In vitro activity of free and liposomal nystatin against Leishmania species in comparison with several amphotericin B and azolic formulations.

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Received: 30.10.2003 Accepted: 15.12.2003

Abstract: The sensitivities of promastigote stages of three species of Leishmania were determined in vitro to nystatin, liposomal formulations of nystatin, other amphotericin B formulations and antymycotic azole drugs. Liposomal formulations of nystatin were more active than free nystatin against L. braziliensis strains but less active against L. infantum and L. tropica.

Key Words: Nystatin, Leishmania, Leishmanicidal activity.

1. Introduction

Leishmaniasis is a desfiguring and sometimes fatal protozoan disease caused by different species of protozoan parasites of the Leishmania genus. Over fifteen species of Leishmania are known to cause disease in humans with a wide clinical spectrum from visceral to cutaneous leishmaniasis. This protozoan parasitic disease is endemic to the American, African and Asian tropical countries and affects human, some 12 million people around the world with an estimated number of 1.5-2 million new cases occurring annually and 350 million live on risk of becoming infected (Desjeux & UNAIDS, 1998).

Drugs available for the treatment of leishmaniosis remain unsatisfactory, the drug of choice are pentavalent antimonials (Sb') which presents renal and cardiac toxicity, require long-term administration and may display poor activity in immunosuppressed patients.

There is an obvious need for new drugs and nature has a been a source for important antiparasitic drugs, most of these are plant derived but an increasing number have been isolated from microorganism.

Certain polyene antibiotic compounds like amphotericin B and nystatin (mycostatin) are known for their fungicidal activity. Amphotericin B is used since long as antileishmanial drug as well and previous results suggest that nystatin has a very good anti leishmanial activity in vitro about Leishmania major (Ali et al., 1997). The mode of action proposed for this drug is the same as for amphotericin B as both of these polyene compounds interact with ergosterol creating a pore in the membrane and thus killing the parasite.

Liposomal nystatin (Nyotran) is a multilamellar liposomal formulation and a systemic antifugal agent against Aspergillus and Candida strains (Arikan & Rex, 2001; Johnson et al., 1998; Oakley et al., 1999). Systemic use of nystatin is limited by toxicity and it is not absorbed from the gastrointestinal tract. A liposomal formulation of nystatin has been developed to facilitate
intravenous administration and reduced toxicity (Mehta et al., 1987; Wallace et al., 1997).

In present study the in vitro antileishmanial activities of liposomal nystatin, Nyotran, was compared with nystatin, other amphotericin B formulations and antymycotic azole drugs (ketoconazole, fluconazole and itraconazole) against different strains of Leishmania braziliensis, L. infantum and L. tropica.

2. Materials and Methods

2.1 Parasites

Four strains of Leishmania spp. were tested in the present study: L. braziliensis (MHOM/PE/95/LQ7 and MHOM/PE/95/LQ8), which were isolated in the province of La Convención, Cuzco, Peru, L. tropica (MON 58/LEM 2578) and L. infantum (MON 183/LEM 2592) promastigotes were adapted for culture in RPMI 1640 liquid medium (Gibco-BRL) supplement with 20% heat inactivated fetal bovine serum, vitamins and amino acids, at 22°C. Logarithm phase cultures of promastigotes were used for experimental purposes.

2.2 Drugs

Pharmaceutical preparation of nine agents were obtained from the respective manufacturer: liposomal nystatin (Nyotran) and nystatin were supplied by Aronex Pharmaceuticals, Inc (The Woodlands, TX, USA), amphotericin B deoxycholate (Fungizone) from Squibb Industria Farmaceutica (Barcelona, Spain), amphotericin B colloidal dispersion (Amphotec) was obtained from Sequus Pharmaceuticals, Inc (Menlo Park, CA, USA), amphotericin B lipid complex (Abelcet) from The Liposome Company, Inc (Princeton, NJ, USA), liposomal amphotericin B (Ambisome) from Vestar Deutschland GmbH (Braunsleweig, Germany), itraconazole from Jansen Pharmaceutica (Beerse, Belgium), fluconazole from Pfizer (Sandwich, UK) and ketoconazole from Janssen Pharmaceutica (Barcelona, Spain). The pharmaceutical preparations were reconstituted according to the manufacturer’s instruction and further dilutions were made with RPMI 1640 medium.

2.3 In vitro assays

In vitro susceptibility assay was determined as described (Tincusi et al., 2002). Parasites (5 x 10^4 promastigotes/well) with the drug concentration to test were performed in sterilised microtiter plates of 24 wells (CorningTM). The final volume employed was 500 µl in each well. Different concentrations were tested and parasite determination was performed after 48 h by density determination on a Coulter Counter model Z1. Resistance was determined by measuring of the 50% inhibitory concentration (IC50), using linear regression analysis with 95% confidence limits.

Tests were performed at least by triplicate on three different days in order to verify the results. The final IC50 was calculated as the arithmetic mean of the IC50 obtained.

