Therapeutic efficacy of caspofungin alone and in combination with amphotericin B deoxycholate for coccidioidomycosis in a mouse model

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Objectives: The therapeutic efficacy of caspofungin alone and in combination with amphotericin B deoxycholate was evaluated in treatment of murine coccidioidomycosis.

Methods: Survival and tissue burdens of the spleens and livers were used as antifungal response markers. In a monotherapy study, caspofungin was injected intraperitoneally at 0.1, 0.2, 0.5, 1 and 5 mg/kg per day on days 2 through 15. Amphotericin B deoxycholate was given at 0.1, 0.2 and 0.5 mg/kg intravenously and 1 and 5 mg/kg intraperitoneally three times per week for 2 weeks. In a combination therapy study, amphotericin B deoxycholate at 0.1 mg/kg was administered intravenously three times per week for 2 weeks, respectively, with and without caspofungin intraperitoneally given at 0.1, 0.5 and 5 mg/kg daily on days 2 through 15 post-infection.

Results: The study shows that caspofungin and amphotericin B deoxycholate at ≥0.5 and ≥0.1 mg/kg, respectively, were significant in both prolongation of survival and reduction of the tissue fungal burdens of mice compared with controls. No sterilization of either organ was observed with caspofungin doses. In combination therapy, any combination of caspofungin (0.1, 0.5 and 5 mg/kg) with amphotericin B deoxycholate (0.1 mg/kg) improved the period of survival and significantly reduced spleen and liver counts compared with controls.

Conclusions: This study indicates that caspofungin has efficacy against systemic coccidioidomycosis in a murine model given in combination with amphotericin B deoxycholate.

Keywords: combination therapy, AMB, echinocandins

Introduction

Coccidioides spp. are the aetiological agents of coccidioidomycosis. The fungus lives in the soil of arid regions of the United States, Mexico and other countries of Central and South America. Coccidioidomycosis is a systemic infection and is frequently refractory to treatment. Amphotericin B deoxycholate, a polyene macrolide, has been the agent of choice for the treatment of severe cases of coccidioidomycosis for 40 years. The drug has the broadest spectrum of activity of any available agent, but its use is limited by its narrow therapeutic index and its poor tolerability profile. Caspofungin, an antifungal agent in the echinocandin family, has displayed potent in vitro and in vivo activities against Candida species, Aspergillus species and other clinically important moulds. In a prior study, we reported the in vitro and in vivo activities of caspofungin against clinical isolates of Coccidioides immitis. Two clinical isolates for which the caspofungin MICs were different were selected for determination of minimum effective concentration (MEC) and the same strains were used for animal studies. The results showed that caspofungin doses of ≥0.5 mg/kg significantly prolonged survival and reduced organ fungal load to a level lower than those of controls. At the same time, we displayed a limited association between in vitro testing with the MIC as the

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endpoint and antifungal treatment in the animal model. A better in vitro—in vivo correlation was noted when we used the MEC as the endpoint in antifungal susceptibility testing. Caspofungin makes possible the opportunity for combination therapy because different fungal structures can be attacked in the fungus at the same time; in this way, the antifungal therapy is making the most of its effects. Combination therapy could be an alternative option to monotherapy for patients with invasive coccidioidomycosis and for some patients who fail to respond to conventional treatment. The increase in available antifungal compounds has raised the number of potential combinations; a therapeutic resource that could be exploited clinically. In the present study, we evaluated the therapeutic efficacy of caspofungin alone and in combination with amphotericin B deoxycholate in a murine model of systemic coccidioidomycosis.

Materials and methods

Animals

Outbred male ICR (Institute Cancer Research) mice that were 4 weeks old (25 g) were purchased from Harlan-Mexico. Ten mice were included in each treatment or control group for each survival and tissue burden study. They were housed in cages of five mice each. Mice were provided food and water ad libitum. All animal research procedures were approved by the Ethics Committee of our University. Care, maintenance and handling of the animals were in accordance with Mexico government licence conditions for animal experimentation.

