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# Caspofungin and Liposomal Amphotericin B Therapy of Experimental Murine Scedosporiosis

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**Immunosuppressed mice were infected intravenously with conidia of *Scedosporium prolificans*. Treatment was begun 1 day later with liposomal amphotericin B, caspofungin, or both drugs initiated concurrently. Amphotericin B and caspofungin were each effective, but combined therapy did not appear to offer advantages over liposomal amphotericin B alone.**

*Scedosporium apiospermum* and *S. prolificans* cause up to 10% of invasive mycelial fungal infections in predisposed patients (4, 7). *S. prolificans* is resistant in vitro to virtually all antifungal agents and has responded poorly to voriconazole, with only 3 of 10 patients improving (1–3, 8–10). A recent review of scedosporiosis in solid organ transplant patients reported only four survivors among 18 patients with *S. prolificans* infection (5).

In this setting, we evaluated a new echinocandin, caspofungin, in the treatment of *S. prolificans* infection. We compared the changes in survival and CFU (as measurements of tissue burden) in neutropenic mice undergoing an acute infection with *S. prolificans*. In addition to caspofungin, we evaluated liposomal amphotericin B alone and in combination with caspofungin.

*S. prolificans* strain 95-2409 was cultured on potato flake agar. Conidia were washed, filtered, counted in a hemacytometer, and suspended in sterile saline. The MIC was determined using the CLSI (formerly NCCLS) method adapted for mycelial fungi, along with the minimal effective concentration according to the method of Kurtz et al. (6). The MIC of caspofungin and liposomal amphotericin B was 8 μg/ml at 24 and 48 h of incubation. The minimal effective concentration of caspofungin was 8 μg/ml at 24 and 48 h.

Male outbred ICR mice were made neutropenic by a single intraperitoneal (i.p.) dose of cyclophosphamide at 200 mg/kg of body weight 1 day prior to infection. Mice were infected intravenously (i.v.) with conidia in a 0.2-ml volume. Beginning 1 day after infection, control mice were treated with sterile water i.p., liposomal amphotericin B (Astellas Pharma Inc., formally Fujisawa) i.v. at 10, 20, or 30 mg/kg once daily, or caspofungin (Merck & Co., Inc) i.p. at 5, 10, or 20 mg/kg once daily. For survival studies, groups of 10 to 12 mice were treated from day 1 through day 10, with observation through day 21 or day 25. Mice which appeared moribund were sacrificed and considered to have died the next day. For studies of tissue burden, mice were treated from day 1 through day 7 after infection and sacrificed on day 8 for quantitative tissue cultures of kidneys. The log rank test was used for comparison of survival studies. The Mann-Whitney U test was used for comparison of CFU counts. Because of multiple comparisons, a $P$ value of $\leq 0.02$ was required for significance.

Survival following infection with a low inoculum dose of *S. prolificans* is shown in Fig. 1A. Liposomal amphotericin B at a dose of 10 or 20 mg/kg significantly prolonged survival over that of controls ($P < 0.0003$), but 30 mg/kg appeared toxic. As shown in Fig. 1B, caspofungin at 5 mg/kg was ineffective, but 10 or 20 mg/kg ($P = 0.0041$) prolonged survival. Survival following administration of a higher dosage of inoculum is shown in Fig. 1C (liposomal amphotericin B and caspofungin) (Fig. 1D). In Fig. 1C, liposomal amphotericin B was ineffective for all three doses ($P > 0.07$). However, as shown in Fig. 1D, caspofungin was ineffective at 5 mg/kg ($P = 0.0452$) but effective at 10 and 20 mg/kg ($P = 0.0032$). Figure 1E shows a survival study with the low dosage of inoculum. Caspofungin prolonged survival over that of controls ($P = 0.0041$). Combined caspofungin and liposomal amphotericin B was similar to a high dose of liposomal amphotericin B alone at 30 mg/kg. There was no significance relative to control values.

Figure 2A shows quantitative tissue cultures for the higher doses of caspofungin and liposomal amphotericin B given individually or beginning together concurrently. Liposomal amphotericin B at 30 mg/kg, but not caspofungin at 20 mg/kg, significantly lowered the fungal counts. Combined caspofungin and liposomal amphotericin B also lowered the fungal counts. Finally, as shown in Fig. 2B, mice were infected with a low dose of inoculum, treated with caspofungin and/or liposomal amphotericin B, and sacrificed at day 8 for tissue counts. Liposomal amphotericin B at 20 mg/kg significantly reduced numbers of CFU, but caspofungin at 20 mg/kg and liposomal amphotericin B at 10 mg/kg were ineffective. The combinations were similar in result to those with liposomal amphotericin B alone.

