Safety and Efficacy of High-Dose Infusions of a Preformed Amphotericin B Fat Emulsion for Treatment of Indian Visceral Leishmaniasis

S. Sundar,* A. Singh, D. Agarwal, M. Rai, N. Agrawal, and J. Chakravarty
Kala-Azar Medical Research Center, Department of Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India; Kala-Azar Medical Research Center, Muzaffarpur, India

Abstract. Amphotericin B deoxycholate is used as a first-line drug for visceral leishmaniasis (VL) in India. Its major drawbacks are prolonged hospitalization of treated patients and toxicity. An open label phase II study with pre-formed amphotericin B lipid emulsion (ABLE) was conducted to evaluate safety and efficacy of four regimens of 15 mg/kg each administered in 1–2 doses. Regimen 1 was 7.5 mg/kg/day on day 1 and day 3, and regimen 4 was a single bolus infusion of 15 mg/kg. The safety profile was excellent with mild infusion reactions seen in 38% of the patients. Definitive cure was achieved in 100% of the patients treated with regimen 4. The overall cure rate was 87% (95% confidence interval = 75–94%). In this study, ABLE was safe and had excellent efficacy when given as a bolus of 15 mg/kg. More studies with larger number of patients and higher doses are needed to establish acceptable, safe and efficacious regimen.

INTRODUCTION

Amphotericin B deoxycholate is used as the first-line drug for treatment of visceral leishmaniasis (VL) in North Bihar, India, because widespread resistance to antimony.1-3 This drug shows excellent efficacy.4,5 However, toxicity and long duration of therapy are its major drawbacks. Lipid formulations of amphotericin B are devoid of serious toxicity. This finding has made it possible to administer high doses of lipid formulations of AB over short durations of 1–5 days.6 Of several lipid formulations of amphotericin B deoxycholate commercially available, liposomal amphotericin B is available at a 90% reduced preferential price of US $20.00 per 50-mg vial. However, it is still unaffordable and beyond the means of patients in this region of India.

Several attempts have been made to provide patients with inexpensive lipid-containing amphotericin B deoxycholate by mixing commercially available lipid emulsions with this drug. However, these preparations lacked uniformity of components of mixtures and quality control. A commercial standardized product of preformed amphotericin B deoxycholate with lipid emulsion (ABLE; Bharat Serum and Vaccines Limited, Mumbai, India) that contains egg lecithin and soybean oil and has an average particle size of less than 1 µM has been developed.7 This product is licensed for use in India for treatment of visceral leishmaniasis. In preclinical studies, mice tolerated 80 times more ABLE than conventional amphotericin B deoxycholate AB (data from the manufacturer).

In a Phase II study, this product was well tolerated and cured 91.1% of the patients tested (total dose range = 9–15 mg/kg).7 The present study was conducted to determine whether 15 mg/kg of amphotericin B emulsion could be safely given as a single infusion or if it needed to be given in a divided dose.

METHODS

Study design. The study was a prospective, open-label, dose-escalation phase II clinical trial to evaluate the efficacy and safety of four short course regimens of amphotericin B emulsion in the treatment of patients with VL. The product is available in a liquid formulation that can be stored below 25°C, and is stable at 40°C up to 6 months.

Amphotericin B at a total dose of 15 mg/kg has shown a cure rate of 97–100%.8,9 Moreover, in our previous phase II study, amphotericin B emulsion at a dose of 15 mg/kg showed an excellent safety profile.7 Encouraged by the results of this study, we used four regimens, each containing a total dose of 15 mg/kg of amphotericin B emulsion, in which the dose of amphotericin B emulsion was sequentially escalated on day 1: regimen 1, 7.5 mg/kg/day on day 1 and day 3; regimen 2, 10 mg/kg/day on day 1 followed by 5 mg/kg on day 3; regimen, 3–12.5 mg/kg/day on day 1 followed by 2.5 mg/kg on day 3; and regimen 4, a single bolus infusion of 15 mg/kg over a ≥4 hour-period on day 1.

