Combination Therapy with Amphotericin B Lipid Complex and Caspofungin Acetate of Disseminated Zygomycosis in Diabetic Ketoacidotic Mice

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We studied the combination of amphotericin B lipid complex (ABLC) and caspofungin in mice with disseminated *Rhizopus oryzae*. Combination therapy improved survival compared to that of mice given monotherapy and that of untreated controls (*P* < 0.05) but did not improve organ clearance. In addition, prophylactic combination therapy was not more effective than prophylactic ABLC alone.

Despite aggressive therapy, mortality due to zygomycosis exceeds 50% (2, 3, 7–9). It is therefore imperative to look for new antifungal therapies to treat invasive zygomycosis, the most common cause of which is *Rhizopus oryzae* (11). Recently lipid formulations of AmB, such as amphotericin B lipid complex (ABLC), have been used in the treatment of zygomycosis because of their decreased nephrotoxicity compared to that of amphotericin B deoxycholate (6). In contrast, echinocandins have not been widely used to treat zygomycosis. We have found that caspofungin acetate (CAS) has activity in the diabetic murine model of hematogenously disseminated *R. oryzae* infection (4). We therefore tested ABLC and CAS in our murine model to determine the potential for combination therapy to improve outcomes compared to those achieved with monotherapy.

**Organism and animals.** *R. oryzae* 99–880 (brain isolate) and 99–892 (lung isolate) were obtained from the Fungus Testing Laboratory, University of Texas Health Science Center at San Antonio. The MICs of amphotericin B deoxycholate and CAS were 0.25 and >16 μg/ml for both isolates by the NCCLS M38A liquid broth microdilution assay. Spores were collected as previously described (5). Pathogen-free male BALB/c mice (20 to 23 g) were obtained from the National Cancer Institute (Frederick, Md.). All procedures involving mice were approved by the institutional animal use and care committee, in accordance with the National Institutes of Health guidelines for animal housing and care.

**Induction of diabetes and infection.** Mice were rendered diabetic with a single intraperitoneal injection of 210 mg of streptozocin per kg of body weight as previously described (5). Ten days after streptozocin administration, mice (9 to 17 per group in two separate experiments) with confirmed glycosuria and ketonuria were infected via the tail vein with 5 × 10³ spores of the brain isolate or 10⁴ spores of the lung isolate of *R. oryzae* (on the basis of pilot studies indicating decreased virulence of the lung isolate).

**Treatment and primary assessment.** Mice received 4 days of ABLC (5 mg/kg/day in 5% dextrose in water), CAS (1 mg/kg/day in distilled water), or a combination of the two administered via the tail vein starting either 24 h postinfection (delayed) or 24 h prior to infection (prophylactic) (5). Positive control mice were diabetic and infected but not treated, where-

![FIG. 1](http://aac.asm.org/...)

FIG. 1. Overall survival during delayed therapy (initiated 24 h postinfection) of diabetic ketoacidotic mice infected with the *R. oryzae* lung isolate (9 per group) (a) or brain isolate (17 per group) (b). *P* < 0.05 by log rank test compared to mice given CAS monotherapy (a) or compared to uninfected controls and mice given CAS and ABLC monotherapies (b).
as negative control mice were diabetic but not infected. Moribund mice (i.e., those unable to ambulate) were sacrificed by pentobarbital overdose. The primary endpoint was the Kaplan-Meier time to death. Median survival time and tissue fungal burden (see below) were secondary endpoints. Tissue fungal burdens in the brains and kidneys (the primary target organs) (1, 4) were determined by quantitative PCR (qPCR) as previously described (1, 4). All qPCR results are expressed as log10 spore equivalents per gram of tissue.

Statistics. Kaplan-Meier curves were compared pairwise by the log rank test. Median survival times and tissue fungal burden results were compared with the nonparametric Wilcoxon rank sum or Steel test for multiple comparisons (10), as appropriate. P values of ≤0.05 were considered significant.

