Short-Course Treatment of Visceral Leishmaniasis with Liposomal Amphotericin B (AmBisome)


We evaluated liposomal amphotericin B (AmBisome; Vestar, San Dimas, CA) administered to 88 immunocompetent patients (56 children) with visceral leishmaniasis (VL) caused by *Leishmania infantum*. Thirteen patients received 4 mg/kg on days 1–5 and 10 (total dose, 24 mg/kg), and all were cured; 42 received 3 mg/kg on days 1–5 and 10 (18 mg/kg), and 41 were cured; 32 received 3 mg/kg on days 1–4 and 10 (15 mg/kg), and 29 were cured (amastigotes were not cleared from 1 child, and 2 relapsed). One adult was cured with a total dose of 12 mg/kg. The four children who were not cured received 3 mg/kg for 10 days; none had further relapses. There were no significant adverse events. For VL due to *L. infantum*, we recommend a total dose of AmBisome of ≥20 mg/kg, given in ≥5 doses of 3–4 mg/kg over ≥10 days.

Visceral leishmaniasis (VL), also known as kala-azar, affects about 1,000 people annually in the Mediterranean countries [1]. Disease prevalence is increasing in many areas, both among immunocompetent people and among those coinfected with HIV. Large numbers of domestic dogs are chronically infected with the causative parasite, *Leishmania infantum*, and so eradication of VL is likely to be impossible.

We have recently shown that liposomal amphotericin B (L-AmB; AmBisome, Vestar, San Dimas, CA) is a highly effective alternative to traditional treatment of VL with the pentavalent antimony (SbV) compounds sodium stibogluconate and meglumine antimoniate [2]. Total doses of L-AmB of 21–30 mg/kg, given as daily doses of 1–3 mg/kg over 10–21 days, were uniformly effective and nontoxic in 20 immunocompetent patients with Mediterranean VL. We have shown that in mice infected with *L. infantum*, L-AmB is targeted to the liver and spleen—the sites of infection in VL—and that after intermittent dosing very high levels of amphotericin B are sustained in these tissues for >14 days [3].

We therefore conducted a dose-ranging study to take advantage of these pharmacokinetic properties, in which the period of hospitalization, and the total dose of L-AmB, would be progressively reduced. We felt it unwise to reduce the overall duration of exposure of *Leishmania* species organisms in infected tissues to <20 days, which is the standard duration of amphotericin B therapy used by others [4]. For this reason, we administered a series of daily doses of L-AmB with a final dose on day 10 in all cases.

Methods

The study was conducted at hospitals experienced in the treatment of VL. The study was approved by each local ethical committee. R. N. D. was study coordinator and L. G. was in charge of the parasitology reference laboratory in Rome, where most aspirates for microscopy and culture and serum specimens for leishmanial serology were sent.

Patients

Patients were eligible for the study if *Leishmania* species organisms were visible in or cultured from an aspirate of bone marrow. Informed consent was obtained from each patient or guardian. Patients were excluded if consent was not obtained or if they were pregnant or had been previously treated with L-AmB or conventional amphotericin B. Immunocompromised patients were defined as those who had coexisting HIV infection or malignant disease; had previously had malignant disease; had another illness such as chronic hepatitis; or were receiving steroid or cytotoxic therapy. These patients were excluded because of the high relapse rates associated with VL in this group [2, 5].
Study Design

Case definitions. A patient was defined as cured if no *Leishmania* species were noted in the smear or culture of bone marrow at day 21 and if no relapse of VL occurred within 1 year after treatment. Drug failure was defined by the presence of *Leishmania* species organisms in the smear or culture of bone marrow at day 21. Relapse was defined as the reappearance of such organisms in the bone marrow aspirate or culture positivity after apparently successful treatment.

This was a dose-decreasing study. We began with a total dose of 24 mg/kg, and for subsequent enrollments we reduced the total doses to 18, 15, and 12 mg/kg. We introduced the next (lower) dosage only when >10 patients in the previous (higher) dosage group had been followed up for >6 months without occurrence of a treatment failure or relapse. If two or more treatment failures or relapses occurred in any group, they were considered to be the result of a suboptimal dose, and then dose reduction would cease. The group receiving a dose immediately above the suboptimal dose would then be expanded. Our intention was to treat a total of 75–125 patients over 30 months in Europe and Brazil.

Treatment Regimens

The L-AmB dosage groups were as follows. Group 24MK received 4 mg/kg on days 1, 2, 3, 4, 5, and 10. Group 18MK received 3 mg/kg on days 1, 2, 3, 4, 5, and 10. Group 15MK received 3 mg/kg on days 1, 2, 3, 4, and 10. Group 12MK received 3 mg/kg on days 1, 2, 3, and 10. The regimen for relapses was 3 mg/kg on days 1–10.

