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Short-Course Treatment Regimen of Indian Visceral Leishmaniasis with an Indian Liposomal Amphotericin B Preparation (Fungisome™)

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Abstract. India bears the burden of about half of global visceral leishmaniasis (VL) cases with emerging problems of stibanate resistance. Liposomal preparations have improved treatment outcome through shorter duration of therapy and lower toxicity compared with conventional amphotericin B. We report the efficacy of two short-course regimens of an Indian preparation of liposomal amphotericin B (Fungisome™) for VL caused by *Leishmania donovani* in India. An open-label, randomized, single-center comparative study was undertaken from 2008 to 2011, involving 120 treatment naive non–human immunodeficiency virus VL patients randomly allocated to two groups. Fungisome™ was given, in groups A ($N = 60$), 5 mg/kg daily for 2 days and B ($N = 60$), 7.5 mg/kg daily for 2 days, as intravenous infusion. Initial cure rate was 100% in both the groups after 1 month posttreatment. At 6 months after completion of treatment, definitive cure rate was group A 90% (54/60, 95% confidence interval (CI): 80.55–95.72%); group B: 100% (95% CI: 95.92–100%); ($P = 0.027$). No serious adverse events occurred in either group. The short-course, 2-day regimen of 15 mg/kg Fungisome™ infusion is easy to administer, effective, and safe for treatment of VL caused by *L. donovani* in India.

INTRODUCTION

Visceral leishmaniasis (VL), a public health problem plagued by serious issues of underreporting, is endemic in six countries representing more than 90% of worldwide cases with India contributing the majority; West Bengal being one of the endemic states of India with 4.76 million population at risk.[1–3] VL remains invariably fatal if untreated. With widespread resistance to pentavalent antimony reported from Bihar, India, various preparations of amphotericin B and miltefosine has become the standard of care.[3–5] The conventional deoxycholate preparation has many adverse effects including nephrotoxicity and requires prolonged hospitalization (approximately 40–45 days for alternate day regimens and 20–25 days on daily regimens). Recently, the liposomal preparation of amphotericin B has improved management outcome through lower toxicity and shorter duration of therapy.[6] Liposomal amphotericin B, branded as AmBisome (Gilead Pharmaceuticals, Foster City, CA), has the highest therapeutic index among current antileishmanial drugs and reduces treatment, emergent toxicities observed with conventional amphotericin B, ensuring administration of higher doses over shorter treatment courses with possibility of reduction of side effects.[4,6] World Health Organization (WHO) recommends a total dose of 10 mg/kg of AmBisome for the treatment of VL in India.[5,6] However, even with the preferential pricing for developing countries,[6,7] which currently stands at $18 per vial, AmBisome is almost 3-fold the price of conventional amphotericin B. To bring down the cost, recent clinical studies to identify minimum effective total dose utilized single infusions of 5–7.5 mg/kg, which however left 10–20% of patients needing further treatment.[8–10] It is prudent to mention here that all the current treatment modalities currently recommended by the WHO provide 6-month cure rates of > 95%.[11–14] A liposomal amphotericin B preparation, developed in India and commercially available as Fungisome™ (Lifecare Innovations Ltd., Gurgaon, Haryana, India), has been used for the treatment of VL.[15] Fungisome is somewhat different chemically from AmBisome in its composition. Each milligram of amphotericin B in Fungisome is encapsulated in liposomes composed of phosphatidylcholine and cholesterol suspended in 1 mL of physiological saline. Fungisome requires sonication for 45 minutes before administration to transform multilamellar vesicles into small unilamellar vesicles. Like AmBisome, Fungisome is also an intravenous infusion (normal saline in contrast to 5% dextrose for AmBisome), and previous experience demonstrated a total dose of 15–21 mg/kg shows an efficacy of 90.9–100% against stibanate responsive and unresponsive cases of VL.[16] We previously used Fungisome in a small cohort of patients in varying doses and demonstrated that a total of 10 mg/kg body weight given over two consecutive days provide 90% definitive cure rate.[17] In this article, we report our experience with two short-course infusion regimens of Fungisome for the treatment of VL, 10 and 15 mg/kg body weight fractionated over 2 days.