Delayed therapy. The brain isolate of *R. oryzae* (Fig. 1b) was more virulent than the lung isolate (Fig. 1a) by both time to death (P = 0.002 by log rank test) and median survival (5 versus 9 days; P = 0.003 by Wilcoxon rank sum test) in untreated mice. In mice infected with the lung isolate of *R. oryzae*, delayed combination therapy nonsignificantly improved the time to death versus that of untreated controls and significantly improved the time to death versus that of mice given CAS monotherapy (P = 0.1 and 0.01, respectively, by log rank test; Fig. 1a). In mice infected with the brain isolate, delayed combination therapy significantly improved the time to death versus that of all other groups (P < 0.05 by log rank test).

The more virulent brain isolate caused a higher brain fungal burden in untreated mice than did the less virulent lung isolate (P = 0.046 by Wilcoxon rank sum test; Fig. 2). All three delayed therapies reduced the brain burden of the less virulent lung isolate compared to that of untreated controls (P = 0.001 by Steel test). There was a strong trend to higher brain burdens in mice treated with combination therapy compared to those given ABLC monotherapy (P = 0.054).

In contrast to the brain, in the kidneys the less virulent lung isolate caused higher fungal burdens than did the more virulent brain isolate (P = 0.046 by Wilcoxon rank sum test). Both delayed ABLC therapy and combination therapy significantly reduced the burden of both *R. oryzae* fungal isolates in the kidneys (P < 0.05 for all comparisons by Steel test). CAS monotherapy did not significantly decrease the burden of either isolate in the kidneys.

Prophylactic therapy. Prophylactic combination therapy and ABLC monotherapy significantly increased the time to death of mice infected with both *R. oryzae* isolates compared to that of untreated controls (Fig. 3; P < 0.04 for both by log rank test). Although prophylactic combination therapy caused a trend to improved time to death and median survival versus ABLC monotherapy in mice infected with the more virulent isolate, these differences were not statistically significant.

While CAS displayed unimpressive in vivo activity on its own during *R. oryzae* infection in mice with diabetic ketoacidosis, delayed combination (ABLC plus CAS) therapy demonstrated synergy against the more virulent brain isolate of *R. oryzae*, resulting in greater survival than that achieved by the sum of ABLC and CAS monotherapies. Because of the diminished virulence of the lung isolate, the favorable trend for combina-

FIG. 2. Burdens of *R. oryzae* brain and lung isolates in mice receiving delayed therapy. Six to 12 mice per group were used in two different experiments. The lower limit of the assay was 1.986 log10 spore equivalents/g. *, P < 0.05 by Steel test compared to untreated control mice.

FIG. 3. Overall survival during prophylactic therapy (initiated 24 h prior to infection) of diabetic ketoacidotic mice (nine per group) infected with the *R. oryzae* lung (a) and brain (b) isolates. *, P < 0.05 by log rank test versus uninfected mice.
tion therapy versus ABLC monotherapy was not significant. Similarly, superiority of combination therapy over ABLC monotherapy was not established in the prophylactic setting, likely because the survival was already high in the ABLC arm.

We observed several differences between the more and less virulent strains of *R. oryzae*. As determined by qPCR (TaqMan assay), the more virulent strain was highly tropic to the brain and less so to the kidneys. Conversely, the less virulent strain demonstrated the opposite tropism. These data suggest that a higher burden of organisms in the brain is responsible for the decreased time to death seen in mice infected with the more virulent strain, and that brain tropism may be a key factor in the virulence of the organism.

ABLC reduced the brain and kidney burdens of both the more and less virulent *R. oryzae* fungal isolates. Combination therapy significantly reduced the fungal burdens of the low-virulence isolate in the brain and kidneys but showed a trend to poorer clearance of the high-virulence isolate from the brain than that achieved by ABLC monotherapy. The significance of this trend to antagonism of combination therapy in the clearance of the more virulent isolate from the brain is unclear. The lack of any antagonism in the clearance of either isolate from the kidneys or the low-virulence isolate from the brain suggests that the trend may be an artifact. Regardless, of greater importance is that the time to death of mice treated with combination therapy was significantly improved versus that of mice given ABLC monotherapy, indicating the superiority of combination therapy in this model.

In summary, combination therapy with ABLC and CAS is more effective than ABLC monotherapy in mice with diabetic ketoacidosis infected with *R. oryzae*. Combination therapy with ABLC and CAS is a promising strategy to improve outcomes during highly lethal zygomycosis infections.

REFERENCES


