Liposomal Amphotericin B (AmBisome) in the Treatment of Complicated Kala-Azar Under Field Conditions

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An open trial of liposomal amphotericin B (AmBisome [L-AmB]; Vestar, San Dimas, CA) for treatment of complicated visceral leishmaniasis was performed in Sudan. Forty-nine patients were treated, and there were six deaths (12% mortality); these were not attributed to therapy. Thirty-seven patients were selected for the trial because of (1) relapse after treatment with a combination of pentavalent antimony (Sb^V) and aminosidine, (2) incomplete parasitological response to Sb^V and aminosidine, or (3) severe illness. Drug regimen 1 (3 doses of 3–5 mg/kg, on days 0, 3, and 10) cured 8 (50%) of 16 patients; regimen 2 (6 doses of 3–5 mg/kg, on days 0, 3, 6, 8, 10, and 13) cured 14 (88%) of 16. For four of 10 partial responders, "rescue" therapy with L-AmB alone (3 mg/kg daily for 10 days) resulted in cure. Twelve less-unwell patients received regimen 3 (4 doses of 4–5 mg/kg, on days 0, 2, 5, and 7); seven of 11 patients evaluated (64%) were cured. The optimal regimen of L-AmB in these circumstances is administration of 4 mg/kg on days 0, 3, 6, 8, 10, and 13.

An epidemic of visceral leishmaniasis has been in progress in Western Upper Nile, Sudan, since 1984 [1–3]. Treatment was established in the region in 1989, and >17,000 patients have been treated by Medecins Sans Frontieres–Holland in temporary treatment centers (J. Seaman, A. J. Mercer, E. Sondorp, and B. L. Herwaldt, unpublished manuscript). Mortality in this epidemic could be 69% [4], and it is estimated that >60,000 people (of a total population of 500,000) may have died. The mortality during treatment was initially ~20%, but it is now <10% (Seaman et al., unpublished manuscript, and [5]). The World Health Organization has recently declared the region a disaster area [6].

Routine treatment is with sodium stibogluconate (pentavalent antimony; Sb^V); 20 mg/kg is intramuscularly injected daily for 30 days [7]. Some 80%–93% of patients are cured by this regimen (Seaman et al., unpublished manuscript, and [5]). The combination of intramuscular aminosidine (15 mg/kg daily) and Sb^V (20 mg/kg daily), administered for 17 days, was introduced to reduce the duration of treatment. This regimen has been shown to clear parasites as effectively during treatment as the longer 30-day course of Sb^V [5], and it has subsequently been used successfully in cases with apparent drug resistance or relapse. However, as the epidemic has progressed, a number of patients have recurrently relapsed despite receiving both Sb^V and aminosidine. Increasing antimonial resistance has been documented in other epidemics [8]. Other patients have severe and complicated visceral leishmaniasis and are at high risk of dying despite treatment (Seaman et al., unpublished manuscript). These patients have reached the treatment centers after a prolonged illness and after journeying for several days by foot (or in the arms of relatives). Permanent treatment centers cannot be safely established in some areas because of the danger created by the civil war. Therefore, there is an urgent need for alternative short-course treatments that have low toxicity.

The presently available alternatives include pentamidine, Sb^V plus IFN-γ; Sb^V plus allopurinol; amphotericin B; and lipid-associated amphotericin B [9]. Pentamidine has substantial toxicity and must be given in prolonged courses [8, 10]. IFN-γ appears less useful [11] than originally thought [12]. Conventional amphotericin B is effective [13] but has significant toxicity. Only lipid-associated amphotericin B appeared to be suitable for the circumstances, which necessitate a nontoxic, effective, short-course treatment [14–19].