Study site. The study was conducted at the field site of the Kala-Azar Medical Research Center in Muzaffarpur, India. The ethics committee of the Kala-Azar Medical Research Center reviewed and approved the study protocol. Written informed consents were obtained from all the patients. The study was conducted in accordance with the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Guidelines for Good Clinical Practice (ClinicalTrials.gov, ID no. NCT00497601).

Inclusion criteria. Patients of either sex and 18–65 years of age (both inclusive) with signs and symptoms of VL (fever, weight loss, and splenomegaly) who had demonstrable parasites in splenic smears were included in this study. Patients who had failed treatment with antileishmanial drugs other than amphotericin B were also included after completion of a washout period of 10 days.

Exclusion criteria. Patients were excluded if they were breast feeding or pregnant, and had positive serologic results for infection with human immunodeficiency virus (HIV). Exclusion criteria also included a granulocyte count < 1,000 cells/mm³; a hemoglobin level < 6 g/dL, a platelet count < 50,000 cells/mm³; levels of hepatic aminotransferases > 2.5 times the upper limits of normal levels, total bilirubin levels > 1.5 times the upper limit of normal ranges; prothrombin time > 5 seconds than that of controls, and serum creatinine levels ≥ 1.5 times the upper limit of normal ranges.
Patients with concomitant life-threatening disease or serious concurrent infection such as malaria, tuberculosis, or bacterial pneumonia, and those allergic to amphotericin B or any other ingredients in the emulsion formulation were also excluded from the study.

**Baseline evaluation.** A baseline screening of complete blood count (CBC); biochemical profile including liver function test, blood urea, serum creatinine and electrolytes; test for antibodies to HIV, urinalysis, chest radiography, and electrocardiography, was conducted. For parasitologic diagnosis splenic/bone marrow aspiration was conducted. The pathologist who read the splenic smears was blinded to the treatment regimen.

**Number of patients.** Sample size was determined after discussion with regulatory authorities for this phase II study and Schedule Y (amended 2005) of Drug and Cosmetic act of India. Four cohorts of 15 patients each were enrolled with a total sample size of 60 patients.

**Dose-escalation strategy.** Recruitment of the subsequent regimen started when the last patient of the previous regimen completed at least seven days after receiving the study drug and if the percentage of patients with dose-limiting toxicity (DLT) was ≤33% in the previous regimen. Recruitment for an ongoing regimen continued only if number of patients with DLT was ≤5 anytime during recruitment. Dose-limiting toxicity was defined as the presence of any drug related nephrotoxicity/hepatotoxicity ≥ grade III and any other drug related toxicity ≥ grade IV except hematologic toxicity. For baseline hematologic parameters in CTC grade III, a reduction of 33%, and a reduction to grade IV for baseline parameters in CTC grades I and II were considered a DLT.

**Treatment protocol.** All patients were hospitalized for at least seven days. Treatment was started within six days of the baseline screening evaluation. Amphotericin B lipid emulsion was diluted with 5% dextrose to a concentration of 1 mg/mL before administration. On day 1, a test dose of 1 mg of ABLE was administered over a 15-minute period and the patient was observed continuously for any allergic or toxic reaction. In the absence of allergic or toxic reactions, the remaining dose was administered over a 4-hour period. Patients in whom severe hypersensitivity or cardiopulmonary complications of hypersensitivity developed were withdrawn from the study. For infusion-related events during the first infusion, premedication (paracetamol, one 500-mg tablet, or an injection of 25 mg of chlorpheniramine maleate) was given 15–30 minutes prior to subsequent injection. On day 3, amphotericin B emulsion was administered as per regimen (except for regimen 4). Patients were examined daily for vital parameters and spleen size. Levels of serum creatinine and electrolytes were measured daily, a CBC was obtained on days 2 and 4 of treatment, and an electrocardiogram (EKG) was obtained on days 2–4. The following tests were conducted on day 7 before discharge: CBC, liver function test, renal function test, serum electrolyte test, chest radiograph, EKG, and urine analysis. Grading of toxicity was done according to the Common Toxicity Criteria for Adverse Events, Version 3 (National Cancer Institute, Bethesda, MD). Patients were withdrawn from the study if they had intolerable toxicity, showed a lack of efficacy, died, withdrew consent, were withdrawn at the discretion of the investigator, were lost to follow-up.

