Efficacy of amphotericin B lipid complex in a rabbit model of coccidioidal meningitis

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Objectives: We compared the efficacy of treatments in a rabbit model of coccidioidal meningitis (CM).

Methods: Rabbits were infected intracisternally with *Coccidioides immitis* and treated with intravenous amphotericin B lipid complex (ABLC), deoxycholate amphotericin B (dAMB), oral fluconazole or diluent [sterile 5% dextrose in water (D5W)]. Survival and cfu in brain, spinal cord and CSF were determined and histology studied. Amphotericin B (AMB) concentrations in serum, CSF and tissue were determined by bioassay.

Results: Fluconazole-treated rabbits and controls lost weight and had decreased mobility. All treatments prolonged survival (P < 0.005) and reduced cfu in brain and spinal cord (P < 0.008); ABLC or dAMB significantly reduced cfu in CNS tissues compared with fluconazole (all P < 0.02). ABLC cleared cfu from CSF faster than dAMB or fluconazole. Histologically, 9/9, 7/8 and 0/24 of the D5W, fluconazole and amphotericin groups, respectively, had severe meningitis. Serum AMB was higher after ABLC at 15 mg/kg than after dAMB (P < 0.02).

Conclusions: Intravenous ABLC was efficacious and could be a treatment option for CM.

Keywords: amphotericin B lipid complex, *Coccidioides immitis*, AMB

Introduction

Coccidioidal meningitis (CM) is the most severe complication of infection with *Coccidioides*. Currently, the standard of therapy for CM is life-long treatment with an azole and/or deoxycholate amphotericin B (dAMB) given intrathecally. Intravenous dAMB therapy is ineffective against CM. However, because azoles are not curative, and intrathecal dAMB is toxic and often unsuccessful, it is necessary to investigate new therapeutic options.

Intravenous amphotericin B lipid complex (ABLC; Abelcet, Enzon Pharmaceuticals, Inc., Bridgewater, NJ, USA) has shown efficacy in models of systemic or meningeval coccidioidomycosis, and has been used for systemic treatment of nonmeningeal coccidioidomycosis in man. We evaluated the efficacy of intravenous ABLC in comparison with oral fluconazole or intravenous dAMB in a rabbit model of CM.

Materials and methods

Infection model

Male New Zealand White (NZW) rabbits (~2.5–3 kg body weight; Myrtles Rabbitry, Inc., Thompson Station, TN, USA) were infected with *Coccidioides immitis* (Silveira strain) by direct cisternal inoculation of 5 × 10⁷ arthroconidia as previously described. ⁶ Samples of blood and CSF were obtained on the day of infection and various times thereafter. Clinical assessments were performed as described previously starting 2 days before infection. ⁶ *C. immitis* cfu in the CSF and tissues were determined as described previously. ⁶
Treatment

Treatment groups consisted of eight animals each, with nine animals in the control group. Beginning 5 days post-infection, controls received sterile 5% dextrose in water (D5W). Treatments were oral fluconazole 80 mg/kg once daily for 19 days (a previously studied dose that produces serum and CSF concentrations similar to those in humans) in PEG200, intravenous infusion three times per week for 3 weeks of dAMB 1 mg/kg in D5W, or ABLC 7.5 or 15 mg/kg in D5W.

Rabbits were euthanized at day 26 or 27, or when they met established criteria for euthanasia. After euthanasia, brain and spinal cord were removed for histopathology and cfu determination as previously described; a small portion of the brain tissue was frozen at −80°C for later determination of amphotericin B (AMB) concentration. All procedures were done with the approval of the Animal Care and Use Committee of the California Institute for Medical Research.

Histology

Microscopic examination of haematoxylin and eosin stained sections was performed in blinded fashion. Meningitis was defined as mild (i.e. rare foci of small meningeal inflammatory infiltrates), moderate (i.e. more numerous and larger infiltrates) and severe (i.e. large numbers of confluent inflammatory infiltrates with numerous granulomas). Parenchymal findings were recorded as described previously.

AMB levels assay

Serum, CSF and brain tissue AMB concentrations were determined by bioassay as described previously. Portions of 200 mg of brain were extracted with 70% methanol; clarified supernatants were dried and residual material suspended in serum. The lower limit of detection for the bioassay was 0.031 μg of AMB/mL.

Statistical analyses

Survival was analysed by log-rank test and tissue burdens were compared by Mann–Whitney U-test using GraphPad Prism 3.03 for Windows.

Results

Clinical

After a transient spike in body temperature 24 h after infection, no significant differences between groups were noted. After day 7 or 8 of infection, D5W- and fluconazole-treated animals progressively lost weight from mean highs of 2.8 or 3.0 kg to lows of 2.6 or 2.4 kg, respectively, at the end of the experiment; dAMB- or ABLC-treated animals gained from 2.9 or 2.8 kg to 3.3 or 3.2 kg, respectively. The mobility and coordination of only the D5W- and fluconazole-treated animals progressively declined, to a mean mobility score of 3.1 or 3.0 out of five, respectively.

Survival

All treatments significantly prolonged survival compared with the controls (P = 0.005); there were no differences in survival among treatment groups (Figure 1a).

Tissue burdens

Animals given ABLC at 7.5 or 15 mg/kg, fluconazole or dAMB had significantly lower cfu in brain (P < 0.0001, < 0.0001, 0.008 and < 0.0001, respectively) and spinal cord (P = 0.002, < 0.0001, 0.003 and < 0.0001, respectively) compared with controls (Figure 1b). No D5W- or fluconazole-treated animals were free of infection from either tissue. ABLC at 7.5 or 15 mg/kg, or dAMB, cleared infection from both tissues in 2, 3 and 2 of the animals, respectively. Animals receiving ABLC
ABLC against coccidioidal meningitis

7.5 or 15 mg/kg or dAMB had significantly lower fungal burdens than those given fluconazole, in brain (\(P = 0.02\), 0.002 and 0.005, respectively) and spinal cord (\(P = 0.015\), 0.0002 and 0.002, respectively). There were no significant differences comparing dAMB with ABLC treatments, nor between the two doses of ABLC; those treated with ABLC at 15 mg/kg had the lowest median cfu (Figure 1b).

