MAJOR ARTICLE

Amphotericin B Treatment for Indian Visceral Leishmaniasis: Conventional versus Lipid Formulations

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In Bihar, India, where visceral leishmaniasis is hyperendemic, amphotericin B deoxycholate is now first-line parenteral treatment. To test the efficacy of amphotericin B deoxycholate versus that of its lipid formulations, Indian patients were randomized to receive treatment with amphotericin B deoxycholate (1 mg/kg on alternate days for 30 days; $n = 51$), liposomal amphotericin B (2 mg/kg per day for 5 days; $n = 51$), or amphotericin B lipid complex (2 mg/kg per day for 5 days; $n = 51$). Infusion-associated reactions were frequent and persistent in subjects treated with amphotericin B deoxycholate. The illness of 3 patients failed to respond to treatment, and 5 patients experienced relapse. Final cure rates were similar. Estimated total treatment costs for a 25-kg patient—$417 for amphotericin B deoxycholate, $872 for liposomal amphotericin B, and $947 for amphotericin B lipid complex—differed as a result of drug cost. Substantial reductions (~60%) in the price of liposomal amphotericin B and amphotericin B lipid complex would make treatment costs comparable to that of amphotericin B deoxycholate, permitting administration of short-course regimens in India.

Up to one-half of the world's 500,000 annual new cases of visceral leishmaniasis (VL; also known as "kala-azar"), a disseminated intracellular protozoal infection, are thought to occur in India. Ninety percent of Indian cases occur in the northeastern state of Bihar, where infection caused by *Leishmania donovani* is epidemic and conventional pentavalent antimony therapy is no longer effective [1, 2]. Recognition of resistance to antimony led to the rediscovery of amphotericin B deoxycholate as a useful treatment in India in the early 1990s [2–4]. Although oral miltefosine was recently approved for VL in India [5], it is not yet available, and injectable paromomycin is still under development [2]. Therefore, in Bihar, amphotericin B is currently the first-line parenteral treatment for VL [6].

In India, amphotericin B deoxycholate regimens include infusions of 1 mg/kg given either daily for 20 days [7] or, because of concerns about tolerability, on alternate days over a 30-day period [4–6]. Efficacy is high (e.g., long-term cure rates of >95% [3–7]); however, prolonged duration, adverse reactions, and the need to monitor renal function and electrolyte levels remain well-recognized drawbacks of amphotericin B deoxycholate treatment.

Lipid formulations of amphotericin B—liposomal amphotericin B (AmBisome; Gilead Sciences), amphotericin B lipid complex (Abelcet; Enzon Pharmaceuticals), and amphotericin B cholesterol dispersion (Amphotec; InterMune)—have also been successfully applied in VL in India and elsewhere (reviewed in [4] and [6]). High cost, however, has put all 3 preparations beyond the reach of patients in regions in developing countries where VL is endemic [4, 6]. Nevertheless, the lipid formulations have particular clinical appeal in the

Received 17 July 2003; accepted 23 September 2003; electronically published 13 January 2004.

Financial support: The Liposome Company; World Health Organization/World Bank/United Nations Development Programme Special Programme in Research and Training in Tropical Diseases (grant 990106); and Sitaram Memorial Trust, Muzaffarpur, India.

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Clinical Infectious Diseases 2004; 38:377–83

1058-4838/2004/3803-0010$15.00

treatment of VL, because they have largely remedied the drawbacks of conventional amphotericin B deoxycholate. These agents are well tolerated and remarkably efficient therapeutically, resulting in 90%–100% cure rates when administered for as brief a period as 5–7 days or even less [4, 6, 8–13].

Although well accepted [2, 6], the preceding conclusions about amphotericin B deoxycholate and the lipid formulations and their relative merits in the treatment of VL have been drawn exclusively from noncomparative trials in which a single agent was tested. Thus, because neither treatment with amphotericin B deoxycholate versus a lipid formulation nor treatment with one lipid preparation versus another has been studied in VL, we filled in this clinical gap by randomizing Indian subjects to treatment with conventional amphotericin B deoxycholate, liposomal amphotericin B, or amphotericin B lipid complex.