**Efficacy criteria.** Clinical improvement on day 15 after completion of treatment was present if there was absence of fever and one or more of the following: an increase in the hemoglobin level by 2 g/dL, compared with the baseline value, an increase in the total leukocyte count by 1,000 cells/mm³ compared with the baseline level, or decrease in spleen size by 33% compared with the baseline size.

Initial cure was considered as a splenic aspirate score of 0 on days 15 or 42. Parasite density was graded by microscopy in a blinded fashion using a conventional logarithmic scale of 0 (no parasites per 1,000 oil-immersion fields) to +6 (> 100 amastigotes/field). A splenic aspirate score ≥ 2 on day 15 was considered as a treatment failure; these patients were given rescue treatment. Those with scanty amounts of parasites (1+) at day 15 had a repeat parasitic assessment on day 42. Absence of parasites was considered as a parasitologic cure, and the presence of parasites was considered as a treatment failure. Patients were followed-up for six months after the end of treatment, and absence of signs or symptoms of relapse indicated definitive cure.

**Statistical analysis.** Statistical analysis was performed using SAS release 9.1 software (SAS Institute Inc., Cary, NC). Safety analyses included all patients who received at least one dose of study drug. Patients were considered eligible for efficacy evaluation if study drug was administered on both days and at least one efficacy assessment was conducted. Data are expressed as mean ± SD. Analysis of variance was used to detect differences among the clinical and biochemical results of the four regimens. Frequency and the percentage of patients who achieved efficacy endpoints (clinical improvement, initial cure, and definite cure) were calculated and compared across the treatment groups by using the Mantel-Haenszel chi-square test or the Mantel-Haenszel chi-square exact test as applicable.

The sponsor of the study had no role in study design, execution, collection, and interpretation of data; preparation of the manuscript; and the decision to publish it. Data collection and analysis was done by an independent contract research organization in collaboration with the authors.

**RESULTS**

A total of 109 patients were screened, and 60 patients were enrolled in the study. Baseline demographic characteristics were similar for all four regimens (Table 1). Males constituted 68.3% of the patients. The mean baseline spleen size was 3.25 cm.

**Safety.** None of the patients had any adverse events to the test dose, or required reduction in dose or interruption of study drug because of adverse events. No patients had nephrotoxicity during the study. Overall 41.7% (25) of the patients reported a study drug-related adverse event. Infusion related pyrexia (38.3%) and chills (28.3%) were the most common drug-related adverse events (Table 2). Pyrexia was observed only on day 1 of the infusion; 65% of these events were of grade 1 severity and 35% were of grade 2 severity.

**Efficacy.** Clinical improvement on day 15 (absence of fever and regression of spleen size) was observed in every patient in all four regimen groups. All except two patients receiving regimen 3 achieved initial cure. Definitive cure was achieved in 100% of the patients receiving regimen 4 compared with 73.3–93.3% of the patients receiving the other three regimens (Table 3). Overall cure rate for the four groups was 87% (95% confidence interval = 75–94%).
The results of this trial confirm the safety of a high-dose infusion of ABLE. It was possible to administer 15 mg/kg as a single bolus dose without any significant adverse events. None of the patients had any reactions to the test dose, a finding similar to what has been previously observed. Thus, it appears that the practice of using a test dose can be abandoned. The low overall cure rate (87%) and failure of initial cure in 2 patients (3.3%) were surprising results, even though the mean ± SD spleen size was 3.251 ± 2.53 cm, which suggested modest disease. In most studies with any formulation of amphotericin B deoxycholate, with a dose of 15 mg/kg, usually a cure rate of approximately 100% is achieved. In treating patients with VL, with any effective regimen, one strives for a cure rate of approximately 95%. In an anthroponotic foci such as India, with lower cure rates, the chance of developing drug resistance is high. Although a cure rate of 100% was achieved with a bolus containing a 15-mg/kg dose (regimen 4), a larger study, possibly with higher doses of ABLE, should be conducted. However, such a study might be less cost-effective.

