Amphotericin B lipid complex versus meglumine antimoniate in the treatment of visceral leishmaniasis in patients infected with HIV: a randomized pilot study

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Optimal treatment for HIV-related visceral leishmaniasis (VL) has still to be established. A pilot clinical trial was carried out in 57 HIV–VL coinfected patients to compare the efficacy and safety of amphotericin B lipid complex (ABLC) versus meglumine antimoniate. The patients were randomized to receive either ABLC 3 mg/kg/day for 5 days (ABLC-5, 18 patients), ABLC 3 mg/kg/day for 10 days (ABLC-10, 20 patients) or meglumine antimoniate 20 mg Sbv/kg/day for 28 days (19 patients). Treatment was considered successful if parasites were not detected in a bone marrow aspirate after treatment. Parasitological cure was attained in 33% (95% CI: 13%–59%) of the ABLC-5 group, in 42% (95% CI: 16%–62%) of the ABLC-10 group and in 37% (95% CI: 16%–62%) of the meglumine antimoniate group (P = 0.94). Eight out of 19 patients administered antimoniate discontinued treatment prematurely following serious adverse events, compared with one in the ABLC groups (P = 0.0006). The efficacy of ABLC is similar to meglumine antimoniate, but the severity of toxicity in the treatment of HIV–VL is lower with ABLC.

Keywords: clinical trials, Leishmania, anti-leishmanial drugs

Introduction

Visceral leishmaniasis (VL) is a severe disease caused by intracellular protozoa of the genus Leishmania. VL is common in patients with HIV infection living in endemic areas, and it is estimated that 2%–9% of patients with AIDS in southern Europe will suffer new or reactivated VL.1 The pentavalent antimony (Sbv) salts, sodium stibogluconate and meglumine antimoniate, have been used for VL treatment both in immunocompetent and immunodepressed patients in most of the world.1–3 However, as evidence of efficacy from clinical trials in patients with HIV is scarce, first-line treatment of VL in this population remains controversial. In a previous clinical trial, the anti-leishmanial efficacy of amphotericin B deoxycholate was shown to be similar to meglumine antimoniate in HIV patients.4 However, both treatments are highly toxic, thus limiting their use.

New lipid formulations of amphotericin B are less toxic than the conventional version5,6 and have shown good anti-leishmanial efficacy in experimental models.7 Some lipid formulations of amphotericin B have also been tested in immunocompetent patients with VL, and low toxicity and high efficacy were usually obtained.8–11 Also, previous results suggest that liposomal amphotericin B provides initially successful therapy of VL in HIV patients.9,12–14 However, no data are available on the efficacy and toxicity of other amphotericin B lipid formulations in HIV patients with VL.

The present pilot study was designed to investigate the efficacy and tolerability of two dosing schedules of amphotericin B lipid complex, as compared to a reference treatment, meglumine antimoniate, in patients with HIV during a first episode of VL.

Subjects and methods

Fifteen major Spanish teaching hospitals took part. The study was approved by the Ethics Committee of each centre and authorized by the Spanish...
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Health Authorities; it was conducted in accordance with Good Clinical Practice Guidelines. Written informed consent was obtained from all patients enrolled in the study.

**Patient population and exclusion criteria**

From August 1997–September 1999, HIV patients who had undergone their first episode of VL were enrolled in the study. Male and female patients aged 18 years or older were eligible for inclusion if they had a diagnosis of HIV confirmed by Western blot, and a first episode of parasitologically confirmed VL. VL was diagnosed in patients showing compatible clinical symptoms and a positive Giemsa-stained smear or culture for *Leishmania* parasites in samples taken from bone marrow, spleen or liver. Exclusion criteria included patients with pancreatitis, prothrombin activity <40%, aminotransferase levels 10× the upper normal limit, myocardopathy, heart failure, a QT corrected interval >500 ms, creatinine levels >twice the upper normal limit, allergy to either ABLC or meglumine antimoniate, concomitant treatment with dideoxycytidine or dideoxyinosine and a life expectancy of <6 months. Women of childbearing potential were excluded if they were pregnant, might become pregnant, or were lactating. Active opportunistic infections were not an exclusion criterion.

**Study design and protocol description**

A multicentre, open-label, blinded, centrally randomized, parallel trial was carried out to compare meglumine antimoniate with two dosages of amphotericin B lipid complex in the treatment of first episodes of VL in HIV patients.

