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LOW-DOSE LIPOSOMAL AMPHOTERICIN B IN REFRACTORY INDIAN VISCERAL LEISHMANIASIS: A MULTICENTER STUDY

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Abstract In this randomized, double-blind, dose-ranging, multicenter trial, 84 patients with visceral leishmaniasis refractory to antimony therapy were administered liposomal amphotericin B (AmBisome) at cumulative doses of 3.75, 7.5, and 15.0 mg/kg for 5 consecutive days. Posttreatment apparent cure and definite cure were assessed at 2 weeks and 6 months after the end of therapy, respectively. Mild to moderate infusion-related fever and rigors were seen in 29 and 44% of patients, respectively. One patient each in the 3.75- and 7.5-mg groups had detectable parasites on splenic smear at posttreatment evaluation. At 6 months' follow-up, however, 2, 1, and 1 patients relapsed in the 3.75-, 7.5-, and 15.0-mg groups, resulting in definite cure rates of 89, 93, and 97%, respectively. There was no significant difference in the cure rates of the 3 groups. Low-dose liposomal amphotericin B given for 5 days can cure most patients with Indian kala-azar

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1. INTRODUCTION

Visceral leishmaniasis (VL), also known as kala-azar, affects a large population (12 million) in Bihar and neighboring states in India, with an estimated annual incidence of 100,000–250,000 cases.1 If untreated, the disease is almost always fatal. Sodium stibogluconate (Sbv) is the first-line drug for its treatment. For the last 2 decades, there has been a steady decline in the response to Sbv ; 37–64% of patients currently fail to be cured by antimony treatment.2–3 Alternative drugs are pentamidine and amphotericin B, but both are toxic. Declining efficacy of pentamidine4 leaves only amphotericin B for these Sbv -refractory patients. Administration of amphotericin B, which is given on alternate days for 1 month or more, is associated with infusion-related chills, rigors, and fever and with cardiac and renal toxicity.5,6 In order to alleviate the side effects, as well as to improve efficacy by targeted delivery, lipid-associated amphotericin B has been used in patients with refractory VL in India and elsewhere.7–14 The high cost of lipid formulations of amphotericin B puts them beyond the reach of most patients in developing countries. In this multicenter,

dose-ranging study, low-dose liposomal amphotericin B (AmBisome, Gilead Sciences, Inc., Foster City, CA) was used for 5 days in an effort to find an affordable and effective regimen for patients refractory to initial Sbv treatment. The aim of the trial was to determine the lowest dose of liposomal amphotericin B able to cure at least 90% of the patient population

2. PATIENTS AND METHODS

Eighty-four patients enrolled in this trial were randomized to receive the following regimens of liposomal amphotericin B infusions: 1) Group A, 0.75 mg/kg per day for 5 consecutive days (cumulative dose, 3.75 mg/kg); 2) Group B, 1.5 mg/kg per day for 5 consecutive days (cumulative dose, 7.5 mg/kg); and 3) Group C, 3.0 mg/kg per day for 5 consecutive days (cumulative dose, 15 mg/kg). Four centers participated, and recruitment of 21 patients (7 per group) at each center was planned. The ethical committees of the Institute of Medical Sciences, Banaras Hindu University; the Kala-azar Research Center, Muzaffarpur; Balaji Uthan Sansthan, Patna; and the Kala-azar Research Center, Darbhanga, approved the protocol, and the trial was conducted as per Good Clinical Practices (ICH E6) and the Declaration of Helsinki. Written informed consent from patients or legal guardians of minors were obtained before the inclusion in the trial, which included consent for the pre- and posttreatment splenic/bone marrow aspirates and testing for human immunodeficiency virus (HIV). Patients of any age or sex were eligible for enrollment into the trial if they had signs and symptoms of VL confirmed by the presence of parasites in splenic or marrow smears if they had failed to respond or VL relapsed after a full course of Sbv treatment. Pregnant or lactating women, HIV-positive patients, and intravenous drug abusers were excluded from the trial. Patients were randomized into preassigned treatment groups by the sealed-envelope technique, and liposomal amphotericin B was administered by an independent coinvestigator who broke the seal of the envelope and prepared infusions. The drug was dissolved in 50–100 mL of 5% dextrose solution and administered for 30–60 min. No premedication was used. Patients, principal investigators, and other workers were blinded to the dose used. Codes were broken after the end of the study, after the completion of 6 months of follow-up for all the patients. Clinical examination and laboratory investigations were performed before treatment, immediately after treatment (Day 5), and 2 weeks after the end of treatment (Day 19). Splenic or marrow smears for parasitological examination were performed before the start of treatment and 2 weeks after the end of therapy (Day 19).

1. Apparent cure.

Evaluation of apparent cure was performed 2 weeks after the end of treatment (Day 19), which was defined as resolution of fever, regression of splenomegaly, and absence of parasites in splenic or marrow smear. Once apparent cure had been established, patients were followed for 6 months. If symptoms recurred within this period, splenic or marrow smears were examined for relapse of disease, and patients with documented relapse were given highdose (25 mg/kg) liposomal amphotericin B as rescue treatment.

