Efficacy of liposomal amphotericin B for secondary prophylaxis of visceral leishmaniasis in HIV-infected patients

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Received 14 March 2007; returned 24 April 2007; revised 30 April 2007; accepted 11 July 2007

Background and objectives: Visceral leishmaniasis (VL) is characterized by frequent relapses in HIV-infected patients, even in those who receive secondary prophylaxis. The aim of our study was to evaluate the efficacy of liposomal amphotericin B (L-AMB) for secondary prophylaxis of VL in HIV-infected patients.

Methods: From January 2001 to December 2005, 17 HIV patients, with at least one previous episode of VL who received L-AMB as secondary prophylaxis for VL, were included in the study. Efficacy was measured as the proportion of patients remaining free (non-relapse) of VL at different time points. Relapses were analysed as time-to-relapse distribution and were evaluated by survival analysis using the Kaplan–Meier method.

Results: Twenty-one episodes of VL were diagnosed and nine relapsed. The median follow-up time was 14 (5–44) months. The probability of remaining free of relapse at 6 months was 89.7% (95% CI, 76.2–100); at 12 months, the probability was 79.1% (95% CI, 61–97.2) and at 24 and 36 months, the probability was 55.9% (95% CI, 30.5–81.3). In the non-relapsing group, patients had a significant increase in CD4 cell levels of 102 (10–174) and 126 (4–159) cells/mm³ at 12 and 24 months, respectively (P = 0.037), whereas in the relapsing group, no significant increase was observed. Prophylaxis with L-AMB was well tolerated and only three patients had a mild impairment of renal function without requiring any change in treatment.

Conclusions: L-AMB is well tolerated and useful for secondary prophylaxis of VL.

Keywords: opportunistic infections, AIDS, AMB

Introduction

Visceral leishmaniasis (VL) is a chronic infection endemic in the Mediterranean basin. The incidence of VL rose during the first years of the AIDS epidemic, and in the pre-highly active antiretroviral therapy (HAART) era, it was estimated that 2% to 9% of all HIV-infected patients had experienced an episode of VL.1 The clinical course of VL in HIV-infected patients is characterized by frequent relapses.2–6 It is well known that intact immunity is the most important factor to reduce the incidence of VL and also to cure the infection. This explains why the incidence of VL has decreased, in the same way as other opportunistic infections, since the introduction of HAART.3,7–9 Nevertheless, despite the availability of HAART, the frequency of VL relapses in HIV-infected patients remains high.2,3 It seems that HAART fails to prevent VL relapses unless the CD4 lymphocyte count reaches a high enough level to protect against them.3,9–11 Therefore, it seems that secondary prophylaxis is necessary until a satisfactory, maintained immunity status is achieved.2–4,11

A previous study by Ribera et al.4 demonstrated that the probability of relapse was significantly higher in HIV-infected patients receiving no secondary prophylaxis for VL than in those receiving antimonial drugs. Since then, various agents have been used for secondary prophylaxis, but the lack of randomized studies and the low number of patients included in the existing published reports do not allow clear recommendations to be made on the best drug choice for this purpose.12–29

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Liposomal amphotericin B (L-AMB) has been used successfully for treating VL even in patients unresponsive to antimonials.\textsuperscript{21–29} The pharmacokinetic properties of L-AMB provide high levels of the drug in bone marrow, liver and spleen, which are the reservoirs of Leishmania; hence, we decided to carry out a non-randomized pilot study to evaluate the efficacy of L-AMB for secondary prophylaxis of VL in HIV-infected patients.

Patients and methods

A prospective, non-randomized, non-controlled study of patients with VL and HIV co-infection was conducted from January 2001 to December 2005 in Hospital Universitari Vall d’Hebron, Barcelona, Spain.

Inclusion criteria

(i) Adult (≥18 years) should be HIV-infected patients with VL.
(ii) Patients should have had at least one episode of VL treated with L-AMB.
(iii) Patients should have received at least one dose of L-AMB for secondary prophylaxis.
(iv) All patients should be under HAART. If patients were not receiving HAART when VL was diagnosed, this treatment was initiated after the fifth dose of L-AMB.

Diagnosis of VL and relapse

VL was diagnosed on the basis of identification of Leishmania amastigotes by direct bone marrow examination or by isolation of promastigotes in bone marrow or other tissues samples, cultured in Novy–McNeal–Nicolle medium at 26°C.

