil, and the other 57% were from the municipalities of São José de Ribamar and Paço do Lumiar, both belonging to the São Luis island (Table 1). Most children in the three groups presented previous or long-term malnutrition: cohort 1) 70% malnourished children (20% mild, 30% moderate, 20% severe), cohort 2) 90% (40% mild, 40% moderate, 10% severe), and cohort 3) 70% (30% moderate, 40% severe) (Table 2). Statistical analysis revealed no difference in the intensity of malnutrition between the groups studied (p = 0.607), or between patients who relapsed after treatment and those who did not (p = 0.619).

Upon inclusion in the study, all patients presented a history of fever and clinical signs of adenomegaly (order of frequency: inguinal, submandibular, cervical and axillary), hepatomegaly (mean of 6.16 cm in cohort, 6.12 cm cohort 2, and 7.10 cm in cohort 3) and splenomegaly (mean spleen size of 9.28 cm in cohort 1, 8.86 cm cohort 2, and 11.51 cm in cohort 3 (Table 2). The mean time between admission and treatment was practically the same for all three cohorts: 6.3 days for cohort 1, 5.4 days for cohort 2, and 5.3 days for cohort 3. No significant differences in the hematological parameters were observed between the three cohorts at the time of inclusion: mean hemoglobin of 8 g/dL, hematocrit of 25%, a leukocyte count below 4000 was only observed for cohort 3, and the mean platelet count ranged from 139,280 in cohort 2 to 180,900 in cohort 3 (Table 2). A splenic aspirate for the diagnosis of the parasite was obtained from three patients (two of group 1 and one of group 3), since no *Leishmania* amastigote forms were detected in the bone marrow aspirates of these patients.

Regarding the therapeutic response, regression of fever was observed in all patients of cohort 1 as confirmed by assessment on day 24. Fever persisted up to day 60 in cohort 2 and up to the last assessment (day 180) in cohort 3. However, these differences were not significant (p= 0.703).

One of the complications observed during the evolution of the patients was pneumonia: in cohort 1, one child on day 10 of treatment; in cohort 2, one child on day 5 and another on day 24 of follow-up; in cohort 3, two patients at the time of hospitalization, with the symptom persisting up to days 60 and 180 in one of the cases.

At the end of follow-up, complete regression of the liver was observed in 4 (40%) patients of cohort 1, 7 (70%) of cohort 2, and 4 (40%) of cohort 3. With respect to the spleen, in cohort 1 an increase in spleen size compared to the measurement at the beginning of treatment was observed in one patient while 5 patients no longer showed splenomegaly. The spleen was nonpalpable in 8 patients of cohort 2 and in 3

Table 1. Sociodemographic characteristics of the 30 patients with American Visceral Leishmaniasis (AVL) included in the study.

Characteristic	**Cohort 1 (N = 10)**	**Cohort 2 (N = 10)**	**Cohort 3 (N = 10)**
Age (years)	4.8 ± 1.03	4.7 ± 1.57	5.4 ± 2.72
Gender			
Male	0.5	0.7	0.7
Female	0.5	0.3	0.3
Race			
White	0.5	0.4	0.4
Mulatto	0.4	0.3	0.6
Black	0.1	0.3	0
Origin·			
São Luis	0.6	0.5	0.5
São José de Ribamar	0.2	0.1	0.3
Paço do Lumiar	0.2	0.4	0.2
Time of permanence in the area (years)	7.08 ± 6.15	3.80 ± 3.11	4.60 ± 3.94
Family income (minimum wage)			
0-1	0.4	0.3	0.5
1-2	0.4	0.5	0.3
> 2	0.2	0.2	0.2

São Luis, Island – Maranhão state, Brazil.

Table 2. Clinical-laboratory parameters of the 30 patients with AVL at the time of inclusion in the clinical trial with liposomal amphotericin B (Ambisome®).

