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Efficacy of Triazoles in a Murine Disseminated Infection by *Candida krusei*▽

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We evaluated the efficacies of posaconazole and voriconazole in comparison with that of amphotericin B in a systemic murine infection by *Candida krusei*. Posaconazole at 50 mg/kg/day and voriconazole at 40 and 60 mg/kg/day prolonged survival and reduced the fungal tissue burden in the kidneys of mice similarly to amphotericin B at 1.5 mg/kg/day and liposomal amphotericin B at 10 mg/kg/day. None of the treatments tested completely resolved the infection.

Candidiasis has become one of the most frequent causes of nosocomial infections. Fluconazole (FLC) is the recommended drug, but several non-*albicans Candida* species such as *Candida krusei* have an intrinsic resistance to FLC. This species is the fifth most common *Candida* species to cause candidemia. An ideal therapy does not yet exist for *C. krusei* infections, and a high mortality rate is reported (15). Currently recommended antifungals for the treatment of disseminated *C. krusei* infections are amphotericin B (AMB) and echinocandins, with voriconazole (VRC) being regarded as an alternative (11, 24). Posaconazole (PSC) is a promising drug, though not yet explored enough in vivo, that shows in vitro MICs against *C. krusei* similar to or lower than those of VRC (12). In this study, we have tested the triazoles VRC and PSC, comparing their efficacies with those of two different formulations of AMB, in an immunocompromised murine model of disseminated infection by *C. krusei*.

Two clinical strains of *C. krusei*, FMR 9728 and FMR 9729, were used. The inocula containing ≥99% of the viable cells for both the in vitro and in vivo studies were adjusted to the desired concentration by counting them with a hemocytometer. The in vitro susceptibilities of both strains were determined using a reference method (7). The minimal fungicidal concentration (MFC) was defined as a 99.9% or greater reduction in the number of CFU/ml (2) (Table 1).

Male OF1 mice were immunosuppressed by a single intraperitoneal (i.p.) injection of 200 mg of cyclophosphamide/kg of body weight, plus a single intravenous (i.v.) injection of 150 mg of 5-fluorouracil/kg on the same day of infection. For the survival studies, the mice received an additional dose of 5-fluorouracil (75 mg/kg) on day 5 after infection, which in previous tests yielded a mortality rate of 100% within 10 days after infection (data not shown). For the survival studies, the mice were challenged with 5×10^7 CFU in 0.2 ml of sterile saline into the lateral tail vein. For the tissue burden studies, the mice were inoculated with 5×10^6 CFU in 0.2 ml of sterile saline, and all the animals survived during the observation period. The procedure standards were approved by the Animal Welfare Committee of the Rovira i Virgili University.

Groups of 10 mice were randomly established for the survival and tissue burden studies. The different groups were treated once daily as follows: AMB deoxycholate (D-AMB) at 1.5 mg/kg of body weight/dose given i.p. (6); liposomal AMB (L-AMB) at 10 mg/kg given i.v. (9); VRC at 10 or 20 mg/kg i.v. (20) and at 40 or 60 mg/kg given orally (p.o.) (22); and PSC at 50 or 100 mg/kg p.o. (19). From 3 days prior to infection, the mice that received VRC were given diluted (50%) grapefruit juice instead of water. The selected doses of VRC have previously been shown to deliver adequate plasma levels in mice when coadministered with grapefruit juice (5, 18, 25). All treatments began 24 h after challenge, and the therapy lasted for 5 days. For the survival studies, the mice were checked daily for 15 days. For the tissue burden studies, the mice were killed 1 day after the completion of the treatment. The spleens and kidneys were aseptically removed, and the entire organs were homogenized in 1 ml of sterile saline. Serial 10-fold dilutions of the homogenates were plated and incubated at 35°C for 72 h. The mean survival time was estimated by the Kaplan-Meier method and compared among groups using the log rank test. Colony counts for the tissue burden studies were analyzed using the Mann-Whitney U test. A P value of <0.05 was considered statistically significant.

For both strains tested, all the treatments significantly prolonged survival relative to the control group ($P < 0.05$) (Fig. 1). No statistically significant differences were observed between the treatments.

For strain 9728, all the drugs except i.v. administered

TABLE 1. In vitro antifungal activity of AMB, VRC, and PSC against the two strains of *C. krusei*[a]

Strain	AMB MIC-0 (μg/ml)	VRC		PSC	
		MIC-2 (μg/ml)	MFC (μg/ml)	MIC-2 (μg/ml)	MFC (μg/ml)
9728	1	0.125	16	0.125	1
9729	1	0.25	>16	0.25	2

[a] MIC-0 corresponds to a 100% inhibition of growth and MIC-2 to a 50% inhibition of growth. MFC corresponds to a 99.9% or greater reduction in the CFU/ml count.

