Efficacy of Abelcet alone, or in combination therapy, against experimental central nervous system aspergillosis

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Background: CNS aspergillosis is the most frequent and devastating manifestation of dissemination and mortality is high.

Methods: Cyclophosphamide-suppressed CD-1 mice were infected intracerebrally with conidia of Aspergillus fumigatus and treated for 10 days with suboptimal doses of Abelcet (4 mg/kg) plus micafungin (1 mg/kg), caspofungin (1 mg/kg), itraconazole (100 mg/kg) or voriconazole (40 mg/kg) and compared with monotherapy. Other groups included conventional amphotericin B (1 mg/kg), Abelcet at 10 or 12 mg/kg or 5% dextrose water (diluent control).

Results: All controls died and all treatment regimens significantly prolonged survival. No monotherapy regimen was superior to another. All dosages of Abelcet and conventional amphotericin B tested were equivalent. Significant enhancement of survival over the respective monotherapies was found only with the combination of Abelcet and voriconazole. Other combinations were not better than Abelcet alone. Recovery of cfu from the brains and kidneys of survivors showed that no regimen was curative. Abelcet and voriconazole showed significantly enhanced efficacy in reducing brain infection. Other combinations showed lower cfu, but no significant enhancement over either drug alone. Dose-escalation of Abelcet alone did not increase reduction of cfu. Recovery from the kidneys showed non-significant reduction of cfu by combinations compared with monotherapies.

Conclusions: Each of the drugs tested had significant efficacy against CNS aspergillosis and Abelcet in combination with voriconazole had enhanced efficacy. Additional studies are warranted.

Keywords: murine models, antifungal therapies, echinocandins, azoles, fungi

Introduction

The introduction of new antifungal agents has improved the therapeutic options available for aspergillosis. However, cures remain elusive, particularly for CNS infection where mortality can be >85%.1 Because of the frequent lack of effectiveness of monotherapy, combination therapy is being examined. Few studies have been performed to date and little is known about optimal drug combinations and effectiveness. We have recently described a murine model of CNS aspergillosis, which has been used to examine therapy with a variety of agents.2–4 In the majority of those studies monotherapy was examined, and although efficacies were found, cure was not obtained.

We have examined the efficacy of combination therapy with Abelcet and caspofungin, which proved no more efficacious than Abelcet alone, although both showed significant efficacy.4 Thus, in the current study we examined other antifungal agents that could be used in combination with Abelcet against CNS aspergillosis.

Materials and methods

Murine model of CNS aspergillosis

A murine model of CNS aspergillosis was established as described previously, utilizing 6.8 × 106 conidia of Aspergillus fumigatus by
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direct intracerebral inoculation. All animal work was performed with the approval of the Institutional Animal Care and Use Committee of the California Institute for Medical Research following the guidelines of the USDA and Office of Laboratory Animal Welfare of NIH.

Therapy studies

For the therapy studies all treatment groups consisted of 10 mice. Therapy was initiated on day 1 post-infection and continued for 10 consecutive days. The study consisted of a dose-escalation monotherapy study with Abelcet and a combination therapy study using an effective but suboptimal dose of Abelcet in combination. Dosages chosen were based on experience in other studies and were those shown to be effective, but not optimal. Doses tested previously were itraconazole (50 mg/kg twice daily or 100 mg/kg once daily), voriconazole (5–40 mg/kg), caspofungin (0.8–10 mg/kg) and micafungin (1–10 mg/kg).

The treatment regimens for the monotherapy arms were as follows: 5% dextrose water controls (D5W), iv once daily; conventional amphotericin B at 1 mg/kg, iv once daily; Abelcet at 4, 10 or 12 mg/kg, iv once daily; caspofungin at 1 mg/kg/day, ip twice daily; micafungin at 1 mg/kg/day, ip twice daily; itraconazole at 100 mg/kg (in 2-hydroxypropyl-β-cyclodextrin), orally once daily.

Figure 1. Cumulative mortality of mice infected intracerebrally with A. fumigatus and given one of the indicated treatments. All treatment groups consisted of 10 mice and treatments were given as described in the text. ABLC, Abelcet; MICA, micafungin; CAS, caspofungin; VCZ, voriconazole; AMB, conventional deoxycholate amphotericin B; ICZ, itraconazole.
and voriconazole in 4% PEG400 at 40 mg/kg orally, once daily, from the manufacturers noted previously. Mice receiving voriconazole were given grapefruit juice 50% in water beginning on day 3 prior to infection. The groups for combination therapy were Abelcet at 4 mg/kg and caspofungin, micafungin, itraconazole or voriconazole.

Determination of fungal tissue burdens

On day 14 of infection, all surviving mice were euthanatized and the brain and kidneys removed aseptically, cfu in these tissues was determined by homogenization of the tissues and plating of serial dilutions. Comparative analyses were carried out by a conservative non-parametric Kruskal–Wallis ANOVA followed by a Dunn’s test for multiple comparisons, or by Mann–Whitney U-tests.

