MAJOR ARTICLE

Single-Dose Liposomal Amphotericin B in the Treatment of Visceral Leishmaniasis in India: A Multicenter Study

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Widespread antimony resistance renders conventional amphotericin B the only option for the treatment of visceral leishmaniasis (VL) in North Bihar, India. Because of its excellent safety profile, a large dose (7.5 mg/kg) of liposomal amphotericin B (L-AmB) was given to each of 203 patients with VL at 4 treatment centers, and the patients were discharged the next day. At initial clinical and parasitological follow-up, performed on day 30 after treatment, evidence of a cure was seen in 195 (96%) of 203 patients (95% CI, 92–98); 4 patients experienced treatment failure. Two patients were lost to follow-up, 2 died (one due to progressive disease and another, 5 months after treatment, due to an unrelated illness), and 12 experienced relapses during follow-up. Thus, 183 patients (90%; 95% CI, 85–94) had obtained final cure 6 months after treatment. Very few adverse events (fever with rigor, in 9.8% of patients) were seen. Single-dose L-AmB (7.5 mg/kg) treatment is safe and effective, and it may be used for the mass treatment of VL in India.

Until recently, conventional therapy for visceral leishmaniasis (VL; also known as kala-azar), a disseminated protozoal infection, consisted of pentavalent antimony given once daily by intravenous or intramuscular injection for 28 days [1]. Since 1990, however, the failure of large-scale antimony treatment in the state of Bihar, India [2], where 40%–50% of the world's cases of VL occur [3], led to the reintroduction of amphotericin B deoxycholate (AmB) as a remarkably active antileishmanial agent [4]. Long-term cure rates of ~97% are routinely induced by intravenous AmB infusions, administered either daily or, more typically, every other day (15–20 infusions of 0.75–1 mg/kg) [5, 6]. However, prolonged courses of treatment, in addition to increasing the hospital burden and cost, often lead to noncompliance or abandonment of the regimen because of associated adverse events. Lipid formulations of amphotericin B have been shown to have high-level efficacy against VL [7, 8], including ⩾90% cure rates when given for just 5–10 days [9, 10], thus making short-course therapy a possibility [10].

We have shown that short-course (5 day) treatment with liposomal amphotericin B (L-AmB; AmBisome, Gilead Sciences), administered daily in infusions of as low as 0.75 or 1.5 mg/kg (total doses, 3.75 mg/kg or 7.5 mg/kg), cured 25 (89%) of 28 patients and 26 (93%) of 28 patients, respectively [11]. In another, follow-up pilot trial, a single total-dose infusion of 5 mg/kg of L-AmB cured 91% of patients [12]. To extend the testing of this clinically appealing, novel approach, we carried out a large, multicenter confirmatory study among children and adults to address 3 interrelated questions: is single-infusion L-AmB therapy effective in cases of VL in India, is an infusion of 7.5 mg/kg well tolerated by patients and a more appropriate dose to use than 5 mg/kg, and, because no additional infusions were

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scheduled, could patients be safely discharged 1 day after treatment? On the basis of the results of our previous multicenter study, which used a 7.5 mg total dose of L-AmB [11], a cure rate of 90% was considered the minimum acceptable for VL in India.

PATIENTS AND METHODS

Patients. In this open-label, noncomparative study involving 4 VL treatment trial centers (2 at Muzaffarpur and 1 each at Patna and Darbhanga) in the state of Bihar, India, a total of 203 patients participated. Patients of all ages and both sexes were potentially eligible if they had symptoms and signs suggestive of VL (i.e., fever with chills, rigor, and splenomegaly) with demonstrable *Leishmania* parasites in splenic or bone marrow aspirate smears. Parasites in splenic smears were graded on a log scale from grade 0 (defined as no parasites per 1000 high-power fields in oil immersion) to grade 6+ (defined as >100 parasites/high-power field) [13]. For consistency, a central laboratory was responsible for all laboratory investigations, and splenic or bone marrow aspirate smears were each read by the same observer in a blinded fashion. Pregnant and lactating women, HIV-positive individuals, and individuals receiving concomitant antileishmanial drugs were excluded from the study. Patients were considered to have prior treatment failure if they had received adequate antileishmanial treatment, consisting of either sodium antimony gluconate (SAG; also known as sodium stibogluconate) at a dosage of 20 mg/kg for 30 days or 15 infusions of AmB administered at a dosage of $\geq$0.75 mg/kg.

Study protocol. This study followed the International Conference on Harmonisation (ICH) Good Clinical Practice guidelines [14] in full compliance with the principles of the Declaration of Helsinki (as amended in Tokyo, Hong Kong, and Somerset West, South Africa). The protocol was reviewed by the institutional review boards of the authors' 4 research centers.

