In vitro susceptibility of Leishmania infantum strains isolated from Spanish HIV-positive patients to Abelcet and Fungizone


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Sir,

In southern Europe, leishmaniasis is an opportunistic infectious disease affecting patients treated with immunosuppressive agents or with the human immunodeficiency virus (HIV) infection, occurring in 2–7% of AIDS patients.1 The number of cases of visceral leishmaniasis/HIV co-infection reported in Spain, France, Italy and Portugal between January 1996 and June 1998 represents 49.8% of the total number of cases these countries have reported since 1990.2 In Spain, c. 100 human cases are reported each year, and there are thought to be a large number of undeclared cases.3 Liposomal amphotericin B is an effective treatment for visceral leishmaniasis in immunocompetent adults and children, including those with severe or pentavalent antimonial-resistant disease.

In this study we report the in vitro leishmanicidal activity of Abelcet (amphotericin B lipid complex) in comparison with that of amphotericin B (Fungizone) in different Leishmania infantum strains isolated from Spanish HIV+ patients.


Promastigotes were cultured in RPMI 1640 liquid medium (Gibco-BRL) with heat-inactivated fetal bovine serum. Parasites were added to sterilized microtitre plates with 24 wells (Corning) at a concentration of 5 × 10⁴/well (500 µL/well), exposed to drugs for 48 h and counted on a Coulter Counter model Z1. The 50% inhibitory concentration (IC50) was determined by linear regression analysis with 95% confidence limits. Tests were performed in at least triplicate on three different days in order to verify the results.

Intracellular amastigotes were cultured in the macrophage cell line J-774, in RPMI 1640 medium (Gibco-BRL) in 24-well (Corning) microtitre plates at a concentration of 50 000 cells/well (500 µL/well), exposed to drugs for 2 days at 37°C in humidified CO2/air (5%/95%). The percentage of infected cells was evaluated microscopically by counting at three distinct places in the well, and each assay was carried out in triplicate. The IC50 was determined from infected cells versus log drug concentration.

These experiments showed that Abelcet has a higher leishmanicidal activity than Fungizone on promastigotes in all strains analysed (Table 1). The strains BCN-143 and BCN-167, Table 1. In vitro susceptibility against promastigote and amastigote forms of L. infantum

<table>
<thead>
<tr>
<th>Strains</th>
<th>IC₅₀ against promastigote (mg/L amphotericin base ± S.D.)</th>
<th>IC₅₀ against amastigote (mg/L amphotericin base ± S.D.)</th>
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<tbody>
<tr>
<td></td>
<td>Abelcet</td>
<td>Fungizone</td>
</tr>
<tr>
<td>MHOM/ES/1990/BCN61</td>
<td>0.12 ± 0.01</td>
<td>11.60 ± 0.7</td>
</tr>
<tr>
<td>MHOM/ES/1996/BCN143</td>
<td>0.05 ± 0.003</td>
<td>3.20 ± 0.1</td>
</tr>
<tr>
<td>MHOM/ES/1996/BCN167</td>
<td>0.08 ± 0.004</td>
<td>10.50 ± 0.3</td>
</tr>
<tr>
<td>MHOM/ES/1997/BCN186</td>
<td>0.32 ± 0.05</td>
<td>11.58 ± 0.02</td>
</tr>
<tr>
<td>MHOM/ES/1999/BCN225</td>
<td>0.30 ± 0.01</td>
<td>72.50 ± 3.8</td>
</tr>
</tbody>
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obtained from the same patient before and after undergoing conventional treatment with Glucantime, showed an increase in the IC\textsubscript{50} value for Fungizone. This fact could indicate that the parasite had acquired some resistance or that in the isolations carried out before and after treatment, different clones were obtained. Carrio et al.\textsuperscript{5} reported that the susceptibility of \textit{L. infantum} strains to SbV [i.e. pentavalent antimonials; Glucantime and Pentostam (sodium stibogluconate)] decreased in patients undergoing treatment with meglumine antimoniate but not in patients treated with amphotericin B. In our study, IC\textsubscript{50} values for Abelcet are very similar in both cases. On the other hand, strain BCN-186 obtained from a patient undergoing Glucantime treatment shows similar behaviour to BCN-167.

Assays with the amastigote form also showed that Abelcet has higher leishmanicidal activity. In all cases the IC\textsubscript{50} values obtained were higher than those obtained in trials with promastigote forms of the parasite (Table 1).

The results of our study show that Fungizone is less effective than Abelcet in the macrophage J-774 model. The parasite’s amastigote stage in all cases showed lower susceptibility to the compounds tested than did the promastigote stage.

The different activities observed in both \textit{in vitro} studies were attributable to differences in size and composition of the particles and interaction with both lipoprotein and host cells by the amphotericin B formulations used in this study.

Previous results also suggest that variation in the activity of amphotericin B formulations against \textit{Leishmania} in macrophage models are attributable, in part, to the type of macrophage model. Yardley & Croft\textsuperscript{6} showed that Abelcet and Amphocil were the most active formulations in the THP-1 \textit{Leishmania donovani} model, with Fungizone and AmBisome having lower activity, albeit similar in nature. In the same study, in a model using peritoneal macrophages from CD1 mice, Fungizone was more active than Abelcet. In our study a similar pattern was observed in the THP-1 \textit{L. donovani} model, where Abelcet was c. 7- to 15-fold more active than Fungizone.

Acknowledgements

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References