A randomization list was prepared using the SAS program, which stratified patients into two groups, depending on the CD4 cell count at inclusion: above or below 200 cells/mm³. If this information was missing at the time of randomization, it was considered equivalent to the lymphocyte count: above or below 1000 cells/mm³. The randomization process was blinded and centralized. Each patient was treated on an open-label basis with one of the following three treatments: ABLC (Abelcet, The Liposome Company, Princeton, NJ, USA) 3 mg/kg body weight daily intravenously (iv) for 5 days, ABLC 3 mg/kg body weight daily iv for 10 days or meglumine antimoniate (Glucantime, Rhône-Poulenc, Madrid, Spain) 20 mg Sb³/kg body weight daily by parenteral route for 28 days as control. Treatment compliance was assessed by counting the number of vials in the returned boxes and by monitoring the clinical history. Treatment with other potentially anti-leishmanial drugs was forbidden during the study. Symptomatic treatment to prevent infusion-related toxicity was carried out under the investigator criteria.

Baseline evaluation included confirmation of HIV infection, VL-related symptoms and physical examination. In addition, a CD4+ T lymphocyte count, a complete blood cell count, a coagulation test and a blood-chemistry panel test were carried out. During the treatment period, clinical evaluation was performed daily, and full blood counts and blood-chemistry tests were performed twice a week. These evaluations were also performed 1 and 5 months after therapy. Electrocardiograms were carried out at baseline, weekly, at the end of therapy and 5 months later. Adverse events were assessed each day during the treatment period, and at 1 and 5 months thereafter. The World Health Organisation (WHO) scale for toxicity was used. A toxicity level on the WHO scale of grade 2 or higher was considered to be an adverse event.

The primary endpoint of the study was parasitological cure. This was defined as the presence of no *Leishmania* parasites in the smear or culture of the tissue biopsy sample, taken from the organ used at inclusion (bone marrow, spleen or liver) between 1 and 7 weeks after the completion of therapy. Therapy failure was defined as parasite persistence in any sample after a complete course of therapy, or the availability of no valid parasitological data after the treatment. VL relapses were studied during a 5 month follow-up period. Bone marrow aspirates or tissue biopsy samples were taken whenever there was a clinical suspicion of VL relapse. It was diagnosed if parasites were observed in tissue samples after the initial parasitological cure. If no relapse symptoms were observed 5 months after the end of therapy, a bone marrow aspirate was performed to confirm the absence of relapse.

**Parasitological tests**

Tissue samples were analysed at the microbiology, haematology or pathology departments of each hospital. In addition, all bone marrow samples were analysed in a central laboratory: the WHO Collaborating Centre of Leishmaniasis, at the Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Madrid. Demonstration of *Leishmania* amastigotes in tissue samples was performed using smears of May–Grünwald-Giemsa stains examined at ×1000 magnification. Bone marrow aspirates were cultured in Novy–McNeal Nicolle medium. Cultures were incubated at 25°C and considered negative if no flagellates were observed after 4 weeks. The microbiologists, pathologists or haematologists who evaluated the tissue samples at the initial cure and relapse were blinded to the therapy received by each patient. In order to ascertain whether relapses were true relapses or reinfections, a genotyping test, using polymerase chain reaction plus restriction fragment length polymorphism was performed in all samples obtained before and after treatment, and is described elsewhere.²³

**Statistical analysis**

Since this was a pilot study, no formal calculation was made of the sample size required. An expected sample of 20 patients per group was considered sufficient to provide a reasonable estimate of treatment effect. The proportions of patients with treatment success were compared among treatment groups by means of the Fisher’s Exact Test, and their 95% CIs estimated using binomial distribution. Safety analysis included all patients who had received at least one dose of drug. Proportions of patients showing adverse events were compared by the Fisher’s exact test.

**Results**

Fifty-seven HIV patients with a first episode of VL were included in the study. Of these 57, 18 were randomly assigned to receive ABLC for 5 days, 20 ABLC for 10 days and 19 meglumine antimoniate for 28 days. Baseline demographics and clinical characteristics are summarized in Table 1. No differences were found in demographic data, history of HIV disease, parasitological data for the diagnosis of LV, concurrent HIV medication, clinical symptoms or analytical data. Only 15 patients were on HAART therapy at VL diagnosis. There were no differences in CD4+ cell counts between patients with or without HAART therapy.