Laboratory animal models of mycelial infections have traditionally involved studies of survival and reduction of tissue burden. Survival studies have been criticized on ethical grounds. However, they enable the investigator to include drug toxicity as well as efficacy in overall responses, and we believe they are essential components of new-drug evaluation. In the case of our studies, mice receiving 10 or 20 mg/kg liposomal

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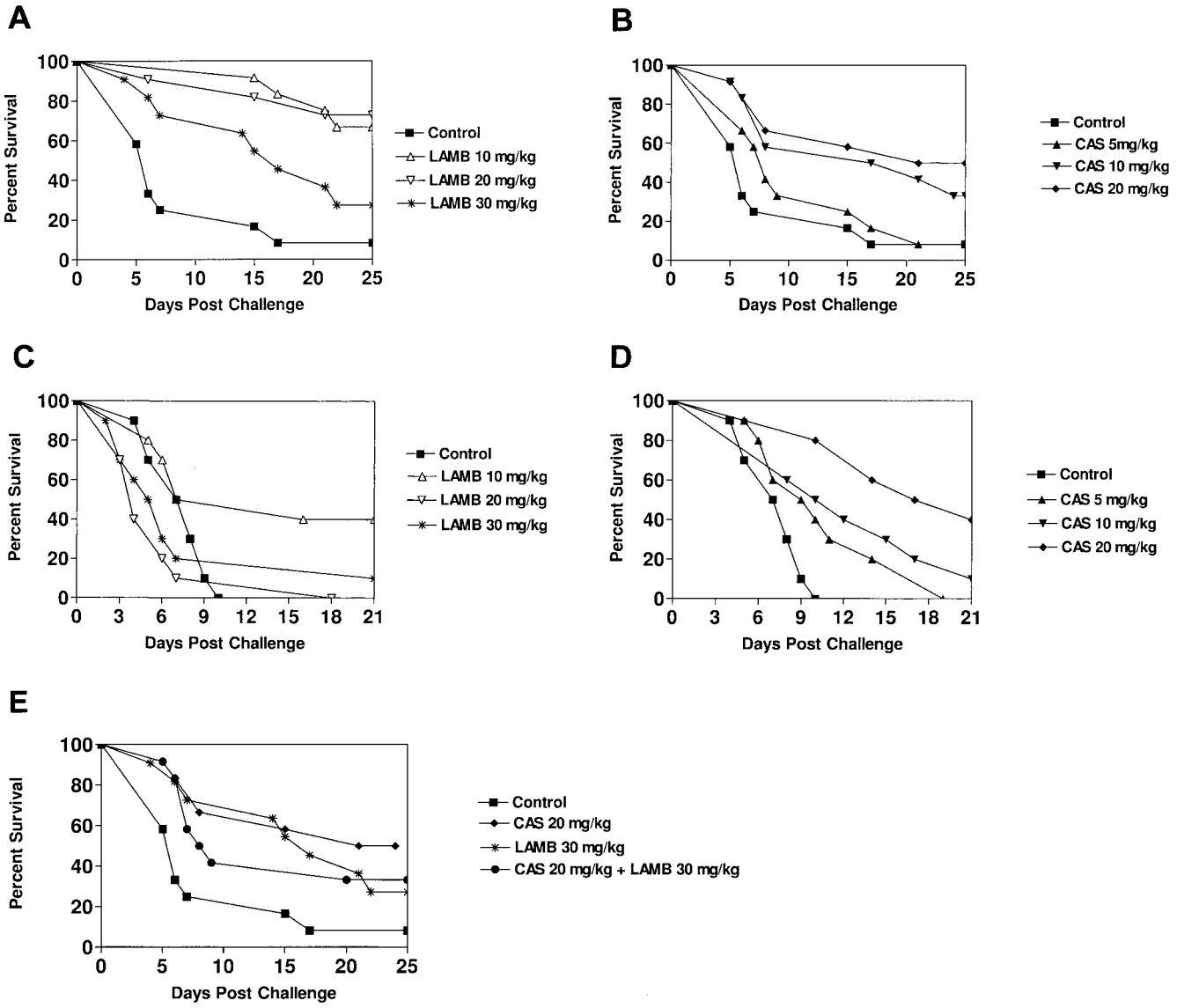


