Introduction

Partricins are semi-synthetic polyene compounds that have been demonstrated to have antifungal efficacy both in vitro and in vivo.1–3 SPA-S-753 (Societa Prodotti Antibiotici, Milano, Italy) is a derivative of partricin A, namely N-dimethylaminoacetyl partricin A 2-dimethylaminoethylamide diaspartate.4 Unlike amphotericin B, SPA-S-753 is water soluble.1–3 This compound has previously been reported to have in vivo activity against Candida albicans2 and Cryptococcus neoformans.1,3 In addition, SPA-S-753 has been reported to have a long half-life in serum (t1/2 = 6.5 h) after ip injection into mice.1 In comparison with amphotericin B, these compounds have been described to have lower levels of toxicity in vivo. This, in conjunction with reported increased efficacy relative to amphotericin B,1–3 make these compounds attractive candidates for further development.

The aim of the present study was to compare the relative efficacy of SPA-S-753 with that of conventional deoxycholate-formulated amphotericin B in a systemic murine model of candidosis using an iv route of infection and drug administration, not previously reported.

Materials and methods

In vitro study

The activity of SPA-S-753 (provided by Intrabiotics Pharmaceuticals, Inc., Mountain View, CA, USA) against C. albicans, strain #5, was determined by macrobroth tube dilution as described previously.5,6 The activity of amphotericin B against this organism has been reported previously.5

In vivo model of infection

The murine model of systemic candidosis was performed under the guidelines of a protocol approved by the Institutional Animal Care and Use Committee of the California Institute for Medical Research. The model was established in 6-week-old male CD-1 mice by intravenous inoculation of 3.2 × 107 yeasts of C. albicans strain #5, as described previously.5,7 Therapy commenced 4 days after infection. Groups of 10 mice received either sterile 5% dextrose in water (diluent controls), amphotericin B or SPA-S-753. Conventionally formulated amphotericin B in deoxy-
cholate (AmB; Pharma-Tek, Inc., Huntington, NY, USA) was given in a volume of 0.25 mL at 0.3 or 1 mg/kg body weight, after dilution in sterile 5% dextrose. SPA-S-753 was given at 0.3, 1, 3 or 10 mg/kg body weight at a relative dose of 5 mL/kg body weight after dilution in 5% dextrose. For the first three doses, the average weight of the mice used was 26.4 g and the SPA-S-753 regimens were administered in 0.13 mL volume. Drug concentrations were adjusted for body weight (average weight 28.4 g) for doses four to six, and therefore SPA-S-753 regimens were administered in 0.14 mL volumes. Ten uninfected mice received the SPA-S-753 regimen at 10 mg/kg. All therapies were given iv on days 4, 6, 8, 11, 13 and 15 post-infection.

Deaths were recorded for 28 days following infection. On day 28, all surviving infected mice were killed by CO2 asphyxia, and the number of *C. albicans* cfu remaining in the spleen and kidneys was determined. The number of cfu in each organ was calculated and expressed as the log_{10} number of cfu per entire organ. The mean number of cfu in each group was determined and expressed as the log_{10} geometric mean number of cfu ± the 95% confidence interval using GB-STAT (version 6.0, Dynamic Microsystems, Silver Spring, MD, USA). Statistical analyses of survival data were performed by Wilcoxon rank sums test by day of death, and comparative cfu in the organs of surviving mice were analysed by Mann–Whitney U-test.

Results

**In vitro study**

The *in vitro* activity of SPA-S-753 was determined against the strain of *C. albicans* used in our model of infection. These results showed that the MIC of SPA-S-753 for this organism was 2.0 mg/L and that the minimum fungicidal concentration (MFC; ≥96% killing of inoculum) was 4.0 mg/L. As reported previously, the MIC of amphotericin B was 0.5 mg/L and the MFC was 2.0 mg/L.

**In vivo study**

The aim of this study was to compare the efficacies of amphotericin B and SPA-S-753 in the treatment of systemic candidosis. The model established proved to be lethal to 90% of the diluent-treated controls (Figure). Previous data from our laboratory indicate that is equivalent to no treatment. The first deaths among the control animals started on day 10, whereas the first death in a treatment group occurred on day 22. Although 90% of the control animals died, only a single animal given 0.3 mg/kg AmB, and two animals given 0.3 mg/kg SPA-S-753, died of infection. No animals given higher doses of AmB or SPA-S-753 died, and all regimens prolonged the survival in comparison with diluent-treated animals (P < 0.001); all treatment regimens were statistically equivalent in the prolongation of survival.