PATIENTS AND METHODS

Eligibility and entry and exclusion criteria. This open-label study, performed between May and July 2001 at the Kala-Azar Medical Research Center (Muzaffarpur, Bihar, India) was approved by the Center's ethical committee. Patients were eligible if they had symptoms and signs of VL (fever, weight loss, and splenomegaly) and if microscopic analysis of a splenic aspirate smear revealed parasites [8, 9]. Patients were excluded if they were pregnant or breast-feeding, were HIV seropositive, or had a serious concurrent infection, such as tuberculosis or bacterial pneumonia. Exclusion criteria also included a granulocyte count of <1000 granulocytes/mm^3, a hemoglobin level of <3.5 g/dL, or a platelet count <40,000 platelets/mm^3; hepatic transaminase or total bilirubin levels that were >3 times the upper limit of normal; a serum creatinine level of >2.0 mg/dL; or a prothrombin time of >5 s above the control time.

Number of patients. A sufficient quantity of liposomal amphotericin B was donated by the manufacturer to treat an estimated 50–55 patients. Therefore, we aimed to enroll 50 patients in each treatment arm. A total of 184 eligible patients were screened; 21 elected not to participate, 10 were excluded by the preceding criteria, and 153 were enrolled (figure 1).

Trial procedures and treatments. Patients completed baseline testing (which included standard hematologic and biochemical profiles, urinalysis, chest radiography, electrocardiography, ELISA for detection of anti-HIV antibody, and a blood smear for detection of malaria parasites) [8, 9] and provided written informed consent (for minors, informed consent was obtained from a parent or guardian). An independent statistician prepared randomization envelopes using a computer-based random number generator. Enrolled subjects were randomly assigned by sealed envelope to receive 1 of the following regimens: 15 infusions of 1 mg/kg of amphotericin B deoxycholate (Fungizone; Sarabhai Chemicals) provided on alternate days after a 1-mg test dose (group A); 5 infusions of 2 mg/kg each of either liposomal amphotericin B (AmBisome) provided on consecutive days (group B); or amphotericin B lipid complex (Abelcet) (group C). In our unit, each of these regimens produces long-term cure rates of ⩾90% [5, 8, 9, 14]. Liposomal