METHODS

The Institutional Ethics Committee of Calcutta School of Tropical Medicine, West Bengal, India, approved the protocol, and the study was conducted in accordance with the ethical principles given in the Declaration of Helsinki, in accordance with the International Conference on Harmonisation Good Clinical Practice guidelines, applicable regulatory requirements, and in compliance with the protocol. All participants including guardians in case of minors provided written informed consent to participate in the study. Lifecare Innovations Ltd. provided Fungisome to all the patients free of cost. The drug-funding source had no access to patient-related data, study design, or analysis. This trial was registered with Clinical Trial Registry–India (CTRI) with registration no. CTRI/2009/091/000764.

This was a prospective, single-center, randomized, and open-label comparative study.

The inclusion criteria were as follows: all patients with symptoms and signs suggestive of VL (fever for more than 2 weeks and splenomegaly) with rK39 immunochromatographic strip

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test (Kalazar Detect™; InBios International Inc., Seattle, WA) positivity and demonstrable amastigotes of *Leishmania donovani* parasites in splenic aspirates between 2008 and 2011. Parasites in splenic smears were graded on a log scale from grade 0 (defined as no parasites/1,000 high-power fields in oil immersion) to grade 6 (defined as ≥ 100 parasites/high-power fields). The following patients were excluded from the study: pregnant and lactating women, human immunodeficiency virus-positive individuals, those previously treated with or receiving concomitant antileishmanial drugs, patients with platelet count below 50,000/μL, white blood cell count below 1,000/μL, serum aspartate aminotransferase and alanine aminotransferase levels more than three times the upper limit of normal, serum creatinine or urea values more than 1.5 times the upper limit of normal, and patients with other major medical illness such as ischemic heart disease, congestive cardiac failure, and stroke.

Eligible patients were randomized (1:1) in to the following treatment groups: group A: Fungisome was administered intravenously at a total dose of 10 mg/kg over a period of 2 days (daily single infusion of 5 mg/kg body weight for two consecutive days) or group B: Fungisome was administered intravenously at a total dose of 15 mg/kg over a period of 2 days (daily single infusion of 7.5 mg/kg body weight for two consecutive days).

Fungisome has been developed with financial support from Department of Biotechnology, Government of India, and Program Aimed at Technological Self Reliance supported by the Department of Science and Industry Research, India. Fungisome is available as 50 mg/50 mL, 25 mg/25 mL or 10 mg/10 mL liquid preparations. According to the manufacturer's guideline, the required total dose was diluted in same volume of normal saline and infused at the rate of 100 mL/hour. Unopened bottle of Fungisome is to be sonicated, with sonicator provided by Lifecare Innovations, before administration. The procedure is 1) fill the sonicator with refrigerated water; 2) clamp the bottle in the stand, the level of water should be slightly higher than the level of the drug in the bottle (two bottles can be sonicated at a time); and 3) switch on the sonicator and run it for 45 minutes (time is preset) (Lifecare Innovations supplies the sonicator when larger numbers of patients are treated or supplies the sonicated drugs on the day of infusion for one or two patients). During study, a coinvestigator following the direction in manufacturer's literature supervised the process of sonication.

In the absence of any untoward clinical events or new abnormality on blood biochemistry, the patients were discharged on the second day. If their clinical conditions are warranted, the patients were kept in the hospital for longer period of observation. Patients who would experience any severe hypersensitivity or cardiopulmonary complications of hypersensitivity were withdrawn from the study.

Initial cure was defined as clinical and parasitological cure at the 30th day of follow-up. Clinical cure was defined as absence of fever or any other constitutional symptoms along with at least one of regression of splenomegaly (≥ 50%) and improvement of hematological parameters (hemoglobin ≥ 10 g/dL) at the 30th day of follow-up. To assess parasitological cure, splenic aspirate (or bone marrow aspirate in whom spleen was not palpable) was performed on day 30 post infusion and grading was done as described previously. Absence of clinical cure or presence of ≥ grade 1 parasite in splenic aspirate on day 30 constituted lack of initial cure. Definitive cure was defined by absence of relapse during 6 months of follow-up. Signs or symptoms suggestive of leishmaniasis, appearing after an initial cure, followed by identification of *L. donovani* bodies in a splenic aspirate, within 6 months, constituted relapse. Treatment failure was defined as either the lack of initial cure or relapse. Patients with treatment failure were to be treated with either liposomal amphotericin B 5 mg/kg on days 1, 3, 5, and 7 or conventional amphotericin B 20 mg/kg body weight over 20–40 days as rescue medication.