Treatment

Each VL episode was treated with 4 mg/kg/day of L-AMB (AmBisome\textsuperscript{4}; Gilead Sciences) intravenously for 5 consecutive days and once per week thereafter for 5 more weeks (total, 10 doses=40 mg/kg). L-AMB was diluted with 5% dextrose injection to a final concentration of 1–2 mg/mL prior to administration. Subsequently, the drug was administered by intravenous infusion, using a controlled infusion device, over a period of ∼120 min. This treatment was designed according to the Infectious Diseases Service protocol approved by our Hospital Committee of Antibiotics.

Follow-up, assessment and analytical methods

For each episode, we recorded variables related to HIV infection, such as demography, CD4 cell count, HIV viral load, antiretroviral therapy at diagnosis of VL and history of previous opportunistic infections. We also recorded the following clinical variables: fever, hepatosplenomegaly, weight loss and blood cell parameters. Finally, we recorded the time between cure of the acute episode and relapse or death.

Patients were assessed every 3 weeks. Physical examination, laboratory tests and adverse event assessment were carried out at each visit. CD4 cell count and serum HIV viral load were measured every 4 months. Parasitological monitoring was performed by peripheral blood mononuclear cell (PBMC) culture, as described previously.\textsuperscript{30}

Definition of cure

Four weeks after treatment of the acute episode, cure was documented by a combination of clinical and parasitological criteria. Clinical criteria for cure required resolution of fever and improvement of the haematological parameters according to the normality values in our hospital (erythrocytes 4.4–5.5 \times 10^{12}/L, haemoglobin 13.1–16.3 g/dL, leucocytes 4.9–9.3 \times 10^{9}/L and platelets 150–386 \times 10^{9}/L). Parasitological criteria for cure required either an absence of parasites in bone marrow aspirate or negative PBMC culture, both performed 1 month after completing treatment of the acute episode. Assessment of cure by bone marrow aspiration was decided by the attending physician.

Prophylaxis

Once cure had been determined, all patients received 5 mg/kg of intravenous L-AMB every 3 weeks as secondary prophylaxis, applying the same reconstitution and administration conditions as used for treatment.

Statistical analysis

The primary endpoint was the proportion of patients remaining relapse-free at several time points (6, 12, 24 and 36 months). Each episode was analysed separately. Continuous variables were compared with the Mann–Whitney U-test and expressed as the median and range. Categorical variables were compared with the $\chi^2$ test, or Fisher’s test when the expected frequency was ≤5. The Kaplan–Meier method was used to estimate the time to relapse.

In addition, we compared the time to relapse between our patients treated with L-AMB and a historical cohort of HIV-infected patients with VL diagnosed in our hospital, who received secondary prophylaxis with pentavalent antimonials. These data have been published previously.\textsuperscript{4} The curves of both series of patients were compared by the log-rank and Breslow tests.

A $P$ value ≤0.05 was considered to indicate statistical significance. Statistical analyses were performed with the SPSS statistical package (version 12.0).

Results

From January 2001 to December 2005, 29 episodes of VL were diagnosed in 17 HIV-infected patients. Eight episodes were excluded from the study. In four cases, because the acute episode was not treated with L-AMB (two patients received pentavalent antimonial salts and two pentamidine), one patient refused to receive secondary prophylaxis, two patients were lost to follow-up before ending treatment and one patient died from an unrelated cause during treatment of the acute episode. The final study group consisted of 21 episodes of VL in 15 HIV-infected patients, who were treated with L-AMB and also received secondary prophylaxis with L-AMB.
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Patients

There were 14 males and 1 female with a median age of 36 (26–53) years. Thirteen (86.7%) patients were former drug users and 2 (13.3%) acquired HIV infection by heterosexual transmission. Two of fifteen subjects included in the study were lost to follow-up: one at the 8th month of follow-up and the other after the first dose of L-AMB for secondary prophylaxis had been administered. One patient died because of bacteraemic pneumococcal pneumonia at the 5th month of follow-up.

Episodes

In all cases, Leishmania amastigotes were identified in bone marrow and PBMC culture was positive. In 13 of 21 (62%) episodes, patients were receiving HAART at diagnosis of the VL episode. Median CD4 cell count at the time of diagnosis was 100 cells/mm³ (4–300). In 20 of 21 episodes, CD4 lymphocyte count was < 200 cells/mm³. Plasma HIV RNA was < 50 copies/mL in eight episodes (38.1%). An opportunistic disease had been diagnosed previously in 15 (71.4%) episodes.

