Activity of a Heat-Induced Reformulation of Amphotericin B Deoxycholate (Fungizone) against *Leishmania donovani*

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Amphotericin B (AmB) in its commercial formulation Fungizone is the “gold standard” treatment for systemic fungal infections and is the recommended second-line treatment for visceral leishmaniasis (VL) infections when conventional tetravalent antimony (SbV) therapy is inappropriate or ineffective (4). Unfortunately, AmB causes acute side effects following intravenous (i.v.) administration, and these limit its more extensive clinical use. Recently, lipid formulations of AmB have successfully been developed to greatly reduce the toxicity of AmB and so enable higher doses of the drug to be given (see reference 3 for a review). Dose for dose, Fungizone has greater activity than liposomal AmB against fungal infections (9); however, against experimental VL infections, lipid formulations are more active (1, 2, 5, 6, 8, 13, 14, 18, 20–22, 24, 28). Three lipid formulations have been approved for clinical use: AmBisome (NeXstar, Cambridge, United Kingdom), Abelcet (The Liposome Co., Princeton, N.J.), and Amphocil (Sequus Pharmaceuticals Inc., Menlo Park, Calif.). Despite their proven success against leishmaniasis (7, 10, 23), they are not frequently used against the disease due to their expense. The developing tropical and subtropical countries, where leishmaniasis affects 6 million individuals (27), cannot routinely afford expensive medication. Simple heating of Fungizone at 70°C for 20 min is an inexpensive procedure which could be used to improve the therapeutic index of AmB, as shown for the therapeutic index of AmB against candidiasis and cryptococcosis (19), and encourage its more widespread use.

In this study, the in vitro and in vivo antileishmanial activities of Pentostam (SbV), Fungizone, and heated Fungizone were compared. The differences in their relative toxicities to mammalian cells and mice were observed. *Leishmania donovani* MHOM/ET/67/L82 amastigotes were maintained in golden hamsters (Charles Rivers, Margate, United Kingdom). The parasites were harvested from an infected spleen for in vitro and in vivo assays.

A stock solution of Fungizone (Bristol Myers Squibb, La Défense, France) was prepared from a marked bottle by the addition of 10 ml of sterile 5% dextrose (aqueous). Heated Fungizone was prepared as described previously (11). Pentostam (100 mg of SbV/ml) and powdered sodium stibogluconate (NaSbV) were provided by Glaxo Wellcome, London, United Kingdom. NaSbV powder was dissolved in 0.25% methylcellulose for in vivo administration. Drug dilutions were made daily in complete medium for in vitro tests and in 5% dextrose for in vivo tests.

For in vitro assays, peritoneal macrophages were harvested from female CD1 mice (Charles Rivers) 24 h after starch (Sigma) induction and were dispensed into 16-well Lab-tek slides (Nunc Ltd., Chicago, Ill.) at a concentration of 4 × 10^4/well (100 μl/well) in RPMI 1640 medium (Gibco BRL, Paisley, United Kingdom) supplemented with 10% heat-inactivated fetal calf serum (Sera-Lab, Oxon, United Kingdom). After 24 h, the macrophages were infected with *L. donovani* amastigotes at a ratio of 10 amastigotes to 1 cell. After 24 h, the infected cells were exposed to drug for 5 days (the cell overlay and drug were replaced on day 3). Prior to drug administration, Fungizone solutions were incubated for 15 min at 37°C to allow the AmB to bind to the proteins in the serum (26). The role of lipoproteins in the endocytosis of AmB in cells by specific receptors has already been shown (15, 25). Cells were treated with both formulations at concentrations ranging from 1 μM to 0.5 nM. The experiment was terminated on day 5 by methanol fixation and Giemsa staining. The percentage of infected macrophages was evaluated microscopically. The 50% effective doses (ED₅₀) were determined by linear regression analysis (sfit; Microsoft Excel) with 95% confidence limits. *P* values were calculated by Student’s *t* test.

In these assays, no toxicity to macrophages was seen with either formulation at the doses tested. ED₅₀s were found to be 0.035 μg/ml for heated AmB deoxycholate and 0.024 μg/ml for unheated AmB deoxycholate (Table 1). The difference in the activities of the two formulations was not significant (*P* > 0.05). Both Fungizone formulations were more active than sodium stibogluconate. In aqueous solution, AmB exists as a mixture of different species in equilibrium: monomers and soluble and insoluble aggregates (17). Under the conditions of the in vitro experiments, for concentrations of AmB below 1 μM, both formulations were mainly in the monomeric form (12), and this could give an explanation for their similar activities.

For in vivo assays, 8- to 10-week-old female BALB/c mice (weight, 20 g) were infected i.v. with 1.5 × 10^7 *L. donovani* L82 amastigotes and were randomly sorted into groups of five mice. At 7 days postinfection, one mouse was killed to check for the patency of infection and drug administration commenced. Sodium stibogluconate was administered subcutaneously for 5
TABLE 1. In vitro activities of Pentostam and unheated and heated Fungizone against L. donovani MHOM/ET/67/L82 in mouse peritoneal macrophages

<table>
<thead>
<tr>
<th>Formulation</th>
<th>ED90 (µg/kg)a</th>
<th>ED90 (µg/kg)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaSbV</td>
<td>21.13 ± 1.93b</td>
<td>&gt;30b</td>
</tr>
<tr>
<td>Fungizone</td>
<td>0.024 ± 0.0008b</td>
<td>0.067 ± 0.001b</td>
</tr>
<tr>
<td>Heated Fungizone</td>
<td>0.035 ± 0.0024b</td>
<td>0.07 ± 0.015b</td>
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</tbody>
</table>

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REFERENCES


Note: All experiments were performed in a controlled environment with appropriate safety measures.