All patients were hospitalized for the first 4 or 5 days of treatment. L-AmB was prepared according to the manufacturer's instructions and infused over 30–60 minutes in 5% dextrose via a temporary canula in a peripheral vein.

Parasitology

All patients' bone marrow aspirates were, by definition, positive for *Leishmania* (as proved by microscopy or culture) at entry. For the 83 patients treated in Italy, bone marrow aspirates (1–3 mL in EDTA or citrate) were sent by rapid mail to the Istituto Superiore di Sanita, in Rome. On arrival the material was seeded in Evans' modified Tobi's medium and in "sloppy" Evans' medium [6]. Leishmanial stocks isolated were characterized by electrophoretic analysis of 13 isoenzymes (15 enzymic loci): PGM, GPI, GOT1, GOT2, ME, 6PGD, G6PD, MDH, NH1, NH2, MPI, ICD, DIA, GLUD, and FH [7]. Indirect fluorescence was used to detect IgG antibodies to *Leishmania*. The antigen was prepared from promastigotes of the World Health Organization reference strain of *L. infantum* (MHOM/TN/80/IPT1). The threshold titer for positivity was 1:80 [8].

Clinical and Laboratory Assessments

The following data were recorded: age, sex, weight, findings of leishmanial serology, and HIV antibody status. Clinical, hematologic, and biochemical assessments were made on days 0, 4 or 5, 10, and 21 and at months 1, 3, 6, and 12 after treatment. Spleen size was measured (along its longest axis) during quiet breathing. Weight and occurrence of fever (temperature, >37.5°C) during the previous 24 hours were recorded. A second bone marrow aspirate for leishmanial testing was obtained on day 21. Adverse events and their possible relation to L-Amb were recorded. All clinical observations of each patient were made by the same observer. The laboratory that performed the initial parasitological and blood analyses for a patient was used for all subsequent analyses.

Statistical Methods

Patients served as their own controls in all comparisons. Mann-Whitney *U* tests or Student's *t*-tests were used as appropriate. All comparisons were two-tailed.

Results

We enrolled 88 patients from August 1992 to June 1994; 83 were treated in Italy, 3 in Brazil, and 2 in the United Kingdom. All patients' serological tests for *Leishmania* were positive, with immunofluorescent antibody titers in the range of 1:160 to 1:10,240.

*Leishmania* Zymodeme Analysis

Zymodeme analysis was performed on isolates from 45 patients; 28 were found to be Montpellier (MON) 1, the commonest zymodeme of *L. infantum* in the Mediterranean. Seventeen patients from the area near Naples had parasites belonging to *L. infantum* zymodeme MON 72, which is typical in that area [9].

Efficacy of Treatment

Group 24MK. Thirteen patients received L-AmB (4 mg/kg) on days 1, 2, 3, 4, 5, and 10. Ten were children (5 females) aged 14 months to 9 years, and 3 were adults (1 female) aged 16, 18, and 25 years. Ten had been infected in Italy and three in Brazil. Eleven patients were previously untreated, and 2 Brazilian children had previously received 1 and 2 courses, respectively, of SbV for VL. All 10 Italian patients were pronounced definitively cured at the 12-month follow-up visit. The three Brazilian children were initially cured at day 21 but were lost to follow up after 1.1, and 3 months, respectively, having returned from Sao Paulo to a rural area of endemicity.
Group 18MK. Forty-two patients received L-AmB (3 mg/kg) on days 1, 2, 3, 4, and 10 (total dose, 18 mg/kg). Twenty-six were children (12 females) aged 5 months to 15 years, and 16 were adults (6 females) aged 17-63 years. Thirty-eight were probably infected in mainland Italy, 2 in Sicily, 1 in Portugal, and 1 in Malta. Forty-one patients had been previously untreated, and one 19-month-old girl had previously received Sb for VL. All 42 patients were initially cured at day 21. One child (aged 6 years) relapsed 3 months after treatment; one adult left the study after 1 month. There were no relapses among the remaining 40 patients, who were followed up for ≥1 year.

Group 15MK. Thirty-two patients received L-AmB (3 mg/kg) on days 1, 2, 3, 4, and 10. Nineteen were children (7 females) aged 7 months to 15 years, and 13 were adults (7 females) aged 17-82 years. Thirty were probably infected in mainland Italy and two in Sicily. Thirty-one patients had been previously untreated, and a 19-month-old girl had previously received two courses of Sb for VL. There was one treatment failure: a 23-month-old boy’s bone marrow aspirate on day 21 was culture-positive but microscopy-negative for Leishmania. Although he had responded clinically to treatment, he was immediately treated as if he had relapsed. Two other patients, boys aged 15 and 17 months, relapsed at 3 months and were re-treated. The remaining 29 patients were followed up for ≥1 year and remained well.