All episodes included in the study achieved clinical cure and had negative PBMC cultures 4 weeks after treatment. In all episodes in which bone marrow aspiration was performed (12 of 21), parasitological cure was also documented.

Relapses

Nine out of 21 (42.9%) relapses were diagnosed during follow-up; in 8 cases, Leishmania was identified in bone marrow samples and in 1 in a duodenal biopsy. The median time to relapse was 14 months (5–44). Parasitological monitoring showed that peripheral blood culture was positive at each relapse.

The Kaplan–Meier estimate of the probability of remaining relapse-free was 89.7% (95% CI, 76.2–100) at 6 months, 79.1% (95% CI, 61–97.2) at 12 months and 55.9% (95% CI, 30.5–81.3) at 24 and 36 months. No significant differences were found when comparing the evolution of our patients with a historical cohort of 17 patients who received antimonials (Figure 1).

Table 1 shows baseline characteristics related to HIV infection in patients who relapsed and in patients who did not. No significant differences were found between the two groups.

In the non-relapsing group, patients showed a significant increase in CD4 cell counts of 102 (10–174) and 126 (4–159) cells/mm³ at 12 and 24 months, respectively (P = 0.037), whereas in the relapsing group, no significant increase was observed (Figure 2).

All patients who did not relapse achieved a viral load < 50 copies/mL at 24 months of follow-up, compared with 33.3% of patients who had relapses (Figure 3).

Adverse events and toxicity

No adverse events were documented during the period of prophylaxis. A mild impairment of renal function was observed in three episodes (14%) during the treatment period. Creatinine serum levels normalized in all three patients once treatment was completed. No treatment was suspended because of adverse events.

Discussion

The introduction of HAART has led to a sharp reduction in the incidence of VL in HIV-infected patients. Nonetheless, once an HIV-infected patient develops VL, frequent relapses are common unless cellular immunity is restored; thus, secondary prophylaxis seems to be mandatory. In the present study, in

Table 1. Baseline characteristics of HIV-infected patients with and without VL relapse

<table>
<thead>
<tr>
<th></th>
<th>Relapsing group (n = 9)</th>
<th>Non-relapsing group (n = 12)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4 cells/mm³</td>
<td>126 (5–300)</td>
<td>82 (4–210)</td>
<td>0.39</td>
</tr>
<tr>
<td>CD4 &lt; 200 cells/mm³</td>
<td>8 (88.8%)</td>
<td>12 (100%)</td>
<td>0.42</td>
</tr>
<tr>
<td>Viral load &lt; 50 copies/mL</td>
<td>4 (44.4%)</td>
<td>4 (33.3%)</td>
<td>0.67</td>
</tr>
<tr>
<td>HAART</td>
<td>8 (88.8%)</td>
<td>5 (41.6%)</td>
<td>0.06</td>
</tr>
<tr>
<td>Previous opportunistic diseases</td>
<td>8 (88.8%)</td>
<td>7 (58.3%)</td>
<td>0.17</td>
</tr>
<tr>
<td>Risk factors for HIV infection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>former drug user</td>
<td>8 (88.8%)</td>
<td>10 (83.3%)</td>
<td>1</td>
</tr>
<tr>
<td>sexual transmission</td>
<td>1 (11.1%)</td>
<td>2 (16.6%)</td>
<td></td>
</tr>
</tbody>
</table>
come from observational and non-randomized retrospective studies; hence, the current recommendations for the prevention of VL relapses in HIV-infected patients are based on low grades of evidence.

Although the experience with L-AMB for the treatment and secondary prophylaxis of VL is limited,21–29 new lipid formulations of amphotericin, with a better toxicity profile and higher tissue diffusion, make them an attractive option for secondary prophylaxis. In addition, some experimental data suggest that amphotericin could be a better drug for patients with immune deficiency. Experimental models have shown that the antileishmanial activity of pentavalent antimonials is T cell-dependent and that could explain why these drugs have shown little activity in immunodeficient mouse models.32 In contrast, the antileishmanial activity of amphotericin B is T cell-independent and this property offers an additional theoretical advantage of L-AMB over antimonials and pentamidine for treatment and prophylaxis of VL.33,34

With L-AMB, the proportion of patients in our cohort remaining relapse-free at 12 months was 80%. These results are better than those obtained by López Vélez et al.20 in their randomized study with amphotericin B lipid complex, in which only 50% of patients remained free of relapse at 12 months. The most relevant experience with secondary prophylaxis of VL in HIV-infected patients was reported by Ribera et al.4 in 1996. In that study, the benefit of secondary chemoprophylaxis with pentavalent antimony over no prophylaxis or prophylaxis with allopurinol was clearly demonstrated. The proportion of relapse-free patients at 12 months was 93% in that study.