2. Definite cure.

After posttreatment apparent cure, patients were followed for at least 6 months for evaluation of definite cure. Patients were designated as definitely

cured if there was absence of signs and symptoms of VL after 6 months of follow-up.

TABLE 1
Clinical and laboratory values of the 3 groups of patients treated with a amphotericin B†

Group (cumulative dose)	Group A (3.75 mg/kg)		Group B (7.5 mg/kg)		Group C (15 mg/kg)	
	Pretreatment	Posttreatment	Pretreatment	Posttreatment	Pretreatment	Posttrea
Age, year (range)	12 ± 1.74* (8–16)		24 ± 1.15 (18–23)		23 ± 3.0 (17–29)	
Male (%)	60		60		53	
Weight (kg)	25 ± 0.59**	27 ± 0.6	34 ± 0.5	34 ± 0.5	32 ± 0.6	33 ±
Spleen size (cm)	9.1 ± 0.9	2.9 ± 2.5	9.3 ± 0.9	2.6 ± 0.5	9.5 ± 0.9	3.1 ±
Hemoglobin (g/dL)	7.6 ± 0.4	9.8 ± 0.3	7.3 ± 0.3	9.9 ± 0.3	7.7 ± 0.4	9.7 ±
Platelets (×10³/μL)	154 ± 13	275 ± 19	165 ± 23	244 ± 19	142 ± 8	243 ±
Serum creatinine (μmol/L)	0.75 ± 0.05	0.66 ± 0.03	0.75 ± 0.04	0.77 ± 0.04	0.82 ± 0.05	0.80 ±
Alanine aminotransferase (IU/L)	24 ± 4	20 ± 1	31 ± 8	24 ± 2	24 ± 2	22 ±

* $P = 0.002$.
** $P = 0.043$.
† All values are expressed as mean ± standard error of mean. Pretreatment = day 0; posttreatment = Day 19.

3. Statistical analysis.

Comparison of the trial groups was performed by the Kruskall-Wallis test for ordinal/continuous data (and the chi-square test for nominal data). The Conchran Armitage trend test was used to evaluate dose response. Fisher's exact test was used to compare groups by means of pairwise comparison with 95% confidence intervals. The Wilcoxon matched-pair signed-rank test was used to evaluate changes from baseline. The sample size calculation was based on a complete cure rate hypothesis of 90% for the highest dose and 50% for the lowest dose. To detect a difference of 40% in cure rates with a power of 80% and a significance level of 5% in a 2-tailed approach, 25 patients per treatment arm were required.

3. RESULT

Eighty-four patients were treated with this protocol, 28 patients in each of the 3 groups. Seventy-eight (93%) of 84 patients were unresponsive to their initial sodium stibogluconate treatment; the remaining 6 patients (7%) relapsed after initial cure. Clinical and laboratory characteristics of the patients are provided in Table 1. Although randomization and treatment was blinded, Group A was significantly younger, which contributed to the lower mean weight (P 0.043) of this group. Otherwise no significant difference in other clinical or laboratory parameters between groups was seen. At posttreatment evaluation on Day 19, a decrease in spleen size and rise in hemoglobin, white blood cell count, and platelets were seen. Compared with baseline values, changes in these parameters were similar among the 3. groups and was not drug-dose dependent (Table 1). One patient each in Groups A and B had detectable parasites in splenic smears; these patients were considered to have failed to respond to primary treatment. One patient in Group C failed to return for the Day 19 follow-up. Thus, in the initial short-term, posttreatment evaluation, 27 patients (96%) each were free of parasites and were considered apparently cured. Patients were followed up for 180 days. Two patients in Group A, one in Group B, and one in Group C relapsed with recurrence of fever, splenic enlargement, and presence of parasites in the splenic/marrow smears. The missing patient of Group C returned for a final evaluation at Day 180 and was found to be free of disease. Nonresponding patients as well as those who relapsed during the follow-up period were given a high dose of amphotericin B (25 mg/kg cumulative dose) as rescue medication and were cured successfully. None of the patients died during the study.

TABLE 2
Response to amphotericin B therapy in Indian visceral leishmaniasis

Cumulative dose	3.75 mg/kg, *n* (%)	7.5 mg/kg, *n* (%)	15 mg/kg, *n* (%)
Total number of patients	28	28	28
Apparent cure (at Day 19)	27 (96)	27 (96)	27* (96)
Initial failure (at Day 19)	1 (4)	1 (4)	–
Relapse	2 (7)	1 (4)	1 (4)
Definite cure (at Day 180)	25 (89)	26 (93)	27 (96)

* One patient did not come back for apparent cure evaluation; however, at 6 months, he was available for final evaluation.