Clinical parameter	Cohort 1	Cohort 2	Cohort 3
Age (years)	4.8 ± 1.03	4.7 ± 1.57	5.4 ± 2.72
Weight (kg)	15.44 ± 1.81	15.17 ± 2.41	16.89 ± 5.23
Height (cm)	1001.00 ± 4.66	100.00 ± 8.72	100.00 ± 14.29
Hepatomegaly* (cm RCM)	6.16 ± 1.94	6.12 ± 2.41	7.10 ± 1.10
Splenomegaly* (cm LCM)	9.28 ± 2.39	8.86 ± 2.17	11.51 ± 2.88
Fever	0.5	0.5	0.2
Malnutrition			
Mild	0.2	0.4	0
Moderate	0.3	0.4	0.3
Severe	0.2	0.1	0.4
Adenomegaly	1	1	1
Skin-mucosal pallor	1	1	1
Edema	0	0.1	0.4
Time between admission/treatment (days)	6.3 ± 2.75	5.4 ± 3.81	5.33 ± 3.71
Anorexia	0.5	0.8	0.4
Diarrhea	0	0.2	0
Cough	0.4	0.6	0.7
Vomiting	0.1	0.2	0
Abdominal pain	0.2	0.1	0.2
Hair loss	0.2	0	0
Bleeding (epistaxis)	0	0.3	0
Bronchopneumonia	0	0	0.2
Laboratory parameters			
Hemoglobin (g/dL)	8.52 ± 1.32	8.07 ± 1.54	7.8 ± 0.68
Hematocrit (%)	25.31 ± 3.55	24.35 ± 4.14	24.16 ± 2.28
Total leukocytes (10^3/mm^3)	4030 ± 1374	4490 ± 1522	3530 ± 1613
Polymorphonuclear cells (%)	0.27 ± 0.12	0.35 ± 0.11	0.45 ± 0.14
Lymphocytes (%)	0.70 ± 0.11	0.63 ± 0.11	0.48 ± 0.13
Platelets (10^3/mm^3)	153,319 ± 88,001	139,280 ± 71,477	180,900 ± 111,623
Albumin (g%)	2.85 ± 0.63	3.13 ± 0.84	2.58 ± 0.64
Globulin (g%)	4.40 ± 1.62	4.06 ± 1.34	4.39 ± 1.36
Prothrombin activity (%)	83 ± 12	86 ± 13	85 ± 15
AST (IU/mL)	30.56 ± 22.31	35.20 ± 20.92	36.40 ± 18.47
ALT (IU/mL)	19.11 ± 14.84	28.00 ± 22.21	19.00 ± 15.30
Creatinine (mg/dL)	0.48 ± 0.2	0.53 ± 0.31	0.50 ± 0.12
Potassium (mEq/L)	3.20 ± 0.44	3.46 ± 0.50	3.40 ± 0.51

* RCM = right costal margin; LCM = left costal margin.

of cohort 3. The other patients showed a marked reduction in spleen size, with a clear reduction of visceromegaly even being observed for patient 26 (cohort 3) who died on day 41 of follow-up due to others cases. Therefore, 60 days after treatment the spleen had regressed on average 73.2% in cohort 1, 87.2% cohort 2, and 78.1% cohort 3, with no significant difference in the reduction of splenomegaly being observed between the three groups ($p = 0.619$).

Patient 11 of cohort 2, presented an increase in arterial pressure (not exceeding the maximum limits), extrasystole, generalized edema, oliguria, elevated urea and creatinine levels, low potassium, and tachycardia upon administration of the 4th dose of the drug and was switched to cohort 2, receiving the 5th dose on day 10 of hospitalization when marked improvement of the symptoms was observed.

The complications observed during treatment and follow-up were practically the same for the three cohorts studied: pneumonia and diarrhea were observed in all three groups, impetigo and bleeding in cohorts 1 and 2, miliaria in cohort 1, varicella in cohort 3, and gastroenteritis in a patient of cohort 2.

With respect to adverse effects of Ambisome®, we may emphasize fever which was observed in patients of cohorts 1 and 2 but not of cohort 3. Dyspnea, cough and chest pain were noted during infusion in patients of cohorts 1 and 3. At the end of follow-up (180 days), recurrence was observed in 2

Table 3. Clinical and laboratory evolution of the patients with VL included in the study after the end of treatment.