However, this optimistic vision of the efficacy of L-AMB for secondary prophylaxis should be balanced by the fact that ~20% of patients at 12 months and 45% at 24 months still have relapses despite HAART and L-AMB. In the long-term follow-up, the efficacy of antimonials also decreased in the study by Ribera et al.,4 with 24 month relapse rates similar to those obtained in our study with L-AMB.

These failures do not seem to be related to the initial condition of the patient. We examined variables related to cellular immunity, such as the baseline CD4 lymphocyte count, viral load and HAART, and there were no differences between patients who relapsed and those who did not. However, during follow-up, the group of patients who had relapses showed a poorer immunological response, with the CD4 lymphocyte count persistently under 200 cells/mm³. These data confirm the importance of the host immune status to eradicate Leishmania infection.

Perhaps the answer to the high frequency of relapses lies in the parasite itself. Selection of drug-resistant pathogens is a well-known consequence of drug pressure; although there are only two small, inconclusive studies on the emergence of Leishmania strains resistant to amphotericin B in HIV co-infected patients, this possibility cannot be ignored in long-term recipients of the drug.35 Carrió et al.36 recently demonstrated that leishmaniasis relapses in patients treated with L-AMB are associated with high inhibitory concentrations (IC₅₀) and that resistance to this drug increases after secondary prophylaxis. If the in vitro resistance of Leishmania strains is confirmed and has a real clinical correlation, several queries will be open and will offer novel and promising strategies of treatment and secondary prophylaxis of VL. If continuous use of L-AMB results in the development of resistance and L-AMB is not more effective than antimonials in preventing relapses, it would be reasonable to use a different drug for prophylaxis than for treatment.
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Table 2. Comparison of relapses in which parasitological cure was or was not documented by bone marrow aspiration

<table>
<thead>
<tr>
<th></th>
<th>Parasitological cure not determined by bone marrow aspiration</th>
<th>Parasitological cure determined by bone marrow aspiration</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of episodes</td>
<td>9</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Number of relapses</td>
<td>3/9 (33.3%)</td>
<td>6/12 (50%)</td>
<td>0.58</td>
</tr>
<tr>
<td>Time to relapse (median and range)</td>
<td>5, 8 and 37 months, 8 (5–37)</td>
<td>6, 7, 14, 17, 19 and 44 months, 14 (6–44)</td>
<td>0.61</td>
</tr>
</tbody>
</table>

Our study is limited by the small number of cases, which is due to the fact that the incidence of symptomatic VL is low since the implementation of HAART. However, to the best of our knowledge, this is the largest series in which the efficacy of L-AMB chemoprophylaxis and the clinical outcome of the cohort have been investigated.

Another limitation of our study is the fact that documentation of parasitological cure by bone marrow aspiration was only performed in 57.1% (12 of 21) of the cases. It is possible that episodes of relapse in the group of patients in which parasitological cure was not documented by bone marrow study were treatment failures rather than relapses. Table 2 shows the number of relapses and the time to relapse, depending on whether parasitological cure was documented by bone marrow aspiration or not. There were no significant differences in the number of relapses or the time to relapse between the two groups. In the three patients with a relapse, in whom bone marrow aspiration was not performed, the relapse occurred at 5, 8 and 37 months after the primary episode, similar to what occurred in those in whom microbiological cure was confirmed by bone marrow aspiration. This period of time is long enough, in our opinion, to consider these new episodes as relapses rather than acute treatment failures. Moreover, despite not having bone marrow confirmation in 42.8% (9 of 21) of the episodes, all the episodes considered cured had negative PBMC cultures, and all the relapsing episodes had positive PBMC cultures. These results agree with Riera et al., who observed that the presence of viable parasites during post-treatment follow-up increases the probability of relapses. This suggests, in our series, the good clinical correlation of that technique.

In conclusion, L-AMB is well tolerated and useful for secondary prophylaxis of VL. Therefore, L-AMB seems to be an alternative to pentavalent antimonials in preventing new VL relapses. However, an immune reconstitution is also required to warrant an effective and durable response. Randomized trials to compare the efficacy of both drugs and their role either in treatment or in prophylaxis are required.

References


Funding

This study was supported in part by ISCIII-RETIC RD06/006.

Transparency declarations

None to declare.