Tissue samples used for the diagnosis of VL originated from the bone marrow in 54 cases (52 with a positive diagnosis by smears, two by culture and 33 with both techniques) and from the liver in two cases. One patient was incorrectly included with a positive palate biopsy, but *Leishmania* parasites were not detected in others samples.

Patient outcome is shown in Table 2. The intention-to-treat population included 56 patients. Only one patient treated with ABLC-10 was excluded from this analysis for failing to meet a major entry criterion: the patient was randomized without a confirmed VL diagnosis. Thirteen patients were excluded from the on-treatment analysis for the following reasons: (1) Early withdrawal with lack of efficacy data: one patient in each group was lost to follow-up, and eight patients in the antimonal group were excluded prematurely follow-
ing serious adverse events. (2) Major protocol violation: one patient in the antimonial group and one in the ABLC-5 group had their efficacy control performed outside the planned time interval (efficacy control performed the day of treatment discontinuation, efficacy control performed 113 days after treatment discontinuation). The on-treatment population consisted of 43 patients: 16 patients in the ABLC-5 group, 18 in the ABLC-10 group and nine in the meglumine antimoniate group. Intention-to-treat analysis of data showed similar efficacy for all treatments. Six (33%; 95% CI: 13%–59%) of 18 patients in the ABLC-5 group, eight (42%; 95% CI: 20%–67%) of 19 in the ABLC-10 group and seven (37%; 95% CI: 16%–62%) of 19 in the antimonial group were parasitologically cured (*P* = 0.96).

In an on-treatment analysis, six (38%; 95% CI: 15%–65%) of 16 patients in the ABLC-5 group, eight (44%; 95% CI: 22%–69%) of 18 in the ABLC-10 group and seven (78%; 95% CI: 40%–97%) of nine in the antimonial group were cured.

The incidence of at least one treatment-related adverse event was similar between the groups (Table 3). Eight out of 19 patients discontinued meglumine antimoniate treatment following serious adverse events: three patients died (one by sudden death, one—a patient with chronic hepatitis—from upper digestive bleeding and another from severe pneumonia), four patients had clinical pancreatitis and two others renal failure. Conversely, no deaths occurred in the ABLC groups during the treatment period, and only one patient stopped the treatment, as a result of severe septic pneumonia (*P* = 0.0006). Infusion-related adverse events occurred only with ABLC and were mild. A total of 15 (40%) of 38 patients who received ABLC reported shivers and/or fever and/or vomiting with the infusion. However, the total number of patients showing adverse events not related to treatment infusion was higher in the antimonial treatment group (14 of 28, 50%) than in the ABLC groups (ABLC-5: four of 18, 22%; ABLC-10: seven of 20, 35%).

Twenty-two patients initially considered as cured were followed-up for 5 months. Only 19 of them had valid relapse data: seven in the antimonial group, four in the ABLC-5 group and eight in the ABLC-10 group. The percentage of VL-free patients at 5 months of follow-up was 50% (95% CI: 7%–93%) in the ABLC-5 group, 38% (95% CI: 9%–76%) in the ABLC-10 group, and 57% (95% CI: 17%–90%) in the antimonial group.

**Discussion**

This is the first comparative clinical trial testing amphotericin B entrapped in lipids versus a standard treatment in HIV patients with a first episode of VL. The results suggest that amphotericin B lipid complex presents a similar rate of initial cure, no treatment-related
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Table 3. Treatment-related adverse events reported during the clinical trial. Number of patients (%)

<table>
<thead>
<tr>
<th></th>
<th>ABLC-5 group (n = 18)</th>
<th>ABLC-10 group (n = 20)</th>
<th>Antimonial group (n = 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death</td>
<td></td>
<td></td>
<td>1 (5%)</td>
</tr>
<tr>
<td>Shivers and/or fever and/or vomiting with administration</td>
<td>7 (39%)</td>
<td>8 (40%)</td>
<td></td>
</tr>
<tr>
<td>Phlebitis</td>
<td>1 (6%)</td>
<td>1 (5%)</td>
<td></td>
</tr>
<tr>
<td>Vomiting</td>
<td>1 (6%)</td>
<td>2 (10%)</td>
<td>4 (21%)</td>
</tr>
<tr>
<td>Epigastric pain</td>
<td></td>
<td></td>
<td>1 (5%)</td>
</tr>
<tr>
<td>Pancreatitis</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Hyperamylasaemia</td>
<td>2 (11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal failure</td>
<td>1 (6%)</td>
<td></td>
<td>1 (5%)</td>
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<tr>
<td>Itching</td>
<td>1 (6%)</td>
<td></td>
<td></td>
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<tr>
<td>Hypokalaemia</td>
<td></td>
<td>1 (5%)</td>
<td></td>
</tr>
<tr>
<td>Worsening anaemia</td>
<td>3 (15%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arthralgias</td>
<td></td>
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<td>1 (5%)</td>
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</table>