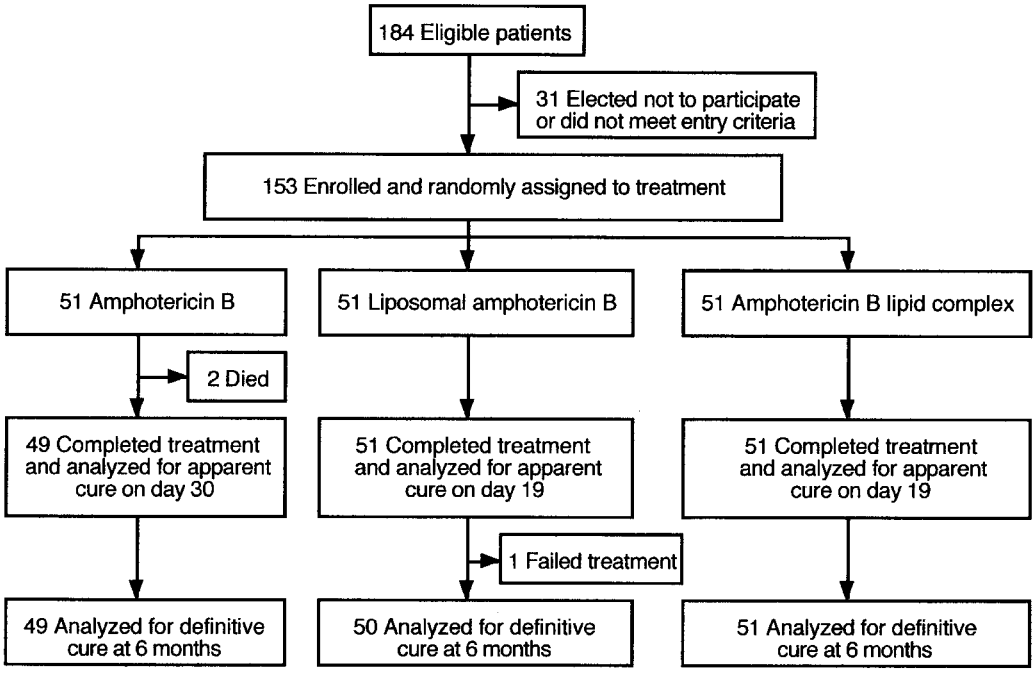

Figure 1. Flow chart illustrating the trial profile

amphotericin B and amphotericin B lipid complex were donated by the manufacturers. The Liposome Company, the manufacturer of amphotericin B lipid complex at the time this study was performed, also provided partial funding used for the purchase of amphotericin B deoxycholate. Neither company had a role in the gathering or interpretation of the data or in deciding whether the study was to be submitted for publication.

Treatment, begun within 3 days after the splenic aspirate was obtained, was administered via a 4-h intravenous infusion. Patients who were treated with amphotericin B deoxycholate, but not those who were treated with liposomal amphotericin B or amphotericin B lipid complex, received antipyretic (paracetamol, 500 mg) and antihistamine treatment (chlorpheniramine, 50 mg) before each infusion. Patients were examined daily. In group A (i.e., those who received amphotericin B deoxycholate therapy), blood counts, serum electrolyte levels, and blood urea nitrogen (BUN) and creatinine levels were measured on days 14 and 30 or when warranted clinically; limited resources precluded more frequent laboratory testing. If the creatinine level increased to >2.0 mg/dL, amphotericin B deoxycholate was withheld until the value decreased to 1.4 mg/dL (i.e., the upper limit of normal). Patients in group A remained in the unit until the last treatment day (day 30); patients in groups B and C remained until day 19, 14 days after the final infusion of liposomal amphotericin B or amphotericin B lipid complex. At these time points (day 30 or day 19), a splenic aspirate was obtained for apparent cure evaluation, and laboratory tests were repeated. Parasite density score for pre- and posttreatment aspirates was graded on the basis of results of microscopic analysis in a blinded fashion using a conventional logarithmic scale of 0, which indicated 0 parasites per oil-immersion field (magnification, ×1000), to +6, which indicated >100 amastigotes per field [8, 9].

Designation of apparent cure on day 19 (groups B and C) or day 30 (group A) required absence of fever, clinical improvement, reduction in spleen size, and a splenic aspirate score of 0 (apparent parasitologic cure) [8, 9]. Definitive cure, assessed after 6 months, required being healthy with no signs or symptoms of relapse [5, 10]. All subjects were provided 400 rupees (US$8) to offset travels costs to the 6-month follow-up visit. Patients who did not return on time were contacted in person. No patient was lost to follow-up.

Statistical analysis. Data are expressed as mean values ± SEM. Analysis of variance was used to detect most differences among the clinical and laboratory results for the 3 patient groups; χ^2 analysis was used to detect differences in sex, previous treatment, and response rates. Paired-sample *t* testing was used to compare pre- and posttreatment values in each group. The Fleiss quadratic method for proportions was used to compute 95% CIs for the individual proportions of patients who responded to each treatment regimen. $P < .05$ was considered significant.

RESULTS

Patient characteristics and initial responses. Clinical features and laboratory results at study entry (day 0) were similar in the 3 patient groups (table 1). Forty-nine patients had been previously treated elsewhere unsuccessfully with sodium antimony gluconate (20 mg/kg per day for ⩾28 days); none had received amphotericin B deoxycholate therapy. Clinically severe VL (i.e., a spleen size of >8 cm, and a hemoglobin level of <7 g/dL [10]) was present in 4, 5, and 2 patients in groups A, B, and C, respectively.