Strain

A clinical isolate of Coccidioides posadasii strain 02-137 was used for the animal studies. We used this species because all the strains isolated in Monterrey, Nuevo Leon, have been identified as C. posadasii. Although there are geographic differences in the distribution of Coccidioides species, there is no apparent difference in the disease produced by these two species. Four-week-old cultures with mycelial phase were maintained on potato dextrose agar plates. Arthroconidia were collected by using the magnetic stir bar technique. They were then filtered through glass wool, washed three times, suspended in sterile saline and counted in a haemocytometer.

Infection model

A previously described model of systemic coccidioidomycosis was utilized. Mice received an intravenous injection of 200 arthroconidia of C. posadasii. Quantitative cultures by serial dilution were used to confirm the inoculum size. The formulations were reconstituted in accordance with the instructions of the manufacturers and administered in 0.2 mL volumes.

Monotherapy study

Caspofungin was injected intraperitoneally at 0.1, 0.2, 0.5, 1 and 5 mg/kg per day on days 2 through 15 post-infection. Amphotericin B deoxycholate was given at 0.1, 0.2 and 0.5 mg/kg intravenously and 1 and 5 mg/kg intraperitoneally. Amphotericin B deoxycholate was given three times per week for 2 weeks, which is a complete therapy regimen of six doses. The control group received sterile distilled water intraperitoneally. Deaths were recorded through to 50 days post-infection. Moribund mice (ruffled fur, hunched posture, weight loss and hypothermia) were terminated, and their deaths were recorded as occurring on the next day. Survivors were terminated at day 50 by methoxyflurane (metofane) inhalation followed by cervical dislocation. The spleens and livers of the dead mice and the survivors that had been terminated were removed aseptically. The organs were homogenized in 2 mL of sterile saline, and the entire volume of homogenate was plated onto potato dextrose agar and incubated at 35 °C for a week to determine the viabilities of the fungi in the organs. For tissue burden studies, the treatment was the same but mice were sacrificed on day 20. Spleens and livers were removed and homogenized in 2 or 5 mL of sterile saline, respectively, and serial 10-fold dilutions were plated onto potato dextrose agar plates and incubated at 35 °C for a week to determine the number of viable cfu in each organ.

Combination therapy study

Amphotericin B deoxycholate at 0.1 mg/kg was administered intravenously three times per week for 2 weeks, respectively, with and without caspofungin intraperitoneally given at 0.1, 0.5 and 5 mg/kg daily on days 2 through 15 post-infection. In addition, one group of mice received caspofungin at 0.1, 0.5 and 5 mg/kg alone, independently. The control group received 5% dextrose intravenously three times per week and sterile distilled water intraperitoneally daily on days 2 through 15 post-infection. The survival study was performed as described above. For tissue burden studies, one group of mice received amphotericin B deoxycholate at 0.1 mg/kg administered intravenously three times per week for 2 weeks with or without caspofungin intraperitoneally given at 0.1, 0.5 and 5 mg/kg daily on days 2 through 15 post-infection. Other groups of mice received only caspofungin at 0.1, 0.5 and 5 mg/kg, independently. The control group received 5% dextrose three times per week and sterile distilled water intraperitoneally daily on days 2 through 15 post-infection. The mice were sacrificed on day 20. Spleens and livers were removed and homogenized in 2–5 mL of sterile saline, respectively. Serial 10-fold dilutions were plated onto potato dextrose agar plates and incubated at 35 °C for a week to determine the number of viable cfu in each organ.

Statistics

For survival studies, the log-rank and Wilcoxon tests were used. The P values for determination of significance varied because of correction for multiple comparisons. For tissue burden studies, Dunnett’s two-tailed t test was used and a P value of ≤0.05 was determined to be significant when the values were compared with those for the controls or for the drugs alone.