After written informed consent was obtained from the study participants, routine screening tests, which included HIV and pregnancy tests, were done. Routine hematological and biochemical examinations, which comprised tests measuring serum albumin, creatinine, electrolyte, alanine and aspartate aminotransferase, bilirubin, and blood urea nitrogen levels, were also performed. For parasitological assessment, splenic aspirate smear examination was done for all but 27 patients (13%) for whom bone marrow aspirate smears were performed in order to obtain a baseline value and 129 patients (65%) for whom bone marrow smears were done for initial posttreatment evaluation. Eligible patients, after enrollment by a central registry of sponsors based in Paris, France, were administered 7.5 mg/kg of L-AmB dissolved in 5% dextrose solution. The total dose was infused over a period of 1 h. Patients were observed during infusion and for a further 24 h for any adverse events. If their clinical conditions so warranted, patients were kept in the hospital for longer periods. Adverse events and laboratory values were graded according to the World Health Organization (WHO) toxicity coding [15]. Patients were subsequently discharged, and, on day 30 after treatment, they were contacted for posttreatment evaluation to determine whether an initial cure had been achieved. The posttreatment evaluation at day 30 consisted of a clinical examination, including liver and spleen size–assessment; an evaluation of laboratory (hematological and biochemical) test results; and an additional splenic or bone marrow smear examination for parasitological assessment. Whether patients had attained a final cure was assessed 6 months after treatment (at day 180) by clinical and laboratory examinations. Parasitological assessment was done only if there were features suggestive of a relapse of the disease.

Definition of response. Initial cure was defined as resolution of fever, regression in spleen size, and absence of parasites in splenic or bone marrow aspirate smears. If scanty parasites (grade 1+, defined as 1–10 parasites per 1000 high-power fields in oil immersion) were still detectable in smears of splenic or bone marrow aspirates obtained at day 30 from patients whose clinical findings and hematological test results showed improvement, these patients were reexamined 4 weeks later, and, if the findings of an additional splenic aspirate smear were negative, they were considered to have achieved an initial cure. Treatment was considered to have failed if the patient had either a parasite grade of >1+ at day 30 or had scanty parasites (grade 1+) at day 30 but detectable organisms at reevaluation, 4 weeks later. Patients were advised to report if and when signs and symptoms of VL relapse were suspected, in which case an additional splenic aspiration was done for confirmation of relapse.

Final cure was defined as negative findings (parasite grade 0) of splenic or bone marrow aspirate smears performed on samples obtained at the initial posttreatment evaluation, and the absence of signs and symptoms of VL at follow-up 6 months after treatment. The intent-to-treat population included all patients who received the trial medication. Those patients who either had treatment failure or who experienced a relapse (i.e., experienced the reappearance of signs and symptoms of VL after achieving an initial apparent cure) were treated with high-dose L-AmB (25 mg/kg administered over 5 days). The sponsor of this study, Gilead Sciences, had no influence on the interpretation of the data, the preparation of the manuscript, or the decision to publish this study.

Statistical analysis. Sample-size calculation was based on the definitive-cure rate of 90%, with a one-sided lower-bound CI of 0.84, a power of 80%, and a significance level of 5%; for this, a minimum of 200 patients were required. The 2-sided Wilcoxon signed rank test was used for comparison of paired

Table 1. Pre- and post-treatment clinical characteristics and laboratory values for 132 male and 71 female patients with visceral leishmaniasis in India.

Variable	Mean value ± SEM		
	Baseline (n = 203)	Day 30[a] (n = 199)	Month 6[a] (n = 183)
Age, years	18.1 ± 1.03[b]	…	…
Weight, kg	28.2 ± 1.01	30.4 ± 1.1	32.78 ± 1.3
Spleen size, cm below costal margin	6.0 ± 0.25	1.29 ± 0.16	0.18 ± 0.04
Serum albumin level, gm/L	30 ± 0.4	40 ± 0.33	42 ± 0.35
Serum creatinine level, μmol/L	74.8 ± 1.8	63.8 ± 1.5[c]	70.9 ± 1.9[c]
Hemoglobin level, g/dL	7.5 ± 1.4	10.5 ± 1.1	10.9 ± 1.7
Platelet count, cells/μL	110,520 ± 4,940	304,090 ± 8,230	204,900 ± 6,060
White blood count, cells/μl	3,960 ± 140	9,550 ± 240	9,190 ± 250

[a] $P < .001$ for change from baseline for all values except as noted.
[b] Range, 2–63 years.
[c] $P = .001$

observations. The Fleiss quadratic method was used for the calculation of CI of proportions.