At entry, fever was present in all subjects except 3 in group A and 1 each in groups B and C. By study day 7, 2 days after completing liposomal amphotericin B or amphotericin B lipid complex treatment, 98% of patients in group B (liposomal amphotericin B) and 76% in group C (amphotericin B lipid complex) were afebrile. In contrast, on the same study day, by which time 4 infusions of amphotericin B deoxycholate had been administered, 4% of those in group A were afebrile ($P < .05$). Overall duration of fever was shorter in group B (3.0 ± 0.2 days) than in group C (6.0 ± 0.2 days) ($P < .05$) and was appreciably shorter in both groups, compared with that in group A (23 ± 1 days) ($P < .05$).

Forty-nine of 51 patients in group A and all patients in groups B ($n = 51$) and C ($n = 51$) completed the assigned treatment. Two patients in group A died on days 9 and 17 after receiving 4 and 9 doses of amphotericin B deoxycholate, respectively. At the time of death, both patients were severely anemic (hemoglobin levels, 4.7 and 5.0 g/dL), and 1 had developed hypokalemia (potassium level, 2.5 mEq/L). Autopsies were not performed.

At evaluation on day 19 (2 weeks after the final infusion of liposomal amphotericin B or amphotericin B lipid complex) or on day 30 (after the final amphotericin B deoxycholate infusion), 150 of the 153 patients were afebrile, had parasite-free splenic aspirate smears, and had fulfilled the criteria for apparent cure (table 2). Compared with baseline results, posttreatment evaluation in all 3 groups (table 1) demonstrated significant decreases in spleen size and increases in body weight, WBC count, hemoglobin level (except in group B), and platelet count.

Adverse reactions. Despite antipyretic premedication, infusion-related reactions typically associated with amphotericin B deoxycholate (higher fever and/or rigors) were near universal and persistent in patients in group A (table 3). In group B and C patients (who received no premedication), 71% and 24%, respectively, had no reactions during any infusion. Liposomal amphotericin B initially induced considerably fewer episodes

Table 1. Baseline and posttreatment demographic, clinical, and laboratory characteristics of Indian patients with visceral leishmaniasis treated with amphotericin B–based therapy.

Characteristic	Group A (n = 51)		Group B (n = 51)		Group C (n = 51)	
	Day 0	Day 30	Day 0	Day 19	Day 0	Day 19
Age, years						
Mean ± SEM	20 ± 2	…	17 ± 2	…	19 ± 2	…
Median (range)[a]	16 (3–50)	…	12 (4–60)	…	13 (1–60)	…
Male sex, %	73	…	63	…	73	…
Prior antimony therapy, no. (%) of patients	17 (33)	…	14 (27)	…	18 (35)	…
Duration of illness, months	2.2 ± 0.2	…	2.2 ± 0.4	…	2.0 ± 0.3	…
Splenic aspirate score	1.7 ± 0.1	0[b]	2.0 ± 0.1	0.02 ± 0.0[b]	2.0 ± 0.1	0[b]
Weight, kg	32 ± 2	34 ± 2[b]	28 ± 2	28 ± 2	32 ± 2	34 ± 2[b]
Spleen size, cm	5.0 ± 0.4	1.0 ± 0.3[b]	4.4 ± 0.4	1.9 ± 0.3[b]	4.8 ± 0.5	1.0 ± 0.3[b]
Hemoglobin level, g/dL	7.1 ± 0.2	8.3 ± 0.2[b]	8.7 ± 1.3	9.1 ± 0.2	6.9 ± 0.3	8.2 ± 0.2[b]
WBC count, $\times 10^3$ cells/mm^3	3.7 ± 0.2	9.7 ± 0.2[b]	4.2 ± 0.3	8.0 ± 0.3[b]	3.7 ± 0.2	7.5 ± 0.3[b]
Platelet count, $\times 10^3$ platelets/mm^3	126 ± 8	275 ± 15[b]	144 ± 14	266 ± 14[b]	112 ± 8	218 ± 12[b]
Blood urea nitrogen level, mg/dL	10 ± 0.3	16 ± 1[b]	11 ± 1	11 ± 0.3	11 ± 0.4	13 ± 1[b]
Creatinine level, mg/dL	0.9 ± 0.1	1.0 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.1

NOTE. Values are expressed as means ± SEMs, unless otherwise indicated. Groups A, B, and C were treated with amphotericin B, liposomal amphotericin B, and amphotericin B lipid complex, respectively.