Group 12MK. Only one man received treatment with this regimen of L-AmB (3 mg/kg on days 1, 2, 3, and 10). He responded clinically and parasitologically and was pronounced cured at the 12-month follow-up visit.

Speed of Response

All patients had a very rapid subjective and objective clinical response, which is summarized in figure 1 and table 1. Typically, appetite and well-being returned by the third day of treatment. Fever was noted on day 0 in 67 patients, on day 5 in 2, and on day 10 in 1. Splenomegaly, pancytopenia, elevated erythrocyte sedimentation rates, and hypoalbuminemia all showed significant change (P < .05) toward normal values by day 5.

Adverse Events

Treatment was well tolerated, and no episodes of phlebitis at the infusion site occurred. Mild and self-limiting adverse events were reported with regard to three patients: enlarged cervical lymph nodes on days 5-17 in a child aged 14 months, mild arterial hypotension after the first dose in a man aged 18 years, and pyrexia (temperature of 40°C) on days 3 and 4 in a child aged 2 years. For no patient was the treatment regimen changed because of adverse events or biochemical toxicity.

Biochemical Toxicity

Electrolytes. Mean serum Na+ and K+ concentrations did not alter significantly during or after treatment in any patient group. Analysis of the pooled data for all patients showed that the mean (±SD) serum K+ concentration fell from 4.38 (±0.54) mmol/L pretreatment to 4.16 (±0.63) mmol/L on day 5 (P < .05), 4.32 (±0.68) mmol/L on day 10 (NS), and 4.37 (±0.71) mmol/L on day 21. There was no significant difference in the proportion of patients with hypokalemia (serum K+ concentration, <3.5 mmol/L) before, during, or after treatment (P > .05, Fisher’s exact test). A serum K+ concentration of 2.4 mmol/L was found on day 10 in one patient in group 24MK. This was the only instance in which a K+ value was <2.5 mmol/L.

Renal function. All groups showed a rise in serum urea level during treatment (figure 1F and table 1), which was statistically significant (P < .05), maximal at day 5, and of small magnitude: the mean rise in urea level was 3.06 mmol/L. The mean change in serum creatinine concentration on day 5 (figure 1E and table 1) was smaller (7.1 μmol/L), suggesting that some of the rise in serum urea concentration was due to increased hepatic urea synthesis. The return to normal of hepatic synthetic function was shown by the rise in serum albumin levels (figure 1A and table 1).

Liver enzymes. There was a sustained, significant decrease (P < .01) in serum alanine transaminase and aspartate transaminase levels in the 18MK and 24MK groups from month 1 of follow-up onward. In a child aged 10 months, liver enzyme levels that were elevated before L-AmB treatment rose and then settled to normal during treatment. In the children in all groups, serum alkaline phosphatase levels rose significantly, beginning on day 21. This probably reflected normal growth, as there was no similar rise among adults.

Discussion

This study confirms that AmBisome is a highly effective, nontoxic form of treatment for VL when administered in a short course (either five or six doses over 10 days). The latter half of this treatment period can be spent outside the hospital, because patients generally experience a rapid defervescence and return of appetite and well-being. We confirmed the impression in case reports [10-12] that L-AmB is safe and effective in young children: 26 of our 88 patients were children ≤2 years of age, and they responded very rapidly to treatment, without toxicity.

In this study, our previous study [2], and published cases [10-13] combined, a total of 112 patients have been treated with AmBisome for Mediterranean VL. All 37 patients who received total AmBisome doses of ≥20 mg/kg have been cured. The only four treatment failures, which are reported here, occurred among 75 VL patients who received a total dose of ≤18 mg/kg. All four patients who required second courses of L-
AmB treatment were children, and all were cured by a total dose of 30 mg/kg.

This suggests that underdosing rather than drug resistance may have been responsible for the treatment failure. The reticuloendothelial tissue mass is greatly increased in cases of VL, and in a murine model of VL, ~50% of the total administered dose of L-AmB can be recovered from the liver and spleen [3]. It may be, therefore, that children require doses based on their reticuloendothelial mass, which may increase out of proportion to their body mass; conversely, it may be that adults could be cured with even lower doses (per kg of body mass) than we administered.