There are three commercial preparations of lipid-associated amphotericin B available. In the preparation of liposomal amphotericin used in this trial (AmBisome [L-AmB]; Vestar, San Dimas, CA), the amphotericin B is incorporated into 1,000-nm spherical liposomes. The drug requires an effective cold chain from manufacturer to field, its reconstitution is complex, it can be given only intravenously, and it is expensive. The other forms of lipid-associated amphotericin B available (amphotericin B lipid complex and amphotericin B cholesterol dispersion) do not share the same structure or composition, and it should

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This study was approved by the Harrow Research Ethical Committee, Harrow, U.K. Permission from local civil authorities (RASS) in Southern Sudan was obtained. Verbal informed consent was obtained from every patient or guardian. The World Health Organization Expert Committee on the leishmaniases knows of the trial and is carrying out similar studies in other centers.

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not be assumed they will have the same spectrum of activity and toxicity. The same consideration applies to solutions of amphotericin in lipid (intralipid). A fourth compound has been used with success in India [19].

An open trial was therefore instituted to determine whether L-AmB would be effective against severe and complicated drug-resistant visceral leishmaniasis and whether short-course treatment would be practical under very adverse field conditions.

Methods

Treatment Centers

Patients were recruited from February 1993 to January 1994 at three treatment centers for visceral leishmaniasis in Western Upper Nile. These centers have no electricity or running water, and there are no buildings other than tents and huts in two of the three centers. Access is by light aircraft only. During the dry season, water in one treatment center was drawn from a shallow swamp shared by wild animals and cattle.

Drug Regimens

L-AmB was maintained between 2°C and 8°C where possible, although some drug was stored at room temperature for several months. Each 50-mg vial was reconstituted with 12 mL of sterile water at room temperature. The reconstituted solution was added via a microfilter to 5–7 times its own volume of 5% dextrose.

Initially, patients were assigned to one drug regimen (regimen 1). This included three doses of 3–5 mg/kg (9–15 mg/kg in total), given on days 0, 3, and 10. This regimen was designed to balance cost, toxicity, and ease of administration. The total dose was based on preliminary experience with Mediterranean visceral leishmaniasis [14, 15], against which L-AmB is effective, and also on the finding that conventional amphotericin B (7 mg/kg) was associated with a 98% cure rate in India [13]. A "rescue" regimen of 3 mg/kg daily for 10 days was to be used in cases in which response to the assigned regimen was only partial.

Regimen 2 arose out of our initial experience with regimen 1 and included twice the dose and a longer duration (i.e., 3–5 mg/kg on days 0, 3, 6, 8, 10, and 13; total dose of L-AmB, 18–30 mg/kg). The same rescue regimen of 3 mg/kg daily for 10 days was used in cases of partial response.

Regimen 3 was four doses of 4–5 mg/kg, given on days 0, 2, 5, and 7 (a total dose of 16–20 mg/kg). This regimen was intended to provide a similar dose to that of regimen 2 within 1 week for less-severely unwell patients who had visceral leishmaniasis but did not fulfill the entry criteria of regimens 1 and 2 with respect to apparent drug resistance or severity. This would be of use in geographically isolated areas without even a temporary treatment center.

Table 1. Probability of death associated with characteristics of patients with visceral leishmaniasis who were severely ill upon enrollment.

Characteristic	Probability of death*
Body mass index of <13.0 (in adults)	.284†
Age of >50 y	.259‡
Bleeding	.183
Intractable vomiting	.238
Moribund condition	Undetermined

* Data are from Seaman, Mercer, Sondorp, and Herwaldt (unpublished manuscript).

† For body mass index between 7 and 11.

‡ For age >45 years.

Patients and Enrollment Criteria

Visceral leishmaniasis was defined by a history of fever for >2 weeks, the finding of splenomegaly, a positive direct agglutination test for leishmania infection (with a titer of >1:25,600), and the presence of leishmanial amastigotes in aspirates from either the spleen or a lymph node. The spleen size was recorded as the vertical length of spleen palpable below the costal margin in the mid-clavicular line.