All patients were monitored for incidence of infusion-related toxicities and other adverse events (AEs) according to National Cancer Institute Common Terminology Criteria AE, version 3 (CTCAE V3).

A sample size of 75 patients per group was sufficient to detect a clinically important difference of 11% between groups in the definitive cure rate at 6 months using a two-sided Z-test with 80% power and 5% significance level.

Patients were randomized to receive either treatment using a computer-generated randomization list. Individual treatment allocations were placed in sealed, opaque envelopes, which were opened after a patient had been entered into the trial. It was not possible to blind patients or treating physicians because of the nature of the intervention.

Data were expressed as means (±SD) for continuous variables and percentages (95% confidence interval (95% CI), Jeffrey's method) for categorical variables. Proportion of patients achieving initial cure and definitive cure were to be compared across the two treatment groups.

Comparison of proportions was done using χ^2 test at 5% level of significance. However, as the expected number of patients not achieving definitive cure in both of the treatment groups was found to be < 5, Fisher's exact test was used. P value < 0.05 was considered as statistically significant.

For safety, the number and percentage of patients experiencing toxicities and AEs (including laboratory abnormalities) across two treatment groups were recorded as per protocol and was presented in a tabular form.

RESULTS

During the study period, we enrolled 150 consecutive Indian VL patients and assessed their eligibility for inclusion in the study at the Calcutta School of Tropical Medicine. Thirty patients were excluded (CONSORT flow chart shown in Figure 1). Baseline clinical characteristics and laboratory values of the patients are shown in Table 1. Of the 120 subjects, 50% (60/120) were male (average age: 17.50 ± 12.94 years) and 36.67% (95% CI: 28.44–45.45) were adults (44/120).

At the end of the treatment, all the patients became afebrile. At 30-day posttreatment, all the patients remained afebrile. There was a reduction of the spleen size and improvement of hematological parameters (details are given in Table 2). Overall more than 10% increment in hemoglobin level occurred in 96.67% (58/60) in group B compared with 86.67% (52/60) patients in group A ($P = 0.048$). In both the groups, more than 33% reduction of splenic size occurred in 95% (57/60), and more than 50% reduction of splenic size occurred in 86.67% (52/60) patients of group A and 91.675% (55/60) patients of group B. Both clinical and

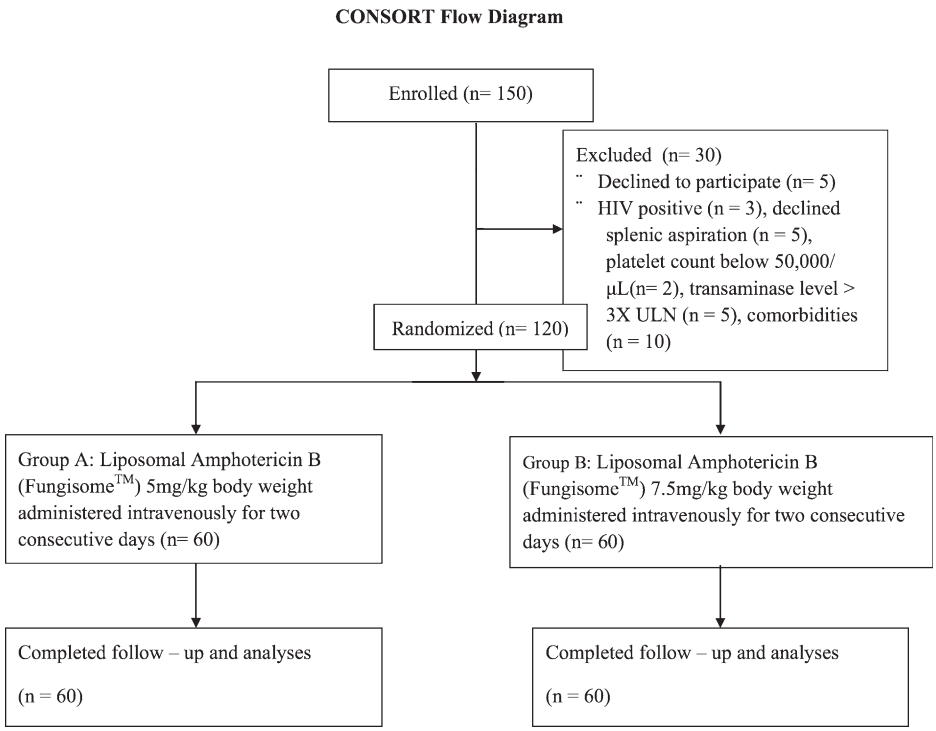

FIGURE 1. CONSORT flow diagram. Details of comorbidities in the excluded patients group are uncontrolled diabetes ($N = 6$), untreated hypertension ($N = 2$), ischemic heart disease ($N = 1$), and dilated cardiomyopathy ($N = 1$).

