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Experimental Therapy with Azoles against *Candida guilliermondii*

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We evaluated the *in vitro* killing activity of voriconazole (VRC) and posaconazole (PSC) against two clinical isolates of *Candida guilliermondii*. The two drugs showed fungistatic activity against both isolates and were effective in reducing kidney fungal burden in a neutropenic murine model of disseminated candidiasis in infected mice. PSC was significantly more effective than VRC against one of the strains. The serum levels of PSC and VRC were above the corresponding MICs for these isolates.

Candida guilliermondii, an emerging pathogen that causes invasive infections mainly in patients who have malignancies and/or intravascular devices, accounts for 1% to 3% of candidemia cases (1) and is the fourth most frequent cause of candidemia in Latin America (2). *Candida guilliermondii* has poor *in vitro* susceptibility to echinocandins, the recommended first-line therapy, with MICs from 2- to 100-fold higher than those for other *Candida* spp. (3, 4), so treatment regimens often fail. Consequently, we need to look for alternative antifungals in the treatment of these infections (5). Numerous studies have demonstrated that posaconazole (PSC) and voriconazole (VRC) are efficacious in experimental infections due to some non-*albicans Candida* species (6–8), but no *in vivo* data exist regarding their effectiveness against *C. guilliermondii*.

We have evaluated the *in vitro* killing activity of PSC and VRC against *C. guilliermondii* and their *in vivo* efficacies in a neutropenic murine model of disseminated candidiasis.

Two clinical strains of *C. guilliermondii* identified by ribosomal DNA (rDNA) sequencing were included in the study. The MICs of PSC and VRC were determined following CLSI guidelines (9) with some modifications (i.e., inocula were adjusted by hemocytometer counts, and viability was assessed by serial plating onto potato dextrose agar [PDA]). The MIC values of PSC and VRC were 0.12 μg/ml and 0.12 μg/ml for strain UTHSC (University of Texas Health Science Center) 11-142 and 0.03 μg/ml and 0.06 μg/ml for strain UTHSC 11-685, respectively.

Time-kill curves of PSC and VRC were generated in duplicate as previously reported (10), and the concentrations for each drug assayed were 0.03, 0.12, 0.5, 1, 2, 8, and 32 μg/ml.

For the *in vivo* studies, 4-week-old male OF1 mice were immunosuppressed by a single intraperitoneal injection of 200 mg/kg of body weight of cyclophosphamide plus a single intravenous injection of 150 mg/kg of 5-fluorouracil (11) 1 day prior to the infection. The mice were infected intravenously via the lateral tail vein with 1×10^8 CFU in 0.2 ml of sterile saline (12, 13). All animal care procedures were supervised and approved by the Universitat Rovira i Virgili Animal Welfare and Ethics Committee.

Six groups (three for each strain) of eight infected animals were established. They received no treatment or 12.5 mg/kg PSC twice daily (BID) or 25 mg/kg VRC once a day (QD), both administered orally by gavage beginning 24 hours after challenge and lasting for 10 days. The doses selected were based on pharmacokinetic studies (14, 15). All the animals received 5 mg/kg ceftazidime subcutaneously once daily until the end of the experiment, and from 3 days before infection, the mice treated with VRC were given diluted (50%) grapefruit juice instead of water (16). Efficacy was evaluated by a reduction of fungal burden in the kidneys on day 11 postinfection. Kidneys from euthanized mice were weighed, homogenized in 1 ml of sterile saline, diluted 10-fold, and placed on PDA plates for determining the number of CFU/g after incubation for 24 hours at 35°C.