[a] In groups A, B, and C, 16, 25, and 19 subjects, respectively, were <12 years old, and 4, 5, and 6, respectively, were <6 years old.

[b] $P < .05$ versus day 0 value.

of fever and/or rigors than did amphotericin B lipid complex (table 3); however, tolerance to both preparations developed rapidly by the time of the third infusion, and only 1 subject in either group reacted to the fifth (final) treatment. In contrast, 45 of 50 patients in group A reacted to the fifth infusion of amphotericin B deoxycholate, and, at the 10th infusion, 31 (63%) of the 49 remaining patients in group A still experienced pronounced reactions.

In group A, laboratory testing, repeated during amphotericin B deoxycholate treatment on day 14 (data not shown) and day 30 (table 1) indicated that 21 patients experienced ⩾1 adverse reaction (for a total of 25 reactions). Nine patients developed hypokalemia (serum potassium level, <3.0 mEq/L) during treatment and required supplementation. Four patients developed an increased BUN level (⩾30 mg/dL) on day 14, and, for 2 of the 4, amphotericin B deoxycholate therapy was withheld for 1 week (as per the protocol) because the creatinine level was also >2.0 mg/dL. Although mean values in group A for hemoglobin increased and for creatinine were unchanged on day 30 versus, compared with mean values on day 0 (table 1), individual patients showed evidence of additional toxicity on day 30. In 9 patients, the hemoglobin level had decreased by >0.5 g/dL below the pretreatment value; 3 others showed nephrotoxicity (⩾2-fold increases over baseline levels of BUN and creatinine, which exceeded the upper limit of normal by >20 mg/dL and >1.4 mg/dL, respectively).

Because 5-day liposomal amphotericin B or amphotericin B lipid complex regimens induced little toxicity [8, 9], laboratory testing was not repeated for group B and C patients until the apparent cure evaluation 2 weeks after initiation of treatment (day 19). Except for a minor increase in BUN level in patients treated with amphotericin B lipid complex, mean potassium levels (data not shown) and creatinine levels in groups B and C were unchanged, and hemoglobin levels increased (table 1). On day 19, 3 patients in group B were hypokalemic, 2 had >0.5 g/dL decreases in hemoglobin level (compared with pretreatment values), and 1 had an elevated creatinine level (2.0 mg/dL); none of these abnormal values were observed for patients in group C.

Outcome and retreatment. During the 6-month follow-up period, relapses, which were confirmed on the basis of detection of parasites on aspirate smears, occurred in 5 of the 150 apparently cured patients (number of patients in group A, 0; in group B, 1; and in group C, 4) (table 2). All other patients in each group were asymptomatic, appeared healthy, and were judged to have shown a definitive cure response. Thus, overall cure rates, which were not significantly different ($P > .05$), were 96% for both amphotericin B deoxycholate (49 of 51 patients; 95% CI, 85–100) and liposomal amphotericin B (49 of 51 patients; 95% CI, 85–100) and 92% for amphotericin B lipid complex (47 of 51 patients; 95% CI, 80–98). In groups B and C, the single patient whose illness failed to respond to therapy and the 5 patients who experienced relapse were retreated with the alternate-day amphotericin B deoxycholate regimen; all re-

Table 2. Response of Indian patients to treatment of visceral leishmaniasis with amphotericin B–based therapy.