parasitological cure rates at 30-day posttreatment were 100% (60/60). By 6 months, after the completion of treatment, 54 of the 60 patients in group A (definitive cure rate: 90%; 95% CI: 80.55–95.72%) and 60 of the 60 patients in group B (definitive cure rate: 100%; 95% CI: 95.92–100%) achieved final cure. The difference was statistically significant ($P = 0.027$).

Summary of AE is detailed in Table 3. None of the patients had any CTCAE grade 3 or higher AEs. Infusion-associated reactions (fever or rigors during drug administration)[18] occurred in all of the 120 patients. Twenty patients (33.33%; 95% CI: 22.39–45.63) in group A and 25 patients (41.67%; 95% CI: 29.87–54.14) in group B had CTCAE grade 2 allergic reaction (P value: 0.34). The only other grade 2 adverse effect was alanine aminotransferase elevation in five patients (4.16%, 95% CI: 1.61–8.89), one in group A and four in group B. All of these reactions responded promptly to either slowing of infusion rates or antiallergic medications and none required temporary interruption of infusions. All of the rest had grade 1 reactions and none had grade 3 or higher reaction. None of these transient infusion reactions warranted discontinuation of treatment.

DISCUSSIONS

With the rising menace of drug resistance and treatment failure with pentavalent antimony in kala-azar, the onus is on amphotericin B to save the day. The amphotericin B

TABLE 1
Baseline characteristics of VL patients

Characteristics	Overall ($N = 120$)	Group A ($N = 60$)	Group B ($N = 60$)	P value
Male sex (%)	60 (50)	31 (51.67)	29 (48.33)	0.85
Age in years (SD)	17.50 (12.94)	15.27 (12.53)	19.74 (13.05)	0.06
Weight in kg (SD)	27.42 (14.31)	29.10 (14.04)	25.73 (14.48)	0.19
Parasite density (SD)	3.95 (1.09)	3.8 (1.25)	4.1 (0.89)	0.26*
Mean splenomegaly in cm below the left costal margin (SD)	11.87 (5.49)	11.62 (5.53)	12.13 (5.51)	0.61
Mean hepatomegaly in cm below the left costal margin (SD)	2.65 (2.53)	2.55 (2.34)	2.75 (2.72)	0.87*
Number of patients with hepatomegaly (%)	114 (95)	56 (93.33)	58 (96.66)	0.68
Mean hemoglobin concentration in g/dL (SD)	8.68 (1.14)	8.82 (1.27)	8.55 (0.99)	0.36*
Mean white cell count × 10^3/mm^3 (SD)	3.21 (1.28)	3.22 (1.28)	3.19 (1.29)	0.94
Mean platelet count × 10^3/mm^3 (SD)	128.93 (42.59)	125.17 (40.19)	132.68 (44.89)	0.34
Mean bilirubin in mg/dL (SD)	0.71 (0.28)	0.79 (0.29)	0.68 (0.29)	0.08
Mean albumin in g/dL (SD)	2.85 (0.48)	2.85 (0.63)	2.86 (0.48)	0.93*
Mean urea in mg/dL (SD)	24.33 (9.42)	23.38 (8.40)	25.28 (10.31)	0.27
Mean creatinine in mg/dL (SD)	0.81 (0.20)	0.81 (0.12)	0.81 (0.25)	0.12*

SD = standard deviation; VL = visceral leishmaniasis.
Comparison of means done with Student's t test and Mann–Whitney U test as appropriate and comparison of proportions done with χ^2 test or Fisher's exact test as appropriate. Group A: Fungisome™ 5 mg/kg body weight given for two consecutive days and Group B: Fungisome™ 7.5 mg/kg body weight given for two consecutive days.
*Statistical significance obtained with Mann–Whitney U test.