	Day 1	Day 24	Day 60	Day 180
Fever				
Cohort 1	0.5	0	0	0
Cohort 2	0.5	0	0	0
Cohort 3	0.2	0.1	0.2	0.2
Pneumonia				
Cohort 1	0	0	0	0
Cohort 2	0	0.1	0	0
Cohort 3	0.2	0	0.1	0.1
Adenomegaly				
Cohort 1	1	0.9	0.7	0.6
Cohort 2	1	0.9	0.8	1
Cohort 3	1	0.9	1	1
Weight				
Cohort 1	15.44 ± 1.81	15.90 ± 2.14	16.80 ± 2.59	17.20 ± 2.14
Cohort 2	15.17 ± 2.41	15.48 ± 2.48	15.55 ± 2.86	17.05 ± 2.62
Cohort 3	16.89 ± 5.23	17.53 ± 5.10	16.46 ± 7.50	16.93 ± 7.59
Hepatomegaly				
Cohort 1	6.18 ± 1.94	4.71 ± 1.44	2.91 ± 2.09	2.42 ± 2.44
Cohort 2	6.12 ± 2.41	4.12 ± 2.20	2.48 ± 2.30	1.04 ± 1.68
Cohort 3	7.10 ± 1.10	3.72 ± 2.57	2.06 ± 3.47	0.75 ± 1.03
Splenomegaly				
Cohort 1	9.28 ± 2.39	4.86 ± 2.12	3.28 ± 3.27	1.87 ± 2.59
Cohort 2	8.86 ± 2.17	4.24 ± 2.68	1.50 ± 3.37	0.94 ± 2.06
Cohort 3	11.51 ± 2.88	6.75 ± 4.24	3.13 ± 3.94	1.80 ± 1.87
Hemoglobin				
Cohort 1	8.52 ± 1.32	6.57 ± 5.72	8.05 ± 5.58	11.00 ± 1.39
Cohort 2	8.07 ± 1.54	7.37 ± 5.15	9.76 ± 3.74	11.40 ± 0.92
Cohort 3	7.80 ± 0.68	8.23 ± 4.46	8.52 ± 4.60	9.36 ± 3.50
Leukocytes				
Cohort 1	4030 ± 1374	6340 ± 4306	7170 ± 3870	7850 ± 3712
Cohort 2	4490 ± 1522	7330 ± 3794	10,180 ± 5419	10,520 ± 3405
Cohort 3	3530 ± 1613	6940 ± 2904	7650 ± 3565	8740 ± 3442
Platelets				
Cohort 1	153,319 ± 88,001	173,000 ± 154,000	214,000 ± 214,000	258,000 ± 110,000
Cohort 2	139,280 ± 71,477	278,000 ± 125,000	349,000 ± 110,000	297,000 ± 75,000
Cohort 3	180,900 ± 11,623	253,200 ± 75,000	180,600 ± 92,000	219,800 ± 141,000
Albumin				
Cohort 1	2.85 ± 0.63	2.72 ± 1.55	4.08 ± 1.58	4.31 ± 2.71
Cohort 2	3.13 ± 0.84	3.57 ± 1.34	4.39 ± 2.11	4.07 ± 1.04
Cohort 3	2.58 ± 0.64	3.84 ± 0.82	3.70 ± 1.48	3.25 ± 1.43
Globulin				
Cohort 1	4.40 ± 1.62	3.35 ± 2.24	2.43 ± 1.55	5.07 ± 3.54
Cohort 2	4.06 ± 1.34	2.76 ± 1.63	3.10 ± 2.00	3.35 ± 3.01
Cohort 3	4.39 ± 1.36	4.86 ± 1.77	3.20 ± 1.54	2.31 ± 1.45

(20%) patients of cohort 1, 1 (10%) patient of cohort 2, and 2 (20%) patients of cohort 3, i.e., 16.6% of the patients treated in the three cohorts showed recurrence of the picture of kala-azar and required Sb^{5+}- based therapy for the conclusion of treatment. The efficacy of treatment is shown in Table 3. Survival analysis showed no difference in the time of survival between the three groups (Table 4).

Discussion

AVL is an infectious disease that mainly affects children of low socioeconomic level and compromised nutritional status, a fact observed in the patients studied here. The signs and symptoms commonly described in the disease such as fever, adenomegaly, pallor, prostration, weight loss and anorexia were observed in most patients, as also reported by

Table 4. Analysis of the time of survival in the three groups of AVL studied.