Characteristic	Group A (n = 51)	Group B (n = 51)	Group C (n = 51)
Completed treatment	49	51	51
Died during treatment	2	0	0
Illness failed to respond to treatment	0	1	0
Apparent cure[a]	49	50	51
Relapse	0	1	4
Definitive cure at month 6	49 (96)[b]	49 (96)[b]	47 (92)[c]

NOTE. Data are no. or no. (%) of patients. Groups A, B, and C were treated with amphotericin B, liposomal amphotericin B, and amphotericin B lipid complex, respectively.

[a] Apparent cure evaluation on day 30 in group A and on day 19 (2 weeks after treatment) in groups A and B.

[b] 95% CI, 85–100.

[c] 95% CI, 80–98.

sponded and were considered cured 6 months after initiation of retreatment.

Treatment regimen costs. On the basis of typical charges for hospital care and laboratory tests for patients with VL in Bihar [4, 6], we estimate that hospitalization costs US$368 for patients treated for 30 days with amphotericin B deoxycholate (e.g., group A) and US$72 for those treated for 5 days with liposomal amphotericin B or amphotericin B lipid complex (e.g., groups B and C). For a 25-kg patient receiving the regimens tested in this study, drug costs were estimated as follows: amphotericin B deoxycholate, US$49 (retail price in India, $6.50 per 50-mg vial); liposomal amphotericin B, US$800 (retail price in India, $160 per 50-mg vial); and amphotericin B lipid complex, US$875 (mean wholesale price in the United States, $175 per 50-mg vial [the drug has not been approved for use in India]) [4, 6, 10]. Thus, total per-patient costs (i.e., hospitalization plus drug) for the treatment regimens used in groups A, B, and C can be calculated at ~$417, $872, and $947, respectively, in a representative 25-kg patient. This example illustrates that drug cost for amphotericin B deoxycholate, liposomal amphotericin B, and amphotericin B lipid complex represent 12%, 92%, and 92% of total treatment costs, respectively. These percentages would be lower (or higher) in patients weighing <25 kg (or >25 kg).

DISCUSSION

Response rates for the amphotericin B–containing regimens tested in this study were high and probably similar. Although we had a limited supply of 1 of the study drugs, we were unable to enroll sufficient patients in each arm to power the study to show that the efficacies of amphotericin B deoxycholate, liposomal amphotericin B, and amphotericin B lipid complex were statistically equivalent. Nevertheless, with satisfactory and apparently comparable cure rates, the highly efficient 5-day liposomal amphotericin B or amphotericin B lipid complex regimens are certainly preferable from a clinical perspective to the 30-day course of conventional amphotericin B deoxycholate regimen.

The preceding conclusion, reasonable for any endemic region, is particularly relevant in Bihar, where VL is epidemic and poverty is extreme [15]. By definition, short-course (e.g., 5-day) regimens produce an abbreviated inpatient stay, which (1) is important to both patients and accompanying families for whom any hospitalization with lost wages is a hardship, (2) increases hospital bed availability in a setting with chronic shortages, (3) reduces laboratory monitoring, and (4) lowers overall hospital-related expenditures. Recent treatment results that use amphotericin B cholesterol dispersion (Amphotec; to-

Table 3. Infusion-associated reactions in Indian patients with visceral leishmaniasis treated with amphotericin B–based therapy.

Reaction	Group A (n = 51)	Group B (n = 51)	Group C (n = 51)
None	1 (2)	36 (71)	12 (24)
Fever and/or rigors (⩾1 episode)	50 (98)	15 (29)	39 (76)
Reactions per patient, mean no. ± SEM	8.4 ± 0.3	0.6 ± 0.1	1.4 ± 0.1
Reactions to successive infusions			
Infusion 1	50 (98)	15 (29)	39 (76)
Infusion 3	47 (92)	6 (12)	8 (16)
Infusion 5	45/50 (90)	1/50 (2)	1/50 (2)
Infusion 10	31/49 (63)	…	…
Infusion 15	9/49 (18)	…	…

NOTE. Data are no. or n/N (%) of patients, unless otherwise indicated. Groups A, B, and C were treated with amphotericin B, liposomal amphotericin B, and amphotericin B lipid complex, respectively. $P < .05$ for all values for group A versus B or C and for all values for group B versus C, except for the number of patients with reactions to infusions 3 and 5.

tal dose of 7.5 mg/kg administered over 6 days) in similar patients in Bihar also indicate a high cure rate (131 (97%) of 135 patients) (S. Sundar, unpublished observations). Thus, a third lipid preparation, previously tested elsewhere [12, 13], also appears to be useful in India as a short-course regimen.