Statistical analyses

Analysis of survival was carried out by log-rank test. For the analyses of comparative organ burdens of *A. fumigatus*, samples missing due to death were assigned a value of 5 log10 cfu, which assures that death is considered as a worse outcome than is survival with any amount of fungal burden. This value approximates the fungal burden just prior to death. Comparative analyses were carried out by a conservative non-parametric Kruskal–Wallis ANOVA followed by a Dunn’s test for multiple comparisons, or by Mann–Whitney U-tests.

Results

All diluent control animals succumbed to infection. In comparison with controls, all treatment regimens significantly prolonged survival ($P = 0.03–0.0001$, dependent on comparison) (Figure 1). No monotherapy regimen proved superior to other antifungal agents at the doses tested.

Dose-escalation of Abelcet showed that all three dosages were equivalent (Figure 1). Both the 4 and 10 mg/kg of Abelcet doses resulted in 60% survival, whereas mice treated with Abelcet at 12 mg/kg had 40% survival. Whether this latter result is reflective of cumulative toxicity or dose-dependent pharmacokinetics remains to be determined. However, there appears to be no dose-responsiveness over this dose range.

The combination of Abelcet at 4 mg/kg plus another antifungal, both given at suboptimal dose, proved useful for each tested combination. Significant enhancement of survival was found only with the combination of Abelcet and voriconazole, where the combination was significantly better than either drug given alone ($P = 0.03$ or 0.04 versus monotherapies). Of the remaining combinations, none was better than Abelcet alone. Mice treated with Abelcet and an echinocandin had better survival than those given Abelcet alone or the echinocandin alone, whereas those given Abelcet and itraconazole had lower survival than those given itraconazole alone (Figure 1). Whether the latter result is due to antagonism or toxicity remains to be determined.

The recovery of cfu from the brains and kidneys of surviving mice are shown in Figure 2. No mouse in any treatment regimen was free of infection in either organ. In the brain, animals treated with Abelcet and voriconazole had the lowest median number of cfu recovered; this fungal burden was significantly lower than that recovered from those given voriconazole alone ($P < 0.05$), but equivalent to those given the Abelcet alone using a Kruskal–Wallis ANOVA and Dunn’s test for multiple comparisons; by Mann–Whitney U-test, the Abelcet and voriconazole combination was superior to both monotherapies ($P < 0.05$). Other combinations did not show significant enhancement of efficacy over each drug given alone, but trended towards having lower cfu than those given monotherapy. The dose-escalation of Abelcet between 4, 10 and 12 mg/kg showed a possible reduced cfu with increased dosage. However, these differences were not significant.

Recovery of *A. fumigatus* from the kidneys showed the same effects as those from the brain. Each combination showed lower cfu from the kidneys than the respective monotherapy (Figure 2b). However, no combinations showed significant enhancement in the reduction of cfu as compared with both monotherapies. Again, no cures were obtained.

Discussion

Abelcet and caspofungin both have efficacy against CNS aspergillosis in our mouse model, as do several other antifungal...
In the current study we have further examined the potential efficacy of Abelcet given alone using dosages higher than used previously, as well as the potential utility of suboptimal dosages of Abelcet in combination with suboptimal dosages of an echinocandin or an azole.

Our previous and the current studies indicate that Abelcet has efficacy against CNS aspergillosis. At equivalent 0.8 mg of amphotericin B/kg of body weight doses, conventional amphotericin B and Abelcet were equivalent in the prolongation of survival and conventional amphotericin B was superior only in the clearance of *A. fumigatus* from the brains; higher doses of Abelcet trended to improved clearance of fungal burden. In comparison, our current studies showed no significant differences in the efficacy of conventional amphotericin B or Abelcet for either prolongation of survival or clearance of fungal burden from the organs. Although Abelcet is efficacious, there appears to be no benefit to daily doses above 4 mg/kg in this model.

Our current results demonstrate that monotherapy, even at suboptimal (non-curative) doses with each of the drugs tested, had significant efficacy against CNS aspergillosis, and that Abelcet in combinations was also efficacious. However, only Abelcet and voriconazole in combination resulted in a significant enhancement of survival and reduction of cfu, better than either drug given alone. These results are similar to studies using a liposomal formulation of amphotericin B.

Our current results also extend those previously reported on potential synergy, where no enhancement was observed using higher doses of Abelcet (8 mg/kg) and caspofungin (8 mg/kg) in combination. In comparison with the previous study, it appears that lower dosages in combination (i.e. Abelcet at 4 mg/kg plus caspofungin at 1 mg/kg) give results similar to the 90% survival and the trend of lower cfu in the organs obtained when using dosages of Abelcet at 8 mg/kg plus caspofungin at 8 mg/kg in combination. Thus, the use of even high doses of Abelcet and caspofungin in combination resulted in no enhancement of efficacy over that obtained with the use of suboptimal doses. Although other dosages of Abelcet and micafungin in combination have not been tested, we would postulate a similar effect; Abelcet and itraconazole may also behave similarly. Of note, we found no evident combinatorial toxicities, nor evidence of antagonism, with the various combinations tested.

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**Transparency declarations**

None to declare.

**References**