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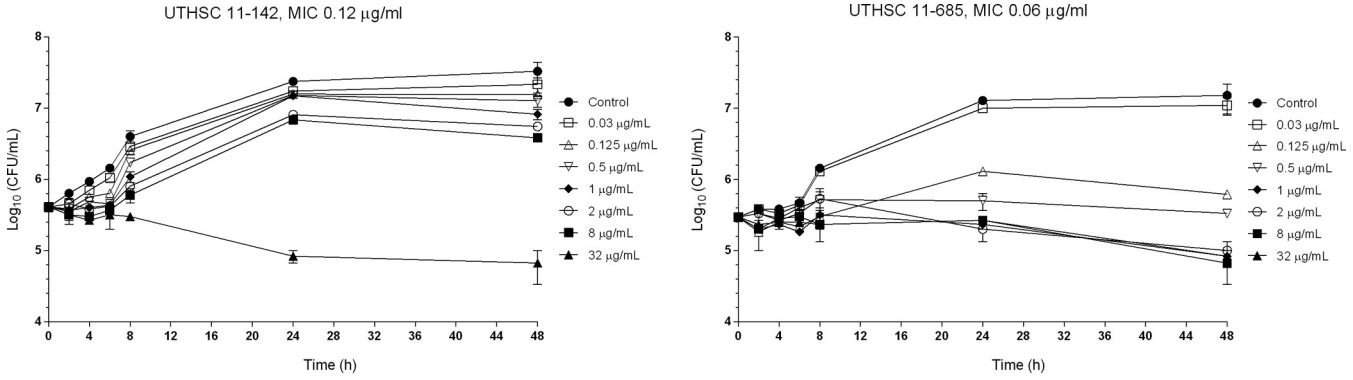

FIG 1 Time-kill kinetic assays of PSC at various concentrations against two strains of *C. guilliermondii*.

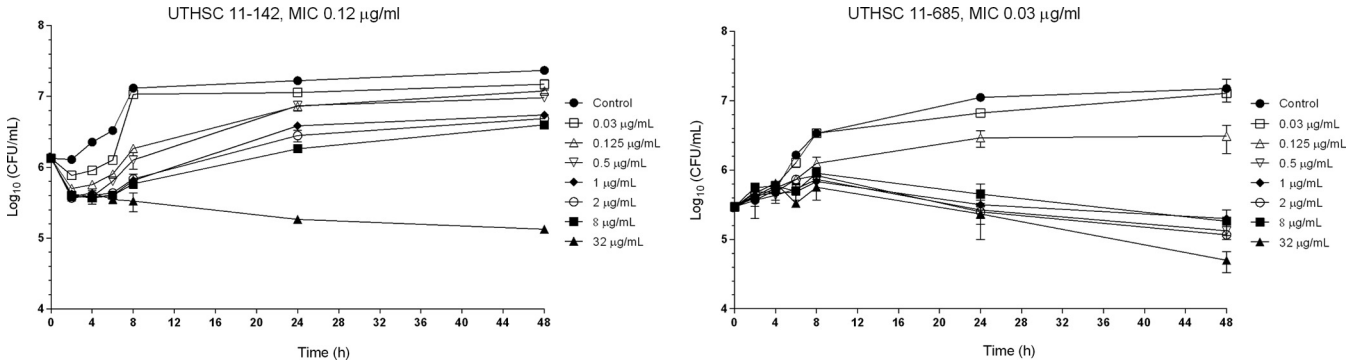

FIG 2 Time-kill kinetic assays of VRC at various concentrations against two strains of *C. guilliermondii*.

Additionally, two groups of 5 mice infected with strain UTHSC 11-685 and treated as described above were anesthetized by inhalatory isoflurane 24 hours after the last dosing, and approximately 1 ml of blood from each mouse was extracted by cardiac puncture. Serum was obtained from centrifuged blood samples and used to determine the drug concentration of each sample by bioassay (17).

Colony counts from the tissue burden studies were analyzed using the Mann-Whitney *U* test, using GraphPad Prism 4.0 for Windows (GraphPad Software, San Diego, CA, USA). Differences were considered statistically significant at a *P* value of <0.05.

Regarding the starting inoculum, PSC and VRC showed fungistatic activities against the two strains, with 0.845- to 0.64-$\log_{10}$ and 0.795- to 1-$\log_{10}$ decreases in CFU/ml, respectively (Fig. 1 and 2).