TABLE 2
Clinical variables at 30th day and 6-month posttreatment

Variable	Group A ($N = 60$)	Group B ($N = 60$)	P value
Mean splenomegaly in cm below the left costal margin (SD)	1.61 (0.80)	1.60 (0.92)	0.92
Mean percentage reduction of splenic size posttreatment (SD)	74.87 (31.52)	77.85 (24.10)	0.56
6-month posttreatment mean body weight in kg	39.36 (16.41)	41.43 (18.37)	0.52
Mean percentage increase in body weight posttreatment (SD)	45.95 (24.12)	64.71 (30.18)	< 0.001
Mean hemoglobin in g/dL (SD)	10.52 (0.94)	10.50 (0.85)	0.92
Mean percentage increase in hemoglobin posttreatment in g/dL (SD)	20.26 (8.36)	23.28 (5.69)	0.16*

SD = standard deviation.
Comparison of means done with Student's t test and Mann–Whitney U test as appropriate. Group A: Fungisome™ 5 mg/kg body weight given for two consecutive days and Group B: Fungisome™ 7.5 mg/kg body weight given for two consecutive days.
*Statistical significance obtained with Mann–Whitney U test.

deoxycholate preparation is disadvantaged by the requirement of long duration of therapy (5–6 weeks), which leads to prolonged hospital bed occupancy, loss of daily wages, along with infrastructure-, manpower-related and human resource–related expenses.[12] Exposure to long duration of potentially toxic drug raises the possibility of serious adverse effects notably nephrotoxicity, thrombocytopenia, anemia, and hypokalemia.[19] Hence, there is a demand to develop short-course regimens that are efficient and have lower risks of adverse effects.

In this study, we report a novel approach toward a short-course therapy. Previous studies have shown that conventional amphotericin B deoxycholate in the dosage of 15 mg/kg body weight provides a cure rate of 97–100%.[20] Previous attempts at short-course therapy of VL used single-dose liposomal amphotericin B infusions at doses of 7.5 and 10 mg/kg body weight with 6-month cure rates ranging from 90% to 95%.[4,21] Although lower dosages result in imperfect cure rate, a large single dose of liposomal amphotericin B might be too toxic. We provided relatively higher doses of amphotericin B, namely 10 and 15 mg/kg body weight in fractionated doses over 2 days. WHO recommendation in 2010 for VL states dose of liposomal amphotericin B to be 3–5 mg/kg per daily dose by infusion given over 3–5 days period up to a total dose of 15 mg/kg by infusion or 10 mg/kg as a single dose by infusion. So total recommended dose comes to 10–15 mg/kg stretched over 1–5 days. We attempted to optimize the duration in 2 days to compare the efficacy of total dose of 10 mg/kg versus 15 mg/kg to find out the minimum effective dose in 2 days. Our apprehension was that a large single dose in one day might be too toxic, as Fungisome has not been used in such doses previously. The landmark study with 10 mg/kg single dose was done with AmBisome, which was proven to be safe. But patients were hospitalized for 2 days to observe any toxicity in second day. We discharged the patients on second day, so duration of hospitalization was same as in 10 mg/kg single dose versus 5 mg/kg for 2 days. Basically, we tried to find out the minimum dose and duration with maximum efficacy and safety in a controlled study with two regimens of 10 mg/kg versus 15 mg/kg total dose as recommended by WHO with a new Indian preparation of liposomal amphotericin B.

Our results are encouraging with final cure rate of 90% with 10 mg/kg body weight dose and 100% with 15 mg/kg body weight regimen in patients fulfilling the inclusion criteria. Previous experiments with 7.5 mg/kg body weight single-dose therapy had shown an initial cure rate of 96% and 90% at 6 months.[4] This dose as a single infusion was established as safe; hence, we designed our study taking 7.5 mg/kg body weight as the highest safe daily dose. A later study compared liposomal amphotericin B 10 mg/kg body weight as a single dose compared with amphotericin B deoxycholate as conventional therapy. Cure rates at 6 months were 95.7% in the liposomal therapy group and 96.3% with conventional therapy. AEs in the liposomal therapy group were more frequent than in the previous study with 7.5 mg/kg body weight in single dose (40% versus 9.8%, respectively, but most of these AEs were minor and inconsequential).[4,21] Liposomal amphotericin B in 5 mg/kg body weight was tried as a part of another trial, which showed a definitive cure rate of 91%.[22] Interestingly this favorable effect of liposomal amphotericin B might have geographic flavor. Berman and others utilized the Davidson regimen,[8] originally designed for European patients (*Leishmania infantum* infection), in different worldwide regions and expectantly demonstrated widely different results.[13] Indian patients with kala-azar require possibly lesser doses compared with patients in other countries.