Informed consent was obtained from the patient or guardian. Routine observations of weight, height, and hemoglobin concentration were made. The body mass index, defined by the formula weight/(height)^2, was used as an index of nutrition. The predominantly Nuer population who are receiving treatment is racially quite distinct, and reliable data about normal values do not exist. Therefore, an index of nutrition based on weight for height is most easily recorded. Patients were reviewed daily, and intercurrent diarrheal illness, malaria, and pneumonia were treated as appropriate. Patients for regimens 1 and 2 were selected on the basis of the following criteria: (1) relapse after treatment with combined Sb^V and aminosidine (many patients had relapsed more than once); (2) incomplete parasitological response to Sb^V and aminosidine; or (3) severe illness, with one or several of the characteristics shown in table 1.

Patients for regimen 3 were not subject to the above enrollment criteria. They were presenting for the first time with visceral leishmaniasis and had received no previous antileishmanial treatment.

All patients received antimalarial treatment with sulfadoxine-pyrimethamine and treatment for giardiasis and amebiasis with tinidazole on admission. Weekly antimalarial prophylaxis with chloroquine was given thereafter. New episodes of fever during treatment were investigated as much as possible and treated empirically. Antipyretic treatment was with paracetamol. Iron, folate, and multivitamins were given daily. All nonpregnant patients received 200,000 IU of vitamin A on admission, except those under the age of 1 year, who received 100,000 IU.

Serology for HIV was not performed for these patients. It is known that the seroprevalence of HIV in this region is low at present [3].

Outcome Definitions

Cure. Cure was defined by the absence of demonstrable parasites in a splenic or lymph node aspirate at the end of treatment. The "test of cure" aspirate was obtained at 21 days in regimens 1 and 2 and at 14 days in regimen 3.

Partial response. Partial response was defined by a decrease in the grade of parasites in splenic aspirate but incomplete clearance at the end of treatment. This category includes primary unresponsiveness (i.e., no decrease in parasite grade of splenic aspirate when the patient was first treated) and secondary unresponsiveness (no decrease in parasite grade of splenic aspirate during subsequent treatment).

Relapse. Reappearance of parasites after apparent cure was considered a relapse. Follow-up was passive, i.e., patients were encouraged to return to the treatment center if they experienced renewed symptoms.

Statistical Tests

The Student's paired *t*-test was used for the analysis of continuous paired variables. Wilcoxon's rank-sum test was used to compare nonparametric unpaired data. Fisher's exact test was used in contingency analysis.

Results

Diagnosis was established by splenic or lymph node puncture and aspirate analysis in 45 of 49 cases, and parasite density was assessed quantitatively by the method of Chulay and Bryceson [20]. In three of the remaining four cases, the diagnosis was made by direct agglutination test, which in conjunction with appropriate clinical findings is known to be reliable in these circumstances [21]. Response to treatment was determined by assessment of splenic or lymph node aspirate in 41 of the 43 cases in which the patients survived. The remaining two patients were clinically cured and the "test of cure" aspirates appeared negative, but they were of insufficient quality for conclusive assessment.

Response to Treatment with L-AmB

Regimen 1. Eighteen patients (8 females and 10 males) with severe and complicated or drug-resistant disease were enrolled according to the criteria above, and 16 survived for assessment. The median age at entry was 8.5 years (range, 0.5–70 years); median hemoglobin concentration, 7.8 g/dL (4.9–11.9 g/dL); median spleen size, 7.0 cm (0–17 cm); and median body mass index, 13.4 kg/m^2 (11.1–17.0 kg/m^2) for adults and 12.1 kg/m^2 (7.7–17.3 kg/m^2) for children. It was necessary to resort to the rescue regimen of 30 mg/kg on 8 occasions; 3 of the 8 patients were cured by this regimen. In the remaining five there was incomplete response to L-AmB alone, and it was necessary to resort to complex and prolonged combinations of both intramuscular and intravenous Sb^V, aminosidine, and L-AmB for cure. Six of 8 patients whose parasite grades were $\geq$4+ at enrollment (vs. 2 of 6 with parasite densities of $\leq$3+) required further treatment. This difference is not statistically significant, however ($P = .23$). Of 16 surviving patients receiving regimen 1, 8 were cured by the initial 3 doses, but the other 8 required further treatment (3 of them were cured by L-AmB alone). Thus, the initial cure rate was 50%, but the rate of cure following rescue with L-AmB alone was 69%.