RESULTS

Patient characteristics. Clinical characteristics and laboratory values for the patients are given in table 1. Of the 203 subjects, 132 of whom were male (median age, 11 years), 103 were children (median age, 7 years; range 2–11 years) and 100 were adults (median age, 30 years; range 12–63 years). Previous antileishmanial treatment had failed for 16 (8%) of the patients (15 had been treated with SAG and 1 had been treated with conventional amphotericin B). Applying an arbitrary cutoff point of a hemoglobin level of <6.0 gm/dL and/or a spleen size of ⩾8 cm below the costal margin, 79 (39%) of the 203 patients were considered to have severe disease. One patient died of progressive disease 3 days after receiving the treatment. Three patients failed to return for posttreatment evaluation on day 30; however, of these, 1 returned for follow-up 6 months after treatment. During the 6-month follow-up period for final cure assessment, 1 patient, who had been showing improvement until 4 months after the initial treatment, developed high-grade fever and breathlessness, and he died 10 days later at his home. Thus, at the end of the study, 199 of 203 patients were available for evaluation.

Efficacy. At initial posttreatment evaluation, performed on day 30 after treatment, all patients had become afebrile, there was a remarkable regression in spleen size (from a mean of 6 cm to a mean of 1.3 cm below the costal margin), and there was significant improvement in clinical findings and hematological test results (table 1). Parasitological evaluation revealed that 192 patients (94.6%) had no detectable parasites in their splenic or bone marrow smears. Seven patients, although they had become afebrile, gained weight, and the size of their spleens had regressed, still had detectable parasites (grade 1+) in their splenic smears. These patients were contacted to undergo an additional clinical and parasitological examination 4 weeks later. On reexamination, no parasites were seen for 3 of these 7 patients, bringing the total number of patients with an initial cure to 195 (96%). The remaining 4 patients still had detectable parasites and were adjudged to have experienced treatment failure, and they therefore received rescue L-AmB treatment.

Twelve patients experienced relapses during the follow-up period, characterized by recurrence of fever, increase in spleen size, and worsening laboratory values. Additional splenic or bone marrow aspirates obtained from these patients demonstrated the presence of parasites. The remaining 183 patients (90%) showed consistent and continued improvement, as measured by clinical characteristics and laboratory values, and they were classified as having achieved a final cure at the 6-month follow-up evaluation (table 2). Of the 15 patients who had been unsuccessfully treated with SAG, 14 (93%) were cured; however, the only patient who had been unsuccessfully treated with AmB experienced failure with this regimen as well.

No statistically significant differences could be identified between the baseline values of the various clinical characteristics and laboratory values for patients whose treatment regimen had failed or who had experienced relapse and those for patients who had attained a final cure (data not shown). Of the 16 patients who experienced treatment failure, 15 were successfully cured by rescue therapy and 1 patient chose to receive treatment elsewhere with conventional AmB.

Adverse events. Treatment with L-AmB was remarkably well-tolerated by both children and adults. A few patients experienced infusion-related fever and rigor (9.8% of patients), chills (3%), vomiting (3.5%), and back ache (1.5%), but in most of these cases the adverse event was mild in severity and

Table 2. Responses among 203 patients to single-dose liposomal amphotericin B (7.5 mg/kg) treatment of visceral leishmaniasis in India.

Outcome	No. (%) of patients	
	Initial evaluation, day 30[a]	Final evaluation, day 180[b]
Cured	195 (96)[c]	183 (90)[d]
Death	1 (0.5)	2 (1)[e]
Primary failure	4 (2)	4 (2)[e]
Relapse	…	12 (6)

[a] Three (1.5%) of the 203 patients in the study group were not present for the initial follow-up evaluation at day 30 after treatment.
[b] Two (1%) of the 203 patients in the study group were not present for the final follow-up evaluation at day 180 after treatment.
[c] 95% CI, 92–98.
[d] 95% CI, 85–94.
[e] The no. of patients shown includes patients whose outcome was determined during initial evaluation at day 30.

did not require any medication. None of these patients showed any evidence of renal insufficiency.

DISCUSSION

The northeastern state of Bihar has emerged as having the most focused concentration of drug-resistant VL in the world. A high proportion of patients experience failure of treatment with pentavalent antimony or pentamidine [2, 16]. Amphotericin B is the only viable treatment option, because the recently-approved oral formulation of miltefosine [5, 17] is yet to become available commercially and is limited by its teratogenic potential.

The long duration of AmB therapy for VL (5–6 weeks) remarkably reduces the availability of hospital beds, and, thus, short-course treatment regimens are sorely needed. Further, treatment with prolonged regimens results in a large proportion of patients not receiving drugs at the dose or for the duration recommended and is one of the important factors responsible for the emergence of drug-resistant strains [18]. With total dose delivery in a single dose, all such possibilities are averted.