FIG. 1. Survival of mice infected with *S. prolificans* and treated with various antifungal agents. (A) Mice were infected i.v. with $2.4 \times 10^5$ conidia/mouse (low dose of inoculum) and treated days 1 to 10 postchallenge. Controls received 0.2 ml sterile water i.p. Liposomal amphotericin B (LAMB) was given i.v. at 10, 20, or 30 mg/kg. (B) Caspofungin (CAS) was given i.p. at 5, 10, or 20 mg/kg. (C) Mice were infected i.v. at $2.3 \times 10^6$ conidia/mouse (high dose of inoculum) and treated from days 1 to 10 postchallenge. Controls received 0.2 ml sterile water i.p. Liposomal amphotericin B was given i.v. at 10, 20, or 30 mg/kg. (D) Caspofungin was given i.p. at 5, 10, or 20 mg/kg. Caspofungin at 5 mg/kg was not effective. (E) In this survival study, mice were infected i.v. at $2.4 \times 10^5$ conidia/mouse (low dose of inoculum). Therapy was administered days 1 to 10 postchallenge. Controls received 0.2 ml sterile distilled water i.p. Caspofungin was given i.p. at 20 mg/kg alone and in combination with liposomal amphotericin B. Liposomal amphotericin B was given i.v. at 30 mg/kg alone and in combination with caspofungin.

amphotericin B and caspofungin gave significant prolongation of survival. In contrast, the 30-mg/kg dose of liposomal amphotericin B, despite reducing fungal burden, showed shortened survival compared with other treatment regimens. This is likely caused by drug toxicity.

While echinocandins have regularly prolonged survival in models of aspergillosis, they have not consistently reduced tissue burden. This may be due to the selective targeting of actively growing hyphal tips of fungi and an overall fungistatic effect. Viewed alone, tissue burden could give a misleading impression of caspofungin inefficacy.

There may also be an effect of the inoculum size on response to antifungal agents. Mice infected with $2.3 \times 10^6$ conidia of *S. prolificans*/mouse had a higher fungal burden than those infected with 1 log fewer *S. prolificans* conidia. Against this more severe infection, liposomal amphotericin B was not beneficial. However, caspofungin remained protective, suggesting that the efficacy of caspofungin may not be dependent on the infecting dose.

Further, we found essentially no interactions of concurrently initiated liposomal amphotericin B and caspofungin at the doses used in this study. Animals treated with both drugs

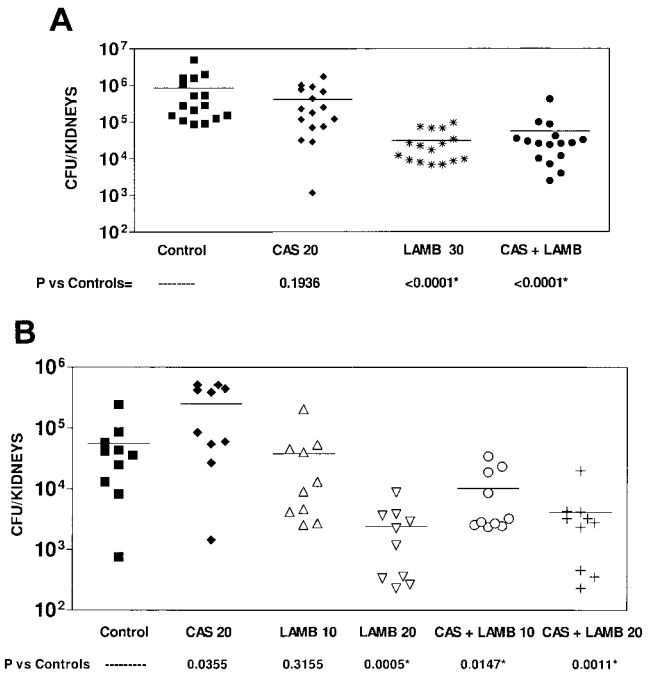


FIG. 2. Burden of *S. prolificans* in kidney tissue treated with various antifungal agents. (A) Mice were infected i.v. with $2.7 \times 10^5$ conidia/mouse and treated days 1 to 7 postchallenge. Controls received 0.2 ml sterile water i.p. Caspofungin (CAS) was given i.p. at 20 mg/kg alone and in combination with liposomal amphotericin B (LAMB). Liposomal amphotericin B was given i.v. at 30 mg/kg alone and in combination with caspofungin. (B) In this study, mice were infected with a sublethal dose of $8.7 \times 10^4$ conidia/mouse and treated with lower doses of liposomal amphotericin B at 10 or 20 mg/kg, caspofungin at 20 mg/kg, or combined therapy.

tended to have both survival and tissue burden in the same range as monotherapy with liposomal amphotericin B alone. Given that caspofungin is effective alone at high inoculation doses, whereas liposomal amphotericin B is not, there may be a good reason for the clinical evaluation of caspofungin alone or even in combination with lipid vehicle forms of amphotericin B in human disease.

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