Results

Monotherapy study

The results of the survival study with dose escalation treatment with caspofungin and amphotericin B deoxycholate are shown in Table 1. All control mice died between days 13–22. Significant prolongation of survival was noted at caspofungin doses of >0.5 mg/kg compared with controls (P < 0.0001). Amphotericin B deoxycholate doses of >0.1 mg/kg significantly prolonged survival compared with controls (P < 0.0001). Fungal burden for the entire livers and spleens of mice that died or for those of mice that survived to day 50 post-challenge were determined.
Combination therapy study

The intention of the caspofungin combination therapy was to evaluate if caspofungin given in combination with amphotericin B deoxycholate showed improved therapeutic activity. We used three different doses of caspofungin (0.1, 0.5 and 5 mg/kg) and the lowest dose of amphotericin B deoxycholate (0.1 mg/kg). Mice in the control group died on days 13–22. Escalating doses of caspofungin alone showed a trend towards efficacy, similar to the results of the initial monotherapy study reported here.

Any combination of caspofungin with amphotericin B deoxycholate enhanced the period of survival compared with that for untreated infected controls as shown in Table 2. The treatment with amphotericin B deoxycholate (0.1 mg/kg) plus caspofungin at 0.1 and 0.5 mg/kg significantly improved the survival compared with animals treated with caspofungin at 0.1 or 0.5 mg/kg alone (P < 0.0001 and 0.0039, respectively). The treatment with amphotericin B deoxycholate (0.1 mg/kg) plus caspofungin at 5 mg/kg was not significantly improved when compared with caspofungin at 5 mg/kg. The effectiveness of amphotericin B deoxycholate alone did not allow demonstration of significance in prolonging survival by the combinations compared with amphotericin B deoxycholate administered alone. There was no sterilization of tissues in any caspofungin plus amphotericin B deoxycholate regimen. Tissue burdens were determined when the mice died or on day 20 post-infection for those that survived; the results are shown in Figure 2. Treatments with some combinations were significantly more effective than other therapeutic regimens in reducing the fungal burden of liver and spleen. Treatment of mice with amphotericin B deoxycholate (0.1 mg/kg) plus caspofungin (0.1 mg/kg) and amphotericin B deoxycholate (0.1 mg/kg) plus caspofungin (0.5 mg/kg) significantly reduced the counts in both organs relative to the counts in the organs of the controls (P < 0.0001) and significantly reduced the fungal load relative to the counts in the spleen and liver of mice treated with caspofungin (0.1 and 0.5 mg/kg) alone (P < 0.0001). There was no significant difference when compared with amphotericin B deoxycholate (0.1 mg/kg) alone. However, the treatment with amphotericin B deoxycholate (0.1 mg/kg) plus caspofungin (5 mg/kg) significantly reduced spleen and liver counts compared with controls, and amphotericin B deoxycholate (0.1 mg/kg) alone and caspofungin (5.0 mg/kg) alone (P < 0.0001).

No sterilization of either organ was observed with caspofungin doses. Ten per cent of the spleens were free of detectable infection when mice were treated with amphotericin B deoxycholate at 1 or 5 mg/kg, intraperitoneally. Positive culture results were obtained for all organs examined from each of the untreated control animals. The tissue fungal burdens of mice were determined when they died or on day 20 post-challenge for those who survived; the results are shown in Figure 1. Treatment of mice with caspofungin at 0.1 and 0.2 mg/kg daily was ineffective in reducing the fungal burden in spleen and liver, and there was no significant difference from the control value. However, treatment of mice with caspofungin at ≥0.5 mg/kg significantly reduced spleen and liver counts in a dose-dependent manner (P < 0.0001). Meanwhile, treatment with amphotericin B deoxycholate at ≥0.1 mg/kg significantly reduced the fungal load of both organs compared with controls (P < 0.0001).