TABLE 3
Summary of AEs in the study population

AEs	Overall ($N = 120$)	Group A ($N = 60$)	Group B ($N = 60$)	P value
Allergy grade 2 (%)	45 (37.5)	20 (33.33)	25 (41.67)	0.34
Fever grade 2 (%)	24 (20)	10 (16.66)	14 (23.33)	0.36
Rigors/chills grade 2 (%)	8 (6.66)	2 (3.33)	6 (1.00)	0.27
Vomiting, all grades	6 (5.00)	1 (1.66)	5 (8.33)	0.21
Diarrhea, all grades	4 (3.33)	1 (1.66)	3 (5.00)	0.62
Aspartate aminotransferase elevation, grade 1	8 (6.66)	3 (5.00)	5 (8.33)	0.72
Alanine aminotransferase elevation, all grades	11 (9.16)	3 (5.00)	8 (13.33)	0.20
Alanine aminotransferase elevation, grade 1	6 (5.00)	2 (3.33)	4 (6.66)	0.68
Alanine aminotransferase elevation, grade 2	5 (4.16)	1 (1.66)	4 (6.66)	0.36
AE leading to treatment discontinuation	0	0	0	–

AE = adverse events.
Gradation of AEs is according to National Cancer Institute Common Terminology Criteria Adverse Events, version 3. Group A: Fungisome™ 5 mg/kg body weight given for two consecutive days and Group B: Fungisome™ 7.5 mg/kg body weight given for two consecutive days.

This was reiterated recently when a multicenter, non-inferiority trial in east Africa comparing efficacy and safety of single dose and multiple doses of liposomal amphotericin B for the treatment of VL was terminated early because of low efficacy of both the regimens.[23]

Previous single-dose regimen was followed by a 24-hour period of in-hospital observation that effectively meant 2 days of hospitalization like in this study. Though the environment of a protocol-based study hardly represents the real-time field condition, if our regimen can be successfully applied under field conditions, this would allow for two daily injections and discharge on second day of hospitalization. It is our hypothesis that keeping the upper limit of daily dose at 7.5 mg/kg body weight maintains safety and avoids greater risks of untoward effects from higher doses in single infusions of liposomal amphotericin B and should be further tested in non-inferiority trials.

It is worthwhile to mention that all previous trials on short-course therapy with liposomal amphotericin B used international brand AmBisome). We used a newly available Indian formulation of liposomal amphotericin B, Fungisome. It was shown to be renal safe in a post-marketing study,[24] though the study design was not optimal and similar safety profile was also reflected in this study.

AmBisome costs $240 per 50 mg vial in the commercial market whereas the equivalent cost of Fungisome is $133; though AmBisome is available through Gilead Sciences AmBisome Access Program at $18 per 50 mg vial for kala-azar elimination program of Government of India.[6,25] Donation is not a sustainable solution for the Indian program, and as such, more cost-effective alternatives, such as Fungisome, should be considered.

Our study suffers from some limitations including the exclusion of previous treatment failure cases and the relatively small sample size especially after the assumption of strict exclusion criteria and need for overnight stay. Interestingly, the mean spleen size of our patients were much greater than recently reported literature.[26] This we believe is due to late presentation of rural patient in the metropolitan tertiary care centers that our study represents. However, despite such late presentation the excellent efficacy of the presented regimen brings hope. It is worthwhile to mention that the Indian National Program for Elimination of Kala-azar list the following regimens in order of preference: single dose 10 mg/kg body weight liposomal amphotericin B; combination regimens (e.g., miltefosine and paromomycin); amphotericin B emulsion; miltefosine and amphotericin B deoxycholate in multiple doses. This study brings another force into the armamentarium against Kala-azar and it remains our belief that the 2-day regimen of liposomal amphotericin B amounting to a total dose of 15 mg/kg body weight should share the place of initial choice with single dose 10 mg/kg body weight liposomal amphotericin B. We believe that our regimen is effective, safe, easy to administer, and thus may be field adaptive. Larger field effectiveness studies are warranted to establish our hypothesis.

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