Determination of *C. immitis* in CSF (Figure 1c) showed a general decrease in numbers of positive cultures and in cfu counts comparing samples from day 15–16 with day 26–27 post-infection. Animals receiving ABLC at 7.5 mg/kg had the fewest. Fluconazole-treated animals had increased positive cultures over the disease course. Significantly fewer rabbits treated with ABLC at 7.5 mg/kg had positive CSF cultures compared with those treated with dAMB (\(P = 0.04\), Fisher’s exact test) at day 15–16. The cfu recovered on days 15–16 post-infection from ABLC at 7.5 mg/kg trended to lower than the dAMB group (\(P = 0.056\)); this ABLC regimen was the only regimen to produce sterilization of the CSF in all animals at terminal sampling.

Histopathology

Severe granulomatous meningitis and/or arteritis were observed in all controls and in seven fluconazole-treated animals (Table 1). Ischaemia and abscesses were found only in DSW- and fluconazole-treated animals. One dAMB-treated animal showed arteritis, whereas no ABLC-treated animals had arteritis. One each treated with ABLC at 7.5 mg/kg or 15 mg/kg had normal brain and spinal cord findings.

**AMB levels**

The median serum concentrations of AMB 2 h after the fifth dose were 0.89, 0.75 and 0.42 mg/L in the ABLC 15 mg/kg, ABLC 7.5 mg/kg and dAMB groups (not significantly different). Twenty-four hours after this dose, median serum concentrations of AMB were 0.50, 0.20 and 0.21 mg/L, respectively. This was a significant difference (\(P = 0.02\)) comparing ABLC at 15 mg/kg with dAMB. At 3–4 days after the last dose, there were significantly higher serum AMB concentrations in animals given ABLC 15 mg/kg (median 0.11 mg/L) or 7.5 mg/kg (median 0.05 mg/L) compared with dAMB (not detectable) (\(P < 0.01\)); ABLC at 7.5 mg/kg and ABLC at 15 mg/kg were not significantly different. AMB was not detected in CSF or brain of any treated animal.

### Discussion

We previously reported greater efficacy of intravenous liposomal AMB (L-AMB) compared with dAMB or fluconazole in treating CM in rabbits. Here, we determined the efficacy of ABLC treatment. Our results show that intravenous ABLC resulted in better therapeutic responses than did fluconazole. Clinical parameters also reflected improved efficacy since ABLC-treated animals showed normal activity and mobility in contrast to fluconazole-treated and control animals, which appeared severely ill and had reduced mobility. Although ABLC showed no statistical advantage over dAMB in survival or tissue fungal burdens, median fungal burdens were lower in ABLC-treated groups. They also showed more rapid fungal clearance from the CSF, and CSF sterilization in all animals in the 7.5 mg/kg ABLC group. Both ABLC and dAMB were better than fluconazole in reducing meningitis severity. Furthermore, one each in the ABLC treatment groups had no histological abnormalities or detectable cfu, indicating cure. In general, these results are similar to those obtained with L-AMB in this model.

Serum concentrations of AMB were higher 24 h after the fifth dose of ABLC at 15 mg/kg and after 3–4 days after nine doses compared with dAMB-treated animals; they suggest the prolonged presence of available drug. Previously, Groll et al. reported concentrations of AMB in plasma (0.84 mg/L), brain (0.35 mg/L) and CSF (0.026 mg/L) in rabbits 30 min after the seventh daily dose of ABLC at 5 mg/kg. In contrast, we did not detect AMB in CSF or brain of any dAMB- or ABLC-treated animals. This may be due to the elapsed time between dosage and sampling, i.e. 3–4 days versus 30 min post-dose, rather than to the level of detection of the bioassay method (0.031 mg/L); this is equivalent to the sensitivity of the HPLC method used by Groll et al., moreover, if the CNS vasculature was not flushed with a drug-free solution prior to processing the parenchyma for drug-level determination, the drug content in the vessels in the parenchyma could have contributed to the values they attributed to the brain. Nevertheless, we show ABLC greatly reduced overall disease severity. In conjunction with other studies showing effectiveness of ABLC in experimental CNS fungal

<table>
<thead>
<tr>
<th>Group (mg/kg/dose)</th>
<th>meningitis</th>
<th>parenchymal findings</th>
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<tbody>
<tr>
<td></td>
<td>absent</td>
<td>mild</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
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</tr>
<tr>
<td>Fluconazole (80)</td>
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<td>0</td>
</tr>
<tr>
<td>dAMB (1)</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>ABLC (7.5)</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>ABLC (15)</td>
<td>1</td>
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</table>

The table summarizes the number of animals showing CNS parenchymal findings and meningitis. Meningitis was scored as mild, moderate or severe as described in the text. Treatment groups consisted of eight animals/group and the control group consisted of nine animals.
infections,\textsuperscript{8–10} the current data indicate that CNS AMB concentrations effective against \textit{C. immitis} are achieved. As both L-AMB\textsuperscript{6} and ABLC demonstrate advantages in this model over dAMB, it would be of interest, in future studies, to compare them.

In conclusion, intravenous ABLC demonstrated efficacy in CM suggesting that ABLC may be a more effective therapeutic option than azoles in CM and avoid the toxicity of intrathecal dAMB.

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Transparency declarations

None to declare.

References