Regimen 2. Nineteen patients (8 females and 11 males) received this regimen; there were 16 survivors. At enrollment the median age was 18 years (range, 1–52 years); median spleen size, 7.0 cm (0–19 cm); median hemoglobin concentration, 8.6 g/dL (6.0–14.7 g/dL); and median body mass index, 15.6 kg/m^2 (11.9–19.0 kg/m^2) for adults and 12.3 kg/m^2 (5.8–29.6 kg/m^2) for children. These patients were therefore significantly older than those in regimen 1 ($P < .01$), but the difference in the adult body mass index is not significant ($P = .13$). Three patients had complicated courses of treatment. One had post-kala-azar dermal leishmaniasis during treatment; although the "test of cure" aspirate was negative, he subsequently received Sb^V and aminosidine for that condition. Another patient received the rescue regimen (3 mg/kg for 10 days) and was cured. Only one patient required drugs other than L-AmB for cure of visceral disease. Thus, the overall cure rate associated with the regimen was 88% (14 of 16) for visceral disease; this rose to 94% (15 of 16) with further doses of L-AmB.

An analysis combining the patients in regimens 1 and 2 showed a significant decrease in mean spleen size (figure 1), from 8.6 cm to 4.4 cm ($n = 30$; $P < .01$), and an increase in mean hemoglobin concentration, from 8.3 g/dL to 9.6 g/dL ($n = 28$; $P < .01$). There was no significant change in weight ($P = .72$).

Regimen 3. Twelve patients (six males and six females) received this regimen. At enrollment the median age was 10.5 years (range, 2–52 years); median hemoglobin concentration, 8.1 g/dL (6.4–11.0 g/dL); median spleen size, 6.8 cm (4–16 cm); and median body mass index, 14.0 kg/m^2 (11.8–15.9 kg/m^2) for adults and 12.8 kg/m^2 (10.8–29.7 kg/m^2) for children. Seven of the 11 surviving patients were cured. Three patients showed partial response to this regimen of L-AmB and were cured by routine further treatment with Sb^V. There was a similar, significant decrease in spleen size during treatment, from 7.4 cm to 2.1 cm ($P < .01$), and a rise in hemoglobin level, from 8.1 g/dL to 9.0 g/dL ($P < .05$).

Adverse Events

Toxicity could be monitored only clinically because of the lack of facilities. The same drawback would apply to the study of any other drug used in these conditions.

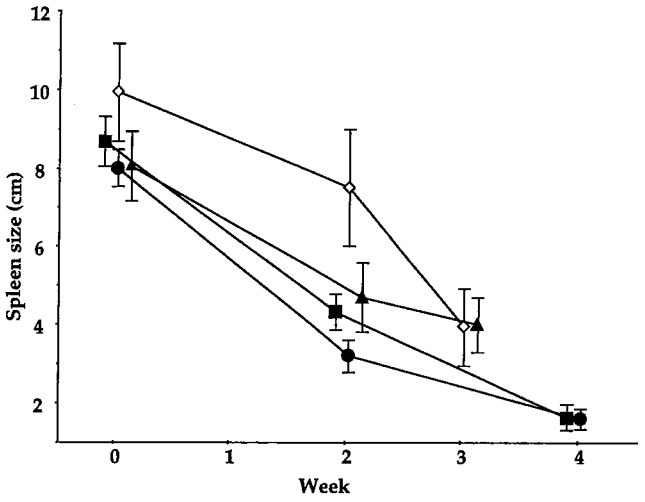

Figure 1. Spleen size (± SEM) during treatment for patients with visceral leishmaniasis (● = 67 patients treated with sodium stibogluconate in an earlier trial [5]; ■ = 20 patients cured by L-AmB in a Mediterranean trial [15]; ▲ = 30 patients cured by initial L-AmB therapy; and ◊ = 8 patients who required further therapy). Significant differences in spleen size (from that on day 0) were noted in all groups on all subsequent days on which such data were recorded ($P < .01$). There was no difference between the response of patients cured by L-AmB and that of those requiring further treatment, a finding indicating that spleen size is not a good predictor of outcome.