This study is one of the few big multicenter clinical trials of treatment for VL and was conducted strictly following ICH guidelines. Both children and adults, irrespective of the severity of illness (39% of patients had severe disease), responded successfully to the single-dose L-AmB treatment. This high-dose infusion of L-AmB was safe and well tolerated by our patient population.

This ultra-short regimen is likely to be extremely popular, as prolonged hospitalization means loss of daily wages, not only for the patient, but also for other members of the patient's family, who might be needed for tasks such as nursing. Because it was possible to discharge these patients after 24 h, use of this regimen also resulted in an ~30-fold increase in the availability of hospital beds. This is highly important in a part of the world like Bihar, India, where, due to paucity of beds, patients await admission for several months, and some even die before being admitted to the hospital. On the basis of our previous experience with single-dose treatment of VL [12, 19], it was considered safe to discharge patients after 24 h of observation. Application of this strategy during severe epidemics of VL will make it possible to treat a large patient population in a very short time.

Even though patients with prior treatment-failure constituted only a small proportion of the participants in this study, all 4 study centers draw patients from regions where maximum SAG resistance occurs [20]. A treatment failure rate of 8% might imply an increase in AmB resistance as a result of using this regimen; however, as discussed above, this has to be viewed against the historical lack of compliance seen with conventional treatments. With the prevailing situation being one of irregular drug-use, the chances of inducing drug resistance are far greater with conventional regimens than with single-dose therapy. For patients who experience initial treatment failure, close follow-

Table 3. Estimated costs for the treatment of visceral leishmaniasis in Bihar, India, by drug regimen.

Expense	SAG, 20 mg/kg q.d. for 30 days	AmB, 1 mg/kg q.o.d. for 30 days	ABLC, 2 mg/kg q.d. for 5 days	L-amB, 5 mg/kg in 1 dose	L-amB, 7.5 mg/kg in 1 dose	Miltefosine, 100 mg q.d. for 28 days
Initial cost per patient, US$						
Drugs	21	58.5	580	390	585	100[a]
Hospitalization	330	368	72	22	22	310
Total	351	426.5	652	412	607	410
Treatment failure,[b] % of patients	60	3	10	10	10	6
Final cost per patient,[c] US$	615	439	696	422	651	454

NOTE. All costs given are for a 30-kg patient. SAG, sodium antimony gluconate; AmB, amphotericin B; ABLC, amphotericin B lipid complex; L-AmB, AmBisome.
[a] Estimated cost (exact cost not available).
[b] All patients who experience treatment failure are retreated with conventional amphotericin B.
[c] Calculated as (initial cost per patient × 100 patients) + (cost of retreating failures)/100.

up and retreatment with either conventional AmB or higher-dose L-AmB of will minimize the rate of ultimate treatment-failure and the emergence of drug resistance.

In contrast to the strains of *Leishmania* responsible for VL in Mediterranean regions, Brazil, and, to some extent, Sudan, which requires a high dose of AmB [7, 21–23], the strains of *L. donovani* responsible for VL in India is remarkably sensitive to low doses of AmB, and even more sensitive to lipid preparations of AmB [8, 12]. Thus, conclusions drawn from this or other studies from India are applicable only to VL occurring in this region.

In the developed world, the cost of treatment may not be such an important issue, and, thus, high-dose L-AmB treatment can be used to obtain higher cure rates (~100%) [21–22]. However, in regions where VL is endemic, affordability is an important factor in deciding on application of a particular regimen. Single-dose treatment not only reduces the hospital cost (though this is not very high in India) to a minimum, but also drastically decreases the wages lost to the family.

When the cost of the study treatment regimen is considered, it is 48% higher than that of treatment with conventional AmB (table 3); however, the increases in safety, ease of administration, and hospital bed availability strongly favor adoption of the single-dose regimen. Further, because cure rates obtained with a dose of 7.5 mg remained similar to those obtained during our previous pilot study with a dose of 5.0 mg [12]—the cost of which is comparable to conventional AmB—an equivalence trial (7.5 mg vs. 5.0 mg) might suggest that the lower dose has efficacy similar to that of the higher dose and might permit wider application of the single-dose regimen.

In regions with high treatment failure rates, SAG treatment is not cost-effective, and the cost of treatment with oral miltefosine is comparable to that of treatment with AmB (table 3). If the price of L-AmB could be brought down through the adoption by the drug manufacturer of a dual-pricing policy for economically deprived regions and through subsidies from the government of India or from the WHO, this highly attractive and useful regimen could be used by the local government of Bihar, India, for a program aimed at elimination of this disease.

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