Survival analysis	**Variables: Time of survival for the three groups** **Censored variables: censored cases are marked with +** **X^2 = 1.06442 *P* = 0.58731**				
Survival (days)	**Group**	**Score**	**Sum of scores**	**Percent censored**	**Sample size**
59.000	1	-26.0000	-28.0000	0.00000	10
59.000	1	-26.0000			
181.00	1	-18.0000			
59.000	2	-26.0000	28.0000	0.00000	10
59.000+	3	4.0000	0.0000	10.00000	10
151.000	3	-20.0000			
59.000	3	-26.0000			

other investigators[3, 28]. Evolutive complications of the disease such as jaundice and cachexia were not detected in the present patients because these conditions are indicators of a late phase of the disease and are generally associated with more severe pictures of longer duration, a fact that does not apply to the present study because of the exclusion criteria adopted here[13, 33, 34].

According to Seaman *et al*.[25], the fever observed during the use of liposomal amphotericin B (Ambisome®) in our patients might have been due to the ability of the drug to release IL-1 and TNF-α from monocytes, suggesting a pyrogenic mechanism. Fever, chills and moderate headache have also been reported by Dietze *et al*.[9], who used another formulation of liposomal amphotericin B (Amphocil®) at a total dose of 14 to 20 mg/kg in Brazilian patients with kala-azar.

The most important complication observed in the present patients was pneumonia, as also reported in the study of Dietze *et al*.[9]. Other complications such as varicella, impetigo, intestinal infection, miliaria and diarrhea were also noted. These complications were attributed to the compromised immune status of the patients not only as a result of the disease but also due to malnutrition[2]. Bleeding (epistaxis) was observed in 3 (10%) of our patients, a complication that occurs during the evolution of the disease[2, 9]. However, no correlation was observed between a larger number of complications and recurrence.

The cost of one Ambisome® ampulla is high for the Brazilian population a fact that renders the treatment with this drug too expensive and its indication should therefore be carefully evaluated. However, this high cost may not be true if more adequate criteria for the evaluation of the cost of hospitalization are used[8, 9, 29]. Thus, a cost-benefit analysis should be included in an eventual comparative study between Ambisomeâ and pentavalent antimonial[6, 18, 29].

In contrast to Ringden *et al*.[21], who had to interrupt the use of Ambisome® in 6 (3%) of their 197 patients, treatment was well tolerated in the present investigation, with no need for discontinuation due to marked toxic or undesired effects. The only exception was a change in the programmed total dose for patient 11 of cohort 1 in order to reduce the drug dose and, consequently, the adverse effects observed in this patient such as a discrete elevation in arterial pressure, extrasystole, edema exacerbation, oliguria, tachycardia and hypopotassemia. Other side effects included fever in patients of cohorts 1 and 2, dyspnea and cough in patient of cohort 1, and chest pain in cohort 3.

Despite reports of a sudden onset of tachypnea, tachycardia and hypertension, accompanied by acute metabolic acidosis and congestion of pulmonary vessels, seen immediately after infusion of liposomal amphotericin B[6, 9], no cardiorespiratory or electrocardiographic changes were observed in the present study. The low toxicity of Ambisome® is partly due to the formation of a lipophilic complex between distearoylphosphatidylglycerol and amphotericin B[17].

Sampaio *et al*.[23] reported problems with the use of Ambisome® in their series when performing campimetry at the end of treatment, with one patient showing restriction of the visual field, a finding not observed in the present study or in other investigations[6, 21, 25].

Ambisome®, at the total doses of 14, 10 and 6 mg/kg, was found to be relatively effective in the treatment of kala-azar, showing rates of cure of 70% in cohort 1, 90% in cohort 2 and 80% in cohort 3. No significant difference in terms of toxicity, tolerability or efficacy was observed between the three therapeutic regimens tested. Tolerability was considered to be good, with only discrete adverse effects that did not require discontinuation of the drug. All patients were asymptomatic after a period of 48 months, except for one who died one year after treatment of AVL due to pulmonary tuberculosis.

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