There were six deaths; in five of these cases treatment was not complete. Two patients receiving regimen 1 died on day 3 of treatment, after receiving two doses. There were three deaths in regimen 2, on days 2, 3, and 9. The first two of these patients had intercurrent severe illness, including hematemesis and jaundice, and one was subsequently shown to be positive for IgG to hepatitis E. The third patient had severe dehydration as a consequence of diarrhea and died after receiving three doses. In regimen 3, one child died after completing treatment but before being checked for parasite clearance. His death was thought to be due to malaria.

Four of 274 infusions went paravenous, giving rise to painful swelling in one case. Three patients received a total of 10 vials of L-AmB that had inadvertently been reconstituted in sterile water containing 0.5% phenol as a preservative. The in-line filters were seen to clog up, but no other adverse effect was noted. When the mistake was realized, the doses were readministered; the contaminated doses are not included in the analysis.

Two patients had seizures during the period of treatment (although not during the actual infusion). One of these seizures was thought to be hypoglycemic in origin. Two patients had jaundice early in treatment with regimen 3 and were switched to regimen 2. However, the subsequent appearance of jaundice in a number of patients at the same treatment center who had not received the drug suggests that the condition was not related to therapy.

Intercurrent Events

Twenty-two patients (45%) had severe diarrhea necessitating treatment with antibiotics in addition to the routine prophylaxis. A field survey of patients in one of the treatment centers (B. L. Herwaldt, Centers for Disease Control and Prevention, personal communication) demonstrated that about 20% were infected with at least one potentially pathogenic parasite. Patients in whom diarrhea developed during treatment were more likely than newly admitted patients to have many leukocytes in their stool specimens, a finding that suggests that bacillary dysentery may account for much of the nosocomial diarrhea.

Fifteen patients (31%) had clinical signs of severe pneumonia (manifest by tachypnea, fever, sputum production, and focal signs), necessitating treatment with antibiotics. Twenty-seven patients (55%) had prolonged vomiting. Six patients (12%) had bleeding during treatment. Tuberculosis was diagnosed in one patient after completion of therapy. One patient had marked residual hepatosplenomegaly despite cure and was therefore treated for presumed *Schistosoma mansoni* infection, which is endemic in the region.

Discussion

Conventionally, cure of visceral leishmaniasis is assessed at 6 months to 1 year. The final recruitment for this trial was in December 1993. Because of the circumstances of the trial, follow-up was passive (i.e., patients were encouraged to return to the center if they experienced new symptoms). We acknowledge that this is a potential weakness of the protocol, as patients may have relapsed or died following treatment. However, 17 (46%) of 37 patients in regimens 1 and 2 attended the clinic for treatment on at least a second occasion, and 9 (24%) of these came on at least a third occasion. Four of the 37 treated with L-AmB returned because of relapse during the course of the trial. For 41 of 43 patients, parasitological confirmation of cure followed treatment. A number of patients subsequently were seen informally and reported that they were well. There are no other reliable treatment facilities in the region, and the presence of Medecins Sans Frontieres is well known by the local population. We therefore hope that the relapse rate has not and will not be substantially underestimated.

