Liposomal Amphotericin B, and Not Amphotericin B Deoxycholate, Improves Survival of Diabetic Mice Infected with Rhizopus oryzae

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The efficacies of liposomal amphotericin B (LAmB) and amphotericin B deoxycholate (AmB) were compared in a diabetic murine model of hematogenously disseminated Rhizopus oryzae infection. At 7.5 mg/kg of body weight twice a day (b.i.d.), LAmB significantly improved overall survival compared to the rates of survival in both untreated control mice (P = 0.001) and mice treated with 0.5 mg of AmB per kg b.i.d. (P = 0.047). These data indicate that high-dose LAmB is more effective than AmB in treating murine disseminated zygomycosis.

Zygomycosis is a frequently fatal infection that occurs in patients with elevated available levels of iron in serum, such as those treated with deferoxamine, or in patients immunocompromised by diabetic ketoacidosis, organ transplantation, or neutropenia (2, 12). The therapy for invasive zygomycosis includes reversal of the underlying predisposing factors, emergent surgical debridement, and antifungal chemotherapy (5, 10, 12). Although prospective clinical studies are lacking, amphotericin B deoxycholate (AmB) remains the antifungal therapy of choice for invasive zygomycosis (5, 12), largely because of a historical lack of alternative cidal therapies. Because the fungus is relatively resistant to AmB, high doses are required, frequently resulting in nephrotoxicity and other adverse effects (12). Even when surgical debridement is combined with high-dose AmB, the mortality associated with zygomycosis exceeds 50% (12). This mortality rate approaches 100% in patients with disseminated zygomycosis, possibly because surgery to remove the infected foci is not feasible (2). These data emphasize the critical need for more effective antifungal chemotherapy for this lethal infection.

The lipid formulations of AmB allow the administration of higher drug doses due to their limited toxicities (1, 4). Scattered case reports have demonstrated successful outcome in patients with zygomycosis treated with lipid-associated AmB (7, 8). Since diabetic ketoacidosis represents a major risk factor for the development of zygomycosis infection (5, 12), we used a diabetic mouse model to compare the efficacy of high doses of lipidic AmB (LAmB) against that of AmB in treating hematogenously disseminated zygomycosis caused by Rhizopus oryzae, the most common etiologic pathogen of zygomycosis (11).

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R. oryzae 99-880 was obtained from the Fungus Testing Laboratory, University of Texas Health Science Center, San Antonio. This strain was isolated from a brain abscess of a diabetic patient with rhinocerebral zygomycosis. Spores were collected by flooding potato dextrose agar plates (PDA) with 7 ml of endotoxin-free phosphate-buffered saline (PBS) containing 0.01% Tween 80 and gently scraping the aerial mycelium.

Male BALB/c mice (≥24 g) were rendered diabetic with a single intraperitoneal (i.p.) injection of 210 mg of streptozocin per kg of body weight in 0.2 ml of ice-cold citrate buffer 10 days prior to fungal challenge (13). Glocosuria, as determined by the use of keto-Diastix reagent strips, was confirmed in mice 7 days after streptozocin treatment. Suspensions of R. oryzae spores in 0.2 ml of endotoxin-free PBS were injected into the lateral tail vein. AmB (Fungizone) and LAmB (Fujisawa Healthcare, and Gilead Sciences) were administered via the lateral tail vein in 5% glucose solution, with the first dose starting 24 h postinfection. Survival data were analyzed by the nonparametric log-rank test. Median survival times were compared by using the nonparametric Steel test for multiple comparisons. Comparisons with P < 0.05 were considered significant.

Preliminary studies indicated that inocula of ≥5 × 106 spores would not be suitable for subsequent efficacy studies, because the majority of the deaths at these high inocula occurred within 4 days of infection, which would not allow time to administer a complete 4-day course of treatment (Fig. 1). Consequently, an inoculum of 103 spores was chosen for antifungal efficacy experiments. Intravenous challenge of diabetic mice with R. oryzae resulted in hematogenous dissemination of the infection to all organs examined, including brain, kidneys, lungs, liver, spleen, and heart, as indicated by detection of fungal growth from organs cultured on PDA plates.

Prior to evaluation of efficacy, we studied the toxicities of once-daily AmB at 1 mg/kg/day and LAmB at 15 mg/kg/day for 4 days in uninfected mice (treatment beyond 4 days was impossible due to profound sclerosis of the tail veins). The once-daily AmB regimen caused severe toxicity, resulting in 60% mortality in uninfected mice. This AmB-mediated toxicity was likely infusion related, because the mice expired within minutes of intravenous administration of the drug. In contrast, the once-daily LAmB regimen did not result in any deaths. Because of the toxicity of once-daily AmB, we chose to administer both drugs twice daily (b.i.d.) at the same total dose (i.e., AmB at 0.5 mg/kg b.i.d. and LAmB at 7.5 mg/kg b.i.d.). This dosing regimen completely elim-
There is a developing consensus that high doses of lipid formulations of amphotericin B (AmB) up to 15 mg/kg/day (3, 6, 14). Although AmB is nephrotoxic than AmB and can be administered at higher doses. Several case reports of patients with zygomycosis document success.

Conversely, AmB mediated no survival benefit over that of untreated controls, and lower doses of LAmB (2.5 and 5 mg/kg b.i.d.) also did not significantly improve the survival of infected mice. Collectively, these data emphasize the critical importance of administering high doses of drug for efficacy against R. oryzae and indicate that LAmB may be the preferred clinical treatment, given its diminished toxicity profile, which allows administration of doses ≥15-fold above those of AmB.

We now report that in murine disseminated zygomycosis, LAmB was significantly less toxic than AmB, allowing administration of 15-fold-higher doses of LAmB than AmB. Furthermore, high-dose LAmB (7.5 mg/kg b.i.d.) was more efficacious than AmB (0.5 mg/kg b.i.d.), significantly improving both the median survival time and overall survival of infected mice.

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