Permanent cure of VL relies upon the recovery of cellular immune responses to *Leishmania* species. Our patients were

Figure 1. Response of patients with visceral leishmaniasis to treatment with liposomal amphotericin B (AmBisome). Mean (±SEM) values for hemoglobin, serum albumin, erythrocyte sedimentation rate, and spleen size are shown. For serum urea and creatinine values, mean (±SEM) change from pretreatment value is shown (ESR = erythrocyte sedimentation rate; ▲ = group 24MK; ■ = group 18MK; ○ = group 15MK [see text for corresponding dosages]).
Table 1. Changes in mean hematologic and biochemical values and spleen size for 88 patients with visceral leishmaniasis, in the days following treatment with liposomal amphotericin B (AmBisome).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean (±SEM) value on indicated day following initiation of treatment</th>
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<tbody>
<tr>
<td></td>
<td>0</td>
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<tr>
<td>Hb (g/dL; normal, 12–17)</td>
<td>8.08 ± 0.16</td>
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<tr>
<td>WBCs (×10^9/L; normal, 5–10)</td>
<td>3.94 ± 0.29</td>
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<tr>
<td>Platelets (×10^9/L; normal, 200–400)</td>
<td>108.21 ± 8.82</td>
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<tr>
<td>Spleen size (cm)</td>
<td>7.13 ± 0.4</td>
</tr>
<tr>
<td>Albumin (g/L; normal, 35–55)</td>
<td>32.57 ± 0.57</td>
</tr>
<tr>
<td>ESR (mm first hour; normal, &lt;7)</td>
<td>90.04 ± 3.2</td>
</tr>
<tr>
<td>Urea (mmol/L; normal, 2.8–7.7)</td>
<td>8.5 ± 0.37</td>
</tr>
<tr>
<td>Creatinine (μmol/L; normal, 80–170)</td>
<td>66.07 ± 3.27</td>
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NOTE. ESR = erythrocyte sedimentation rate. *P < .05.
†P < .01.

not severely cachectic and did not have underlying immunosuppression due to HIV or other causes. In cases of severe and complicated VL in war-torn south Sudan—where patients are malnourished, suffer from coexisting parasitic, bacterial, and viral infections, and have VL in an extremely advanced state—we found different dosage regimens of L-AmB to be effective for 33 of 49 patients, most of whom would have been expected to die [14]. However, in such difficult circumstances, treatment should be given over at least a 14-day period. For patients with VL who are coinfected with HIV, the situation is, again, different: whilst AmBisome has little toxicity, eventual relapse may be severe.

Two other particulate, lipid-complexed, amphotericin B preparations have undergone studies with regard to treatment of leishmaniasis. These should not be considered equivalent to AmBisome, the formulation of L-AmB we used; the pharmacokinetics are dissimilar, and both their toxicity and efficacy in cases of VL are likely to be different [15]. Amphocil (amphotericin B cholesterol dispersion; Zeneca, London, UK, and Liposome Technology, Menlo Park, CA) has been used in Brazilian patients with VL. A regimen of 2 mg/kg for 7 or 10 days cured all 20 patients [16], and 2 mg/kg for 5 days cured 9 of 10 patients [17]. Adverse events, including fever, chills, and respiratory distress, were common in children <3 years old. The adverse effects of Amphocil were partly preventable by pretreatment with nonsteroidal antiinflammatory drugs. Abelcet (amphotericin B lipid complex, previously called ABLC; Liposome Co., Princeton, NJ) has been used in 61 Peruvian patients with mucocutaneous leishmaniasis, in whom it was less toxic but also less efficacious than amphotericin B desoxycholate [18].

The chief obstacle to the use of L-AmB for VL in developing countries is cost. The retail drug cost (in American dollars) of treating a 30-kg VL patient with sodium stibogluconate (Pentostam; Wellcome, London, UK) is ~$200; with meglumine antimoniate (Glucantime; Specia, Paris, France), ~$100; with sodium antimony gluconate (Albert David, New Delhi, India), ~$25; and with L-AmB, ~$1,500 – $2,500. Some manufacturers will provide their drug at far lower prices than these for humanitarian reasons.

Pharmacologists in India have successfully treated a patient with VL with their own formulation of liposomal amphotericin B [19]. A simpler approach has been to mix intravenous-feeding lipids and amphotericin B; however, the mixture appears to be no less toxic than amphotericin B desoxycholate [20]. Reports on several large studies of amphotericin B treatment of Indian VL have recently been published [4, 21, 22]. These studies found that amphotericin B, given at a dosage of 1 mg/kg daily or on alternate days (to a total dose of 20 mg/kg), resulted in only mild toxicity and a higher cure rate than with SBV.
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References