The pharmacokinetics of L-AmB are unusual. It is selectively concentrated in reticuloendothelial tissue, the site of disease in cases of visceral leishmaniasis. In mice given 3 mg/kg on days 0, 1, 2, 3, 4, 5, and 10, 30% of the dose of amphotericin B administered can be recovered from the liver and spleen on day 11 and 15% on day 25 [22]. It may be that L-AmB has to saturate a given reticuloendothelial mass and that the ideal dosage will provide sufficient drug for achievement of this goal. Although the data do not reach statistical significance, there is an impression that those patients in regimen 1 with larger spleens and higher parasite densities were most likely to require further treatment. Thus, the ideal dosage may be determined by the mass of infected reticuloendothelial tissue and the intensity of infection rather than by the body weight. It is important to note that this trial was not designed to compare regimens 1 and 2, nor to compare L-AmB with Sb^V. Regimen

2 was adopted when it became apparent that there was an unacceptable rate of partial response. This regimen appears to be the best in complicated cases, defined by the criteria discussed above. Sb^V remains the drug of choice for new cases, of which we treated ~1,000 during the period of the trial.

The relative lack of efficacy of regimen 1 was surprising, as a regimen of conventional amphotericin B (7-mg/kg doses) was sufficient to cure 98% of antimony-unresponsive cases in India [13]. The patients in that trial had hemoglobin concentrations and spleen sizes at enrollment that were similar to those of our patients, but their average age and weight were much higher (24.3 years and 35.4 kg, respectively). Thus, the proportionate mass of infected reticuloendothelial tissue in our patients was higher and may have required a greater dosage of amphotericin. The possibility also exists that conventional amphotericin is more effective, although the selective concentration of L-AmB within macrophages, the site of leishmanial replication, would appear theoretically to favor use of the liposomal preparation. Amphotericin B cholesterol dispersion at a dosage of 14 mg/kg or 20 mg/kg was sufficient to cure 19 of 20 Brazilian patients [18]. However, these patients were previously untreated. In addition, although the hemoglobin concentrations at enrollment were similar, the method of determination of spleen size in that study (using a scale starting from −3 cm) was dissimilar to ours; therefore, a direct comparison is difficult. Further comparative trials of different preparations of amphotericin B are desirable for investigation of both relative efficacy and toxicity.

The six (of 32) surviving patients in regimens 1 and 2 who ultimately required treatment with drugs other than L-AmB may have been infected with parasite strains that have become resistant to amphotericin B. This has not previously been reported, although it is suspected to have occurred in patients with HIV–leishmania coinfection who have received multiple courses of AmBisome in Europe [23]. The severe malnutrition seen in our subjects may have increased their immunosuppression and contributed to this treatment failure. It is also possible that the low initial dosage and prolonged half-life contributed not only to the early relapse but also to the development of in vivo drug resistance. Another possibility is that exposure to high ambient temperatures may have caused the drug to deteriorate during storage; this possibility warrants further investigation.

When Dietze et al. conducted a trial of amphotericin B cholesterol dispersion in Brazil [18], chills and fever were not infrequently noted, and in three children under the age of 3 years a marked increase in respiratory rate was related to infusion [18]. Thirteen of the patients in the present study were children aged ≤3 years. We did not record any similar increase in respiratory rate or note the occurrence of chills in our patients, and these effects have not been seen in European patients [15]. Although we administered no systematic premedication, many of our patients did receive paracetamol (acetaminophen) early in treatment. It is interesting that Dietze et al. [18] reported that the subsequent use of diclofenac sodium seemed to reduce the severity of the problems they noted. The same European trial documented no significant change in urea or creatinine values during treatment or 6 months later.

Administration of L-AmB is suitable as treatment for drug-resistant visceral leishmaniasis. Our present regimen of choice in the field is administration of 4 mg/kg on days 0, 3, 6, 8, 10, and 13. The fact that no toxicity was seen in this trial and in more detailed European trials [15] makes it possible to give L-AmB to extremely sick patients. Sb^V remains the drug of choice for effective treatment of the majority of new cases. In addition, the cost of treatment with L-AmB (~U.S.$800 per patient cured) is much greater than that with Sb^V, which also is expensive when resources are scarce. However, we believe the results of this trial conducted in very difficult circumstances warrant further comparative trials of L-AmB and also of other lipid-associated amphotericin B compounds.

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