Better tolerability also appears to favor the use of liposomal amphotericin B as well as amphotericin B lipid complex over amphotericin B deoxycholate in cases of VL in India. Infusion-associated inflammatory reactions (higher fever and rigors), which are expected with any amphotericin B–containing formulation, were more frequent and considerably more persistent in patients treated with amphotericin B deoxycholate. Although liposomal amphotericin B and also amphotericin B lipid complex produced initial infusion-related reactions (the latter of which produced more reactions than did the former), tolerance to both agents was near complete by the third infusion. Because just 5 infusions (compared with 15 for amphotericin B deoxycholate) and lower total doses of drug were administered (10 mg/kg, compared with 15 mg/kg for amphotericin B deoxycholate), the opportunity for additional adverse reactions to develop was also likely minimized in liposomal amphotericin B– and amphotericin B lipid complex–treated patients [8, 9]. Such adverse reactions (e.g., renal insufficiency, hypokalemia, and/or decreased hemoglobin level [16]), typically associated with lengthy administration of amphotericin B deoxycholate, developed in 21 (41%) of 51 patients in group A.

Five days of treatment with liposomal amphotericin B or amphotericin B lipid complex reduced the estimated expense of a 30-day hospitalization by ~80%; however, such costs are low to begin with in India. Although a short-course regimen predictably proved cost-effective in a European region where hospital charges are higher [6, 11], in India, resulting savings from an abbreviated hospital stay cannot offset the high drug cost of either liposomal amphotericin B or amphotericin B lipid complex. Therefore, to successfully take advantage of these regimens in India—where they are particularly needed—overall cost (drug plus hospitalization) must logically be brought into line with that estimated for amphotericin B deoxycholate, the current benchmark for parenteral treatment in Bihar [6]. The obvious approach to enhance deployment of amphotericin B lipid complex and liposomal amphotericin B in India is to reduce the price of the drug.

To achieve the preceding objective, we calculate that a substantial reduction (~60%) in the prices of amphotericin B lipid complex and liposomal amphotericin B would be necessary. Reductions in drug price might be feasible by governmental or foundation subsidization, but it would ideally be made by the manufacturers of amphotericin B lipid complex and liposomal amphotericin B. Such a charitable effort would be clearly well placed in Bihar, where 40%–50% of the world's patients with VL are embedded in high-level poverty [15] and both amphotericin B lipid complex and liposomal amphotericin B have a demonstrated record of efficacy [6, 8, 9, 17, 18]. The notion of regional drug price reduction by manufacturers is not unduly optimistic [19] and, in fact, has already been partially undertaken. By an agreement with the World Health Organization, the manufacturer of liposomal amphotericin B provides one 50-mg vial free for every 3 bought by a developing country [2]. If this arrangement for liposomal amphotericin B were altered to 3 vials free for every 2 purchased (five 50-mg vials approximate the adult treatment course used in group B in this study), the necessary price reductions would be achieved. A similar arrangement, if also put into place for amphotericin B lipid complex, would open the door to deployment of 2 well-tolerated, effective, and particularly efficient treatment regimens for VL.

Acknowledgments

We thank Dr. Hetty Waskin (The Liposome Co.), Dr. Regine Buffels (Gilead Sciences), and Dr. Jonathan Berman for their support and advice. The Liposome Co. and Gilead Sciences donated the amphotericin B lipid complex (Abelcet) and liposomal amphotericin B (AmBisome), respectively.

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