3. Results

Table 1 summarizes the in vitro susceptibilities of Leishmania strains to the different compounds tested. Multilamellar liposomal formulation of nystatin, Nyotran, was more active than free nystatin against L. braziliensis strains but less active against L. infantum and L. tropica. In the case of azolic antibiotic all compounds tested were less active than amphotericin B and nystatin preparations evaluated.

<table>
<thead>
<tr>
<th></th>
<th>L. braziliensis</th>
<th>L. braziliensis</th>
<th>L. infantum</th>
<th>L. tropica</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nyotran</td>
<td>0.34±0.11</td>
<td>0.35±0.09</td>
<td>0.28±0.05</td>
<td>1.05±0.025</td>
</tr>
<tr>
<td>Nystatin</td>
<td>3.6±1.15</td>
<td>4.4±0.7</td>
<td>&lt; 0.1</td>
<td>0.33±0.06</td>
</tr>
<tr>
<td>Fungizone</td>
<td>1.25±0.13</td>
<td>1.36±0.29</td>
<td>0.16±0.08</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>Amphotec</td>
<td>&lt; 0.1</td>
<td>&lt; 0.1</td>
<td>0.22±0.01</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>Abelcet</td>
<td>-</td>
<td>-</td>
<td>&lt; 0.1</td>
<td>1.52±0.09</td>
</tr>
<tr>
<td>Ambisome</td>
<td>-</td>
<td>-</td>
<td>0.5±0.11</td>
<td>0.7±0.16</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>33.1±5.36</td>
<td>36.7±4.2</td>
<td>21.3±2.6</td>
<td>41.1±3.7</td>
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<tr>
<td>Itraconazole</td>
<td>54.6±3.9</td>
<td>59.4±8.1</td>
<td>26.3±8.4</td>
<td>-</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>66.2±13</td>
<td>52.1±3.9</td>
<td>54.3±5</td>
<td>-</td>
</tr>
</tbody>
</table>

Free nystatin was as active in vitro as Abelcet against L. infantum but liposomal nystatin and the rest of liposomal amphotericin B as well showed a very good activity.
In the case of *L. braziliensis* strains, Nyotran was more active that nystatin, only more active than azolic compounds in this case.

*L. tropica* was highly susceptible to Amphotec and Fungizone, both compounds were more active than nystatin and Nyotran but, in this parasite species, nystatin was 3 times more effective than Nyotran.

4. Discussion

Nystatin has been a useful antifungal agent since the 1950s but toxic effects following parenteral administration have limited its application to the topical treatment of fungal infections (Richardson & Warnock, 1997). On the other hand Nyotran is a liposomal nystatin formulation and a systemic antifungal agent.

Our results indicated that liposomal nystatin and free nystatin are broad-spectrum leishmanicidal agents which are active *in vitro* against *L. braziliensis*, *L. tropica* and *L. infantum*.

There is only one previous report (Ali et al., 1997) in which susceptibility of *L. major* promastigotes to nystatin *in vitro* was examined and results obtained suggest that nystatin has a very good leishmanicidal activity.

The data from our study shows that liposomal nystatin is more active *in vitro* than free nystatin only against *Leishmania braziliensis* strains examined. In contrast, liposomal nystatin is less active *in vitro* against *L. tropica* and *L. infantum* strains. Many previous *in vitro* studies have shown differences in the sensitivity of *Leishmania* species to different classes of drugs, but our results are the first comparative study of the *in vitro* leishmanicidal activity of liposomal and free nystatin and compared this with other liposomal amphotericin B and azolic formulations.

The micellar Fungizone and the colloidal dispersion Amphocil were 15x and 7x more active than Abelcet and Ambisome against promastigotes of *L. tropica*. A similar pattern was also observed *in vitro* against promastigotes of *L. donovani* and *L. major* in a peritoneal macrophages model (Yardley & Croft, 2000) and against *T. cruzi* amastigotes in a macrophage model (Yardley & Croft, 1999).

Fungizone and Amphocil were also 3 and 10x more active than nystatin and Nyotran against *L. tropica* promastigotes and all were much more active than azolic compound ketoconazol.

In our study Abelcet and nystatin were the most active formulations against *L. infantum* promastigotes, with Fungizone, Amphocil and Nyotran having lower similar activity. Previous studies showed also that Abelcet has a higher leishmanicidal activity than Fungizone on promastigotes of *L. infantum* isolated from Spanish HIV+ patients (Piñero et al., 2002). All azolic compounds showed less leishmanicidal activity than the rest of examined compounds.

Against *L. braziliensis* strains examined Amphocil was the most active compound and we reported an activity ranking of Nyotran > Fungizone > Nystatin >> azolic compounds.

In a previous work have reported results on some of these liposomal amphotericin B formulations against *L. donovani* and *L. major* amastigotes in a murine macrophage model (Yardley & Croft, 2000). Our results confirm a significative difference in activity between the amphotericin B formulations, attributable in *in vitro* experiment to differences in the size and composition of the lipid vehicles.

5. Acknowledgements

We thank Dr. Pilar Arévalo for providing the azolic drugs tested in this work.

6. References