PSC and VRC significantly reduced the fungal load in the kidneys of mice infected with each of the two isolates in comparison to that of the control group ($P = 0.0002$) (Fig. 3). No statistically significant differences were found between the two drugs for strain UTHSC 11-142 ($P = 0.0771$); however, for the UTHSC 11-685 isolate, PSC worked better than did VRC ($P = 0.0002$). The mean (± the standard deviation) serum concentrations of PSC and VRC on day 11, 24 hours after the last dosing, were 5.4 ± 2.06 μg/ml and 5.65 ± 0.95 μg/ml, respectively (i.e., above the corresponding MIC values for the two strains tested).

In a previous experimental murine infection by *C. guilliermondii*, liposomal amphotericin B was the only effective treatment in reducing the fungal load in kidneys, in contrast to the low efficacies of fluconazole (FLC) and anidulafungin (AFG), although both of the tested strains were susceptible to these drugs (13). In this study, using the same fungal isolates, we have demonstrated the good *in vivo* efficacies of VRC and PSC. The MICs of PSC and VRC were within the ranges considered indicative of susceptibility and below the epidemiological cutoff values for *C. guilliermondii* (i.e., 0.5 and 0.12 μg/ml, respectively) (18). To our knowledge, there have been no reports of the efficacies of VRC and PSC in the treatment of invasive infections by *C. guilliermondii* in the clinical setting, but in the few clinical cases that reported infection by other non-*albicans Candida* species, both drugs demonstrated efficacy (19). In our study, the *in vitro* data were good predictors of the *in vivo* outcomes against strains, showing MIC values of PSC and VRC equal to those of the modal MICs reported in a recent multicenter study (18). To our knowledge, this is the first study to have established a relationship between the *in vitro* activities and *in vivo* efficacies of PSC and VRC against clinical isolates of *C. guilliermondii*.

In summary, our study demonstrated that PSC and VRC were experimentally effective in the treatment of an invasive infection by *C. guilliermondii* caused by isolates refractory to FLC and AFG in a neutropenic murine model in infected mice (13).

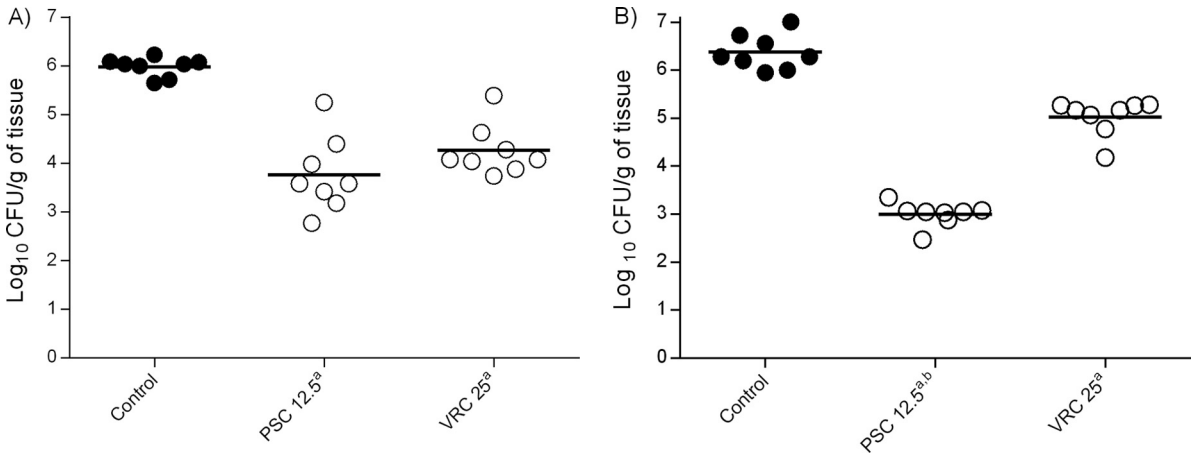

FIG 3 Effects of antifungal treatment on fungal loads in the kidneys of neutropenic mice infected with 1×10^8 CFU/animal of two clinical strains of *C. guilliermondii*, UTHSC 11-142 (A) and UTHSC 11-685 (B), 11 days postinfection. PSC and VRC were administered orally at 12.5 mg/kg BID and 25 mg/kg QD, respectively. [a], $P < 0.05$ versus control; [b], $P < 0.05$ versus VRC at 25 mg/kg QD.

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