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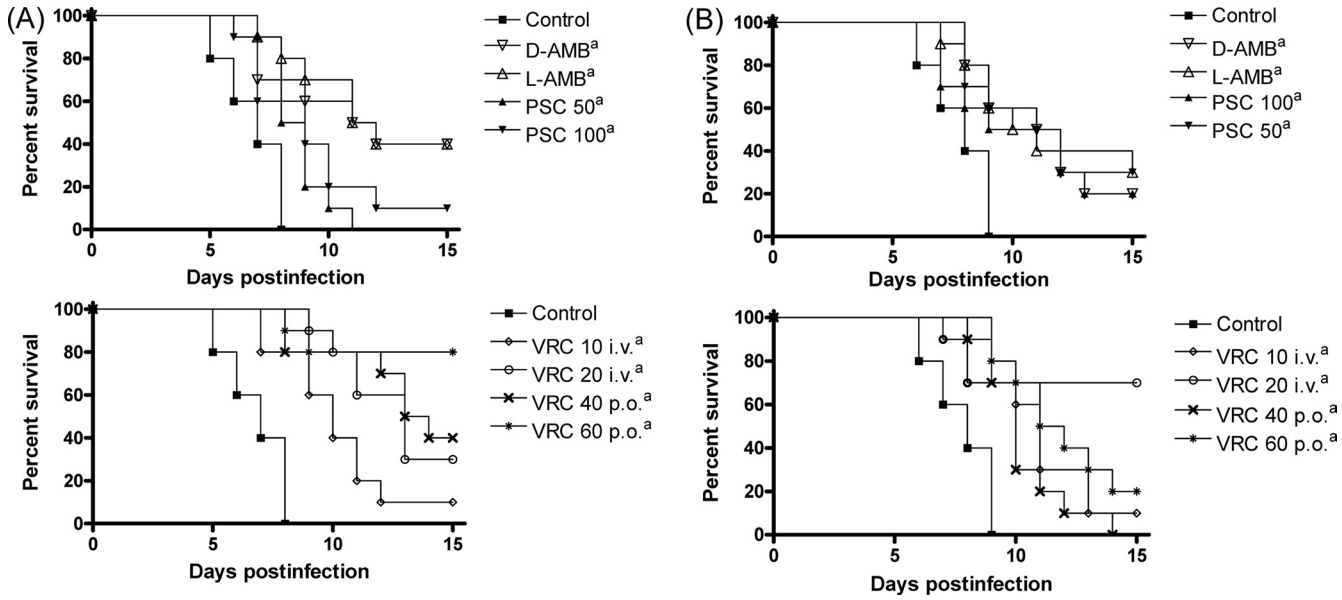

FIG. 1. Cumulative mortality of mice infected with *C. krusei* FMR 9728 (A) and FMR 9729 (B). D-AMB, D-AMB at 1.5 mg/kg/day i.p.; L-AMB, L-AMB at 10 mg/kg/day i.v.; VRC 10 i.v. and VRC 20 i.v., VRC at 10 and 20 mg/kg/day i.v., respectively; VRC 40 p.o. and VRC 60 p.o., VRC at 40 and 60 mg/kg/day p.o., respectively; PSC 50 and PSC 100, PSC at 50 and 100 mg/kg/day p.o., respectively. [a], $P < 0.05$ versus control.