With a cumulative dose of 3.75 mg/kg (Group A), 7.5 mg/ kg (Group B), and 15 mg/kg (Group C) of liposomal amphotericin B, long-term (definite) cure occurred in 89, 93, and 96% patients, respectively.

No significant difference was seen between the cure rates of the 3 groups (Table 2). Overall, liposomal amphotericin B was well tolerated. The most frequent side effects were observed during infusion. Forty-six episodes of infusion-related rigors were experienced by 37 patients (96%); 91% of the episodes were of mild intensity. Thirty patients (36%) experienced rigor only once. Similarly, 49 episodes of fever were recorded in 25 patients (30%), occurring only once in 13 of the 84 patients included in the trial. Elevation of temperature was considered mild in 34 of 49 of the episodes, and 11 episodes were of moderate intensity. Eight patients (30%) experienced back or lumbosacral pain, which in 2 patients was of severe intensity. This occurred in one patient each of Groups B and C. Seven patients (8%) had one episode of vomiting during the treatment period. There was no significant change in renal or hepatic biochemistry, although in 7 patients (8%), there was grade I rise (up to 1.26–2.5 times of upper limit of normal value) in serum creatinine, which had returned to normal by the posttreatment evaluation on Day 19. No hepatic or bone marrow toxicity was detected. Overall, the incidence of adverse reactions was similar among the 3 treatment groups. No serious adverse events related to this formulation of liposomal amphotericin B were recorded.

4. Discusion

The liposomal amphotericin B preparation was effective in curing antimony-unresponsive patients in all 3 dosage groups. In India, conventional amphotericin B has been shown to be remarkably effective at low doses in the treatment of VL. The lowest reported effective cumulative dose of conventional amphotericin B has been 7 mg/kg, with a cure rate of 98%.15 In one study, 84% of the patients, who were treated with a cumulative dose of 5 mg/kg of amphotericin B lipid complex (Abelcet, Liposome Co., Princeton, NJ), were cured of Indian VL.8 Liposomal amphotericin B is approved in several European countries for primary treatment of VL, and the U.S. Food and Drug Administration approved it recently, recommending a total dose of 21 mg/kg16; however, in a pilot trial in Indian VL, liposomal amphotericin B in cumulative doses of 6, 10, and 14 mg/kg cured all 10, 9, and 10 patients, respectively.11 The cumulative dose of 3.75 mg/kg of amphotericin B is by far the lowest total dose of amphotericin B used for the treatment of VL. Nevertheless, even at this low dose, 96% of patients were cured initially, a rate similar to those treated with 7.5 and 15 mg/kg. Given that 2 patients in the 3.75 mg/ kg group and one each in 7.5 and 15 mg/kg group relapsed, a definite cure rate of 89, 93, and 97% was achieved with these 3 groups, respectively. The differences in these cure rates were not statistically significant because the sample size was too small to measure a small effect. Although infusion-related reactions were measured, they were milder than those usually seen with conventional amphotericin B treatment. Although clinical availability of liposomal amphotericin B is a major therapeutic advance in the treatment of leishmaniasis, it may remain out of reach of most patients with VL because of its higher cost. The low-dose regimens evaluated here not only reduce the cost by one third to one sixth of the currently recommended doses, but the shortened duration of treatment reduces the usual hospital stay from 5 weeks or more to just 5 days. The shortened hospital stay will further reduce the hospital cost and the person-days lost for both patients and attendants, and it will increase the availability of hospital beds 6- to 7-fold. Although hospital and other associated costs in India are not high, they can partially offset the higher cost of the drug. The decreased number of infusions from 15 or more to 5 reduces the costs of fluids, tubing, and other hospital supplies. Of course,

patients who either fail to respond to treatment or who relapse after initial cure have to be retreated, and while calculating the cost of therapy, we took into account the cost of retreating this subset of patients. Safety of antileishmanial drugs is a major concern in the treatment of VL, as all available antileishmanial drugs result frequently in drug-induced toxicity. Conventional amphotericin B is frequently associated with moderate to severe infusion-related reactions. Premedications such as antipyretics, antihistamines, and hydrocortisone are often used. Thrombophlebitis and sudden death due to drug-induced myocarditis are other serious problems encountered with conventional amphotericin B.5,6 In this trial, the incidence of adverse reactions was low, and they were minor. In some patients, a mild but reversible rise in serum creatinine was observed. Infusion-induced reactions of a milder nature were only seen in one third of the patients, and these did not require any intervention. Infusion-related side effects with amphotericin B occurred less frequently compared with amphotericin B lipid complex.7 This trial supports other reports in the conclusion that that liposomal amphotericin B can be used at low doses in a short course without compromising its efficacy.17 Although no statistically significant differences where measured between the 3 treatment groups, the lowest effective cumulative dose reaching the definite cure rate level of 90% is 7.5 mg/kg. Liposomal amphotericin B is safe and well tolerated. However, if it is to be used in epidemics in Bihar or elsewhere in the world on a large scale, purchase of this drug needs to be available commensurate with resources of the health care system.

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