Although pharmacokinetic data for human patients receiving this preparation of ABLE is not available, in preclinical studies in male New Zealand white rabbits, the pharmacokinetics of amphotericin B after administration of ABLE showed a peak concentration 0.387 ± 0.176 µg/mL, a clearance time of 16.06 ± 12.5 mL/hr/kg, an apparent volume of distribution of 26.14 ± 18.52 L/kg, and a half-life of 6.622 ± 10.63 hours. The peak level of AB after administration of ABLE was lower than that of conventional formulations. However, levels in liver and spleen were higher than those of the conventional formulation. One explanation of reduced toxicity and efficacy could be lower serum peak levels, although for efficacy, tissue (spleen and liver) levels are more important.

ABLE has been stabilized by controlling critical parameters such as particle size, distribution, and pH. This drug has undergone extensive stability studies as per ICH guidelines. On the basis of this data, ABLE has a shelf-life of two years. In addition to its excellent safety profile, there are several advantages of this formulation. It is already an indigenous production in commercial use in India, and contrary to other lipid formulations, which are prohibitively expensive, this actual market cost of this product is US $10.00 per 50-mg vial, i.e., half the preferential price of amphotericin B deoxycholate. Thus, affordability and availability are not likely to be problems with ABLE. Liposomal amphotericin B cured 89–91% of patients at a dose range of 3.75–7.5 mg/kg. However, in an anthroponotic foci, a higher cure rate is desirable because the likelihood of development of drug-resistant strains increases. Thus, low-dose therapy appears attractive in terms of cost. However, they cannot be used in control programs. Results of a comparative study of ABLE with liposomal amphotericin B will only indicate which of the two regimens is cost-effective. Furthermore, a well-tolerated, affordable, single-dose therapy will have a major impact in reducing the financial burden of these poverty-stricken patients, as well as that of the National Elimination Program. It will help to lessen overcrowding at treatment centers, where patients have to wait weeks to months to secure admission because of a lack of vacant hospital beds.

In a previous phase II study with ABLE, the product showed excellent tolerability, which has been reconfirmed in this study. In a previous study, infusion reactions were
observed in 40% of patients receiving this product compared with 29% receiving liposomal amphotericin B.13 The infusion reactions were mild in nature. None of the patients had nephrotoxicity or a drug-related serious adverse event. This finding also opens up the possibility of administering this compound at peripheral health centers. Phase II–III trials with a single bolus of ≥ 15-mg/kg doses of ABLE with larger sample sizes are needed to establish the safety and efficacy of this preparation.

Monotherapy of diseases caused by microbes is likely to induce drug resistance and treatment failures. Thus, multidrug regimens should be developed for treatment of VL. High cure rates were achieved when low-dose liposomal amphotericin B was used with miltefosine for a short duration.14 Once doses with acceptable efficacies are known, possibilities of using other antileishmanial drugs in conjunction with ABLE can be explored to prevent development of resistance.

Received July 25, 2008. Accepted for publication December 17, 2008.

Financial support: This study was supported by Bharat Serum and Vaccines Limited (Mumbai, India).

Authors’ addresses: S. Sundar, A. Singh, D. Agarwal, M. Rai, and J. Chakravarty, Kala-Azar Medical Research Center, Department of Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221 005, India. N. Agrawal, Kala-Azar Medical Research Center, Rambag Road, Muzaffarpur 842001, India.

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