deads and a lower rate of serious adverse events compared with meglumine antimoniate.

The trial was designed before HAART had become a standard HIV treatment in Spain, but the inclusion of patients started at the beginning of the HAART era. This therapy increases the CD4 cell count and decreases the incidence of opportunistic infections in most patients as a result of the partial restoration of immunity. In fact, HAART, as with other opportunistic infections, reduced the incidence of primary VL episodes. This may have been why recruiting the number of patients scheduled in the protocol was drawn out. In our trial, however, most patients developed VL when they were not being treated with HAART, which could protect them if the induced immunological response is sufficient.

The pentavalent antimonial was selected as the reference compound because it was first-line therapy for VL in HIV patients at the beginning of the trial and is the treatment recommended by the WHO. In a comparative clinical trial with a similar design and dose schedules, we found an initial cure of 66% (95% CI, 50%–79%) using parasitological criteria. We believe that the low response rate attained in the present trial, using pentavalent antimonials, is partially due to a high rate of treatment drop-out due to severe adverse events or early death. In fact, as with our previous trial and using strict parasitological criteria, more than 75% of patients who completed treatment attained a parasitological initial cure.

Tolerability, incidence and type of adverse events reported for antimonial therapy correlated well with those previously described, frequently being pancreatic damage. In HIV patients, both slight and serious antimonial-related electrocardiographic alterations have been reported. In our study, no serious electrocardiographic changes were detected, although one unexpected sudden death was reported after 2 weeks of treatment, possibly due to antimonial toxicity. On the other hand, in the present trial, ABLC treatment was generally well tolerated with few infusion-related adverse events, and only one of 38 patients treated with ABLC showed renal failure.

ABLC has been shown to be a successful treatment in immunocompetent Indian patients with VL. The present trial, ABLC has been tested in a new setting, in severely immunosuppressed patients with VL caused by Leishmania infantum species, resulting in a low response with the doses used. We cannot explain these results. ABLC has not been tested as a treatment for VL in other countries, and we do not know the total doses of ABLC required to produce a high-level response in immunocompetent patients from other areas. Results from the different trials have shown that immunocompetent Indian and Kenyan patients with VL require lower doses of liposomal amphotericin B, as compared with European or Brazilian subjects, to attain parasitological cure. It has been suggested that these differences could be explained by different susceptibilities of the Leishmania species (Leishmania donovani in India and Kenya, L. infantum in Mediterranean VL, and Leishmania chagasi in Brazil) to amphotericin B, differences in the ages of patients and also in the splenic parasitic burden. Moreover, it seems that liposomal amphotericin B could be more efficacious than ABLC in immunocompetent Indian patients, although no formal comparative trial has been made between the drugs. If these results are true, it could be assumed that the total dose of ABLC required to attain a high parasitological initial cure in HIV–VL coinfected Mediterranean patients must be higher than the lowest dose of liposomal amphotericin B with high efficacy in this population.

At present, there is no general consensus about VL treatment in HIV patients in our country. However, pentavalent antimonials remain first-line therapy for new VL cases in most Spanish hospitals, because liposomal amphotericin B is expensive. If the patient develops severe toxicity, intolerance or treatment failure then liposomal amphotericin B is administered. Similarly, liposomal amphotericin B is the preferred drug in VL relapses.

In summary, although both treatments—meglumine antimoniate for 28 days and amphotericin B lipid complex for 5 or 10 days—showed a similar rate of initial cure, ABLC had a lower rate of serious adverse events, and no deaths occurred during the treatment period. Consequently, we believe that ABLC might to be an alternative to meglumine antimoniate therapy for VL–HIV coinfection. However, more clinical trials with higher doses and pharmacoeconomic studies are necessary to support our proposal.

Acknowledgements

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References