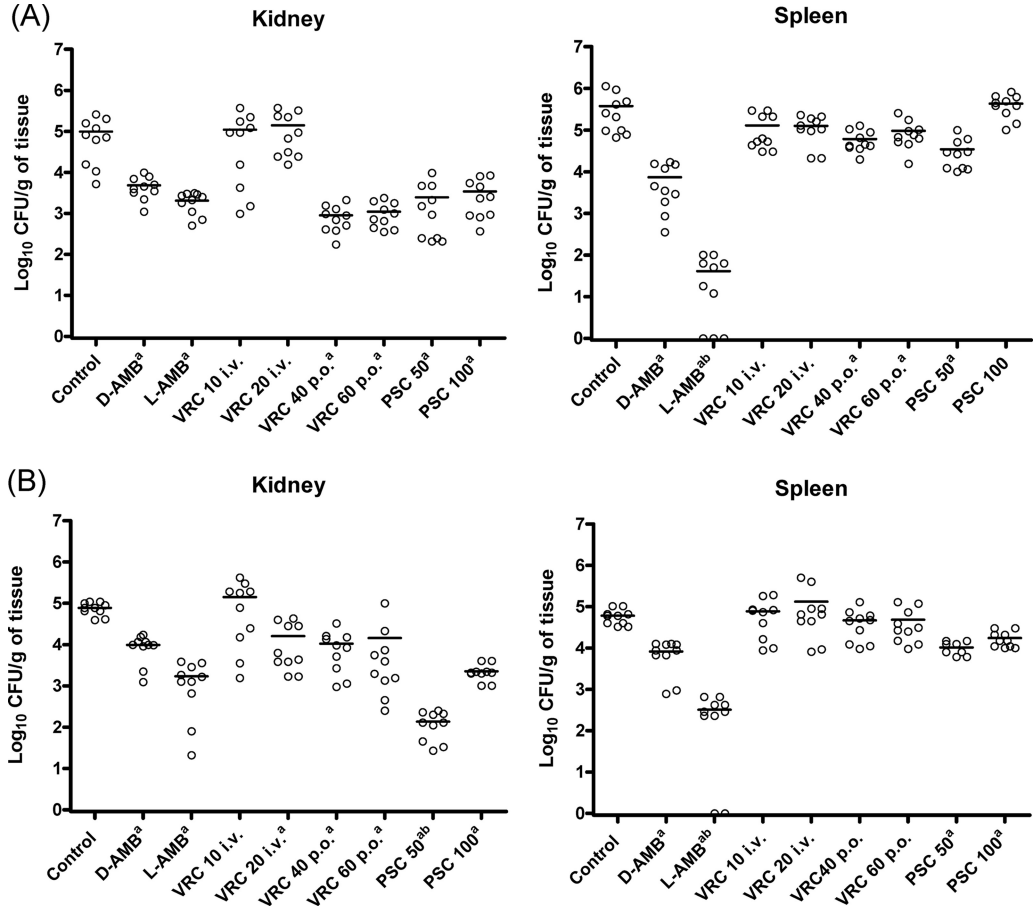

FIG. 2. Effects of the antifungal treatments on the tissue burden of *C. krusei* FMR 9728 (A) and FMR 9729 (B) in the kidneys and spleens of mice. D-AMB, D-AMB at 1.5 mg/kg/day i.p.; L-AMB, L-AMB at 10 mg/kg/day i.v.; VRC 10 i.v. and VRC 20 i.v., VRC at 10 and 20 mg/kg/day i.v., respectively; VRC 40 p.o. and VRC 60 p.o., VRC at 40 and 60 mg/kg/day p.o., respectively; PSC 50 and PSC 100, PSC at 50 and 100 mg/kg/day p.o., respectively. [a], $P < 0.05$ versus control. [b], $P < 0.05$ versus the rest of the therapies. Horizontal lines of scatter plots indicate mean values.

VRC were effective in reducing the fungal burden in the kidneys relative to that of the control group (Fig. 2). In the spleens, the two formulations of AMB, the lower dose of PSC, and both of the p.o. administered doses of VRC were able to reduce the CFU counts relative to that of the control group. For strain 9729, D-AMB, L-AMB, and PSC were effective in reducing the fungal burdens in both organs, while VRC even at high doses was only able to reduce the fungal load in the kidneys. In addition, PSC at 50 mg/kg was able to significantly reduce the fungal burden in the kidneys relative to the other therapies. L-AMB was clearly more effective than the other therapies in reducing the tissue burden in the spleens for both strains.

Despite the relatively high MICs that AMB showed against both strains tested, this drug was effective in vivo. Overall, in our murine model, L-AMB performed slightly better than VRC and PSC, while D-AMB did not outperform the azole treatments.

Although *C. krusei* shows an intrinsic resistance to FLC, no cross-resistance to other azoles has been observed (13, 14). VRC remains active against most strains of *C. krusei* (12, 17), its efficacy being demonstrated in vitro and in clinical trials (3, 10). Several authors have reported a fungistatic effect of VRC (16, 26), while others have stated a fungicidal effect of this drug (1, 21) against *Candida*. Despite the high MFCs observed for both strains, in our study, VRC improved the survival of mice for the two strains tested and reduced the tissue burden greatly for strain 9728 and modestly for strain 9729.

In our murine model, PSC has demonstrated efficacy in the treatment of *C. krusei*-disseminated infection with similar or even improvement of the results obtained with currently recommended treatments such as D-AMB, L-AMB, and VRC. Surprisingly, the lower dose of PSC proved to be slightly more effective than 100 mg/kg in tissue burden clearance. A lack of a dose-effect relationship for this drug has been previously reported with different fungi in mice (4, 19). A decrease in the absorption of PSC at doses higher than 50 mg/kg in mice (8) could easily correlate with a lack of effect increase, although a decrease in drug efficacy is puzzling and merits further investigation. The low MFCs observed and the efficacy of PSC in the survival and fungal burden studies agree with the reported fungicidal activity of this compound against *C. krusei* (23). Despite the lower MFCs observed for PSC with respect to those for VRC, no statistical differences were observed in vivo between the two compounds with the exception of tissue burden reduction in the kidneys for strain 9729.

In conclusion, our results suggest that PSC could be a therapeutic alternative to AMB and VRC for the treatment of disseminated infections by *C. krusei.* Further experimental studies are warranted to confirm our results.

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