Table 1. Survival of mice with systemic coccidioidomycosis and left untreated or treated with caspofungin (CAS) or amphotericin B deoxycholate (DAMB)

<table>
<thead>
<tr>
<th>Treatment group&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Dose (mg/kg)</th>
<th>Survivors (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>none</td>
<td>0</td>
</tr>
<tr>
<td>CAS</td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>10</td>
</tr>
<tr>
<td></td>
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<td></td>
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<td>50</td>
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<td></td>
<td>5.0</td>
<td>80</td>
</tr>
<tr>
<td>DAMB</td>
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<td>90</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>100</td>
</tr>
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<td></td>
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<td></td>
<td>5.0</td>
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</tr>
</tbody>
</table>

<sup>a</sup>Ten mice per group. The mice were monitored through to day 50 post-challenge.

Discussion

Despite current advances in the treatment of invasive fungal infections, therapy of coccidioidomycosis is restricted to polyenes and azoles. Amphotericin B deoxycholate therapy is associated with frequent relapses and both acute and chronic toxicity. Renal insufficiency may be severe enough to lead to either early dose reduction or discontinuation of the drug. Treatment with itraconazole and fluconazole is also associated with frequent post-treatment relapses.<sup>11,12</sup>

In the present study, we evaluated the in vivo interaction between caspofungin alone and caspofungin in combination with amphotericin B deoxycholate against systemic coccidioidomycosis in a murine model. Treatment with caspofungin, particularly at 0.5 mg/kg given daily, was seen to prolong survival. Furthermore, in our experiments, as noted above, treatment with caspofungin at 0.5 mg/kg also reduced the cfu in spleen and liver compared with the fungal load in those tissues of the controls. However, no dose of caspofungin alone displayed sterilization of the organs.

Under the conditions of our experiments, the combination of caspofungin with amphotericin B deoxycholate was significant in prolonging survival and reducing C. posadasii burdens of spleens and livers of infected mice compared with controls. No antagonism was observed with caspofungin plus amphotericin B deoxycholate combinations.

Data on both the in vitro interaction of caspofungin with other antifungal drugs and the in vivo use of caspofungin in combination therapy against Coccidioides spp. are as yet limited. However, the activities of the combination of caspofungin with amphotericin B deoxycholate against Aspergillus spp. have been broadly evaluated in vitro.<sup>13</sup> Interactions between caspofungin and azole drugs have been investigated in vitro and in vivo.<sup>14–16</sup> The studies demonstrated synergistic interaction. Interactions
between caspofungin and azole drugs have also been investigated in vivo in animals.\textsuperscript{17,18}

Combinations of echinocandins and polyene have been studied also in clinical trials.\textsuperscript{19,20} In these studies, it was demonstrated that this combination can be administered safely to high-risk patients with haematological malignancies. These trials are open studies with a retrospective design, with small numbers of patients, poor data quality and, in general, are difficult to interpret. But there remains some interest in conducting a Phase III control prospective randomized trial of amphotericin B plus echinocandins.

The present study expands the potential of combination therapy of coccidioidomycosis. This is of interest especially for the early weeks of treatment, when patients are most severely ill. We have no human data on combination therapy in coccidioidomycosis, but the present murine study should encourage its consideration.

**Table 2.** Survival study of mice with systemic coccidioidomycosis and left untreated or treated with caspofungin (CAS), amphotericin B deoxycholate (DAMB) or CAS/DAMB

<table>
<thead>
<tr>
<th>Treatment group\textsuperscript{a}</th>
<th>Dose (mg/kg)</th>
<th>Survivors (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
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<td>0</td>
</tr>
<tr>
<td>CAS/DAMB</td>
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</tr>
<tr>
<td>CAS/DAMB</td>
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<td>0</td>
</tr>
<tr>
<td>CAS</td>
<td>0.5</td>
<td>40</td>
</tr>
<tr>
<td>CAS</td>
<td>5.0</td>
<td>70</td>
</tr>
<tr>
<td>DAMB</td>
<td>0.1</td>
<td>100</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Ten mice per group. The mice were monitored through to day 50 post-challenge.
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Transparency declarations

None to declare.

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