A single animal in the uninfected group treated with SPA-S-753 at 10 mg/kg died on day 12 of the experiment. This animal had received three doses of SPA-S-753. No other animals in this group showed any overt signs of toxicity or illness, nor were there any changes in organs noted on gross examination of mice treated with SPA-S-753. Moreover, the animal that died did not show any overt symptomatology. Although this death cannot be directly attributed to drug toxicity, it is the probable cause. Additional studies would be required to determine whether this dosage is approaching the lethal toxic limit.

The second parameter used to evaluate the comparative efficacy of each therapy was the burden of *C. albicans*.
Partricin therapy of systemic candidosis

remaining in the spleen and kidneys (Table). With AmB there was dose-responsive efficacy in the reduction of fungal burden, with the mean burden of \textit{C. albicans} recovered from the kidneys of animals treated with 1 mg/kg AmB significantly lower than that recovered from those of mice given 0.3 mg/kg AmB. SPA-S-753 also reduced the fungal burdens in both organs, but did not do so in an entirely dose-responsive manner. In the spleen, the mean burden of cfu recovered from the group given SPA-S-753 1 mg/kg was higher than that in the group given SPA-S-753 0.3 mg/kg. Similarly, in the kidneys the number of cfu recovered from the group given SPA-S-753 3 mg/kg was higher than that of the group given SPA-S-753 1 mg/kg. This lack of sharp dose responsiveness made some of the comparisons between drugs more difficult.

In the spleen, equivalent dosages of AmB and SPA-S-753 were not statistically different in their capacity to reduce fungal burden. The 10 mg/kg dose of SPA-S-753 was superior to 0.3 or 1 mg/kg AmB or SPA-S-753, and equivalent to 3 mg/kg SPA-S-753. AmB 0.3 mg/kg was the least effective in eradicating infection, whereas all regimens of SPA-S-753 and 1 mg/kg AmB cured 40–100% of infection in the spleen; 10 mg/kg of SPA-S-753 cleared all animals of spleen infection (Table). The comparative efficacies indicated that SPA-S-753 was equivalent to AmB (0.3 or 1 mg/kg SPA-S-753 was equally effective as the same doses of AmB).

In the kidney, the key target organ of infection in this model, all treatment regimens were efficacious in comparison with controls. The 0.3 mg/kg regimens of AmB and SPA-S-753 were equivalent and inferior to all other drug treatments. AmB 1 mg/kg and SPA-S-753 10 mg/kg were equivalent and had the lowest mean burdens of cfu (Table). Fewer kidney infections were eradicated than spleen infections. AmB 1 mg/kg cleared three mice of kidney infection and SPA-S-753 10 mg/kg cleared four animals, whereas other treatments cured one or none (Table). The comparative efficacy of SPA-S-753 versus AmB in the kidneys could be estimated to range from equivalent (0.3 and 1 mg/kg dosages of each drug yielded the same results) to three- to 10-fold less potent. The evidence for a three-fold difference in efficacy was that 1 mg/kg AmB was superior to 3 mg/kg SPA-S-753; evidence for the <10-fold difference was that 3 mg/kg SPA-S-753 was significantly better than 0.3 mg/kg AmB; and evidence for the 10-fold difference was that 10 mg/kg SPA-S-753 was equivalent to 1 mg/kg AmB.

With respect to curing both organs, AmB 1 mg/kg cured three mice and SPA-S-753 10 mg/kg cured four mice of infection (Table). No other treatment regimens cured more than a single animal. Considering that SPA-S-753 10 mg/kg cured all animals of spleen infection as well as four of kidney infection, this regimen could be considered, by the criterion of cure, as the most effective treatment overall.

### Table. Recovery of \textit{C. albicans} from the spleens and kidneys of treated mice

<table>
<thead>
<tr>
<th>Treatment group (mg/kg)</th>
<th>No. of mice cured of 10</th>
<th>No. of mice alive of 10</th>
<th>Log10 geometric mean cfu per organ (no. of organs free of infection)</th>
<th>[95% confidence interval]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diluent controls</td>
<td>0</td>
<td>1</td>
<td>4.12 (0)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.13 (0)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Amphotericin B (0.3)</td>
<td>0</td>
<td>8</td>
<td>1.10 (2)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.79 (0)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[0.5–1.7]</td>
<td>[4.6–7.0]</td>
</tr>
<tr>
<td>Amphotericin B (1.0)</td>
<td>3</td>
<td>10</td>
<td>0.58 (5)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.65 (3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[0.08–1.1]</td>
<td>[0.9–4.4]</td>
</tr>
<tr>
<td>SPA-S-753 (0.3)</td>
<td>0</td>
<td>8</td>
<td>0.58 (5)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.58 (0)&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[0.1–1.2]</td>
<td>[5.9–7.3]</td>
</tr>
<tr>
<td>SPA-S-753 (1)</td>
<td>1</td>
<td>10</td>
<td>1.05 (4)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.41 (1)&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[0.2–1.8]</td>
<td>[2.7–6.1]</td>
</tr>
<tr>
<td>SPA-S-753 (3)</td>
<td>1</td>
<td>10</td>
<td>0.34 (7)</td>
<td>4.88 (1)&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[0–0.8]</td>
<td>[3.5–6.3]</td>
</tr>
<tr>
<td>SPA-S-753 (10)</td>
<td>4</td>
<td>10</td>
<td>0 (10)</td>
<td>1.72 (4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[0.35–3.1]</td>
<td></td>
</tr>
</tbody>
</table>

*<sup>P</sup> < 0.01 or 0.001, depending on comparison, versus all treatment regimens.  
*<sup>P</sup> < 0.05 versus SPA-S753 at 3 mg/kg and <sup>P</sup> < 0.01 versus SPA-S753 at 10 mg/kg.  
*<sup>P</sup> < 0.01 versus amphotericin B at 1 mg/kg, SPA-S753 at 1 or 3 mg/kg, <sup>P</sup> < 0.001 versus SPA-S753 at 10 mg/kg.  
*<sup>P</sup> < 0.05 versus SPA-S753 at 10 mg/kg.  
*<sup>P</sup> < 0.001 versus amphotericin B at 1 mg/kg or SPA-S753 at 10 mg/kg, <sup>P</sup> < 0.01 versus SPA-S753 at 1 mg/kg or SPA-S753 at 10 mg/kg.  
*<sup>P</sup> < 0.05 versus amphotericin B at 1 mg/kg, <sup>P</sup> < 0.01 versus SPA-S753 at 10 mg/kg.
Discussion

In the current study, we examined the efficacy of SPA-S-753, a partricin A derivative, for its efficacy in the treatment of experimental systemic candidosis. Previous studies have demonstrated SPA-S-753 to be efficacious for the treatment of candidosis established by an ip route of infection and with drugs administered into the same compartment, as well as extended durations of treatment (e.g. up to 24 days od or bd using doses ranging from 0.015 to 4 mg/kg). In addition, we used only six doses of AmB or SPA-S-753 given three times a week for 2 weeks. SPA-S-753 was also administered at a higher dose (10 mg/kg) than those described above for ip treatment. Thus, with the reduced number of treatments and increased length of time between them, we conducted a rigorous test for establishing efficacy. Overall, we found SPA-S-753 to be an efficacious treatment in our model of systemic candidosis, which further corroborates previous results. Dosages as low as 0.3 mg/kg of the drug gave a significant survival advantage and demonstrated some efficacy in clearing infection from the spleen.

The overall efficacy of SPA-S-753 in the regimens used in this study shows it to be conservatively between equivalent to and <10-fold as potent as AmB. These results contrast with those previously reported, where SPA-S-753 was shown to have greater activity than AmB in a murine model of candidosis. However, these differences are likely to be due to dosing frequency and duration, as well as route of administration. Another source of difference could be the comparative MICs and MFCs of AmB and SPA-S-753 for the strain of C. albicans used in our studies, where AmB was four- and two-fold more active, respectively, than SPA-S-753. It would be of interest to examine additional isolates of C. albicans that had identical MICs and MFCs for AmB and SPA-S-753, to determine whether in vitro activity predicted in vivo efficacy or outcome.

When given ip, SPA-S-753 has been reported to be less toxic than AmB. Similarly, we found SPA-S-753 to be less toxic than AmB when given iv. A single uninfected animal given 10 mg/kg SPA-S-753 died of possible drug toxicity during the course of our study. Considering that 1 mg/kg AmB iv is approaching the level of acute lethal toxicity in mice, we would estimate that SPA-S-753 is at least three- to 10-fold less acutely toxic than AmB. Additional studies are required to define this relationship better. If SPA-S-753 is substantially (<10-fold) less toxic than AmB, then the therapeutic index might be improved. Results published previously seem to indicate that the activity of SPA-S-753 relative to AmB improves with increased frequency of dosing. If a similar tendency was also true after iv administration, there could be a substantial improvement in therapeutic index. Overall, these results are very encouraging and highlight the need for further study of SPA-S-753.

Acknowledgements

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References


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