In Vitro Pharmacodynamic Characteristics of Amphotericin B, Caspofungin, Fluconazole, and Voriconazole against Bloodstream Isolates of Infrequent Candida Species from Patients with Hematologic Malignancies

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Time-kill and postantifungal effect (PAFE) of amphotericin B, caspofungin, fluconazole, and voriconazole were determined against clinical isolates of Candida guilliermondii, Candida kefyr, and Candida lusitaniae. Azoles displayed fungistatic activity and no measurable PAFE, regardless of the concentration tested. Amphotericin B and caspofungin demonstrated concentration-dependent fungicidal activity, although amphotericin B only produced a significant dose-dependent PAFE against all isolates tested.

Invasive fungal infections are important causes of morbidity and mortality in immunosuppressed patients (10). Although C. albicans, C. glabrata, C. parapsilosis, and C. tropicalis account for the majority of Candida bloodstream infections, recent epidemiologic trends indicate a shift toward infections by the less frequently isolated non-albicans Candida (NAC) species (12). Among NAC species, C. kefyr, C. guilliermondii, and C. lusitaniae are rare causes of invasive infections but are increasingly encountered among severely immunosuppressed patients occurring in nosocomial clusters and/or exhibiting innate or acquired resistance to one or more established antifungal agents, often related to intravascular catheters and breaks in infection control precautions (3–5, 11, 12, 14).

Currently, knowledge of the in vitro pharmacodynamic characteristics of C. kefyr, C. guilliermondii, and C. lusitaniae is poor and limited to amphotericin B (AMB) and voriconazole (VRC) only (7, 15). Therefore, we conducted time-kill and postantifungal effect (PAFE) studies with AMB, caspofungin, fluconazole (FLC), and VRC against bloodstream isolates of C. guilliermondii, C. kefyr, and C. lusitaniae from neutropenic patients.

Antifungal agents. Stock solutions of AMB (Sigma-Aldrich SRL, Milan, Italy), caspofungin (Merck Sharp & Dohme Italia SpA, Rome, Italy), FLC (Pfizer Inc., New York, N.Y.), and VRC (Pfizer) were prepared in RPMI 1640 medium (Sigma) buffered to a pH of 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS) buffer (Sigma) and stored at −80°C until use. Antifungals were solubilized in sterile water, except AMB in dimethyl sulfoxide (Sigma).

Test isolates. Six Candida isolates were obtained from the Clinical Microbiology Service, Department of Hematology and Oncology, “Spirito Santo” Hospital, Pescara, Italy, for use in this study: two strains each of C. guilliermondii (337 and 555), C. kefyr (240 and 270), and C. lusitaniae (325 and 447) were selected for testing.

Antifungal susceptibility testing. The MIC for each isolate was determined, in triplicate, by broth microdilution techniques as outlined by the National Committee for Clinical Laboratory Standards (17). The endpoint was defined as 50% inhibition of visible growth for azoles and complete inhibition of visible growth for AMB and caspofungin.

Time-kill. Before the time-kill studies were initiated, antifungal carryover effects were examined. Briefly, 100 µl of a standardized suspension (1 × 10³ CFU/ml) of each isolate were added to either 900 µl of sterile water with (sample) or without (control) antifungal at 4 and 8 times the MIC. Immediately following the addition of the antifungal, 100

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and colony counts were performed at 0, 2, 4, 6, 8, and 24 h after the final wash. PAFE experiments were conducted in duplicate.

Analysis. Fungicidal activity was defined as a $\geq 3 \log_{10}$ (99.9%) reduction in CFU per milliliter from the starting inoculum concentration. To quantify the extent of antifungal activity, maximal effect ($E_{\text{max}}$), the concentration producing 50% of $E_{\text{max}}$ ($E_{50}$), the EC$_{50}$, and the range of the net change ($\log_{10}$ CFU per milliliter) in fungal density were determined for each time point by a sigmoidal Hill three-parameter model with GraphPad Prism (version 4; GraphPad Software Inc., San Diego, Calif.). PAFE was calculated by taking the difference in time required for control and test isolates to grow 1 $\log_{10}$ following drug removal.

Antifungal susceptibility results. Median MICs ranged from 0.5 to 4.0 $\mu$g/ml for AMB, 0.25 to 128 $\mu$g/ml for caspofungin, 0.12 to 2.0 $\mu$g/ml for FLC, and 0.12 to 1.0 $\mu$g/ml for VRC (Table 1).

Time-kill results. No antifungal carryover has been observed with any of the isolates at the concentrations tested. Time-kill plots representative of those noted in this study are showed in Fig. 1. AMB yielded fungicidal activity at concentrations $\geq 4$ times the MIC against C. guilliermondii isolates, reaching fungicidal activity between 1.1 and 3.1 h. AMB exhibited fungici-

### Table 1. Microdilution broth results for study isolates

<table>
<thead>
<tr>
<th>Test isolate</th>
<th>Median MIC ($\mu$g/ml) ($n = 6$)</th>
<th>AMB</th>
<th>Caspofungin</th>
<th>FLC</th>
<th>VRC</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. guilliermondii 337</td>
<td>2</td>
<td>128</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>C. guilliermondii 555</td>
<td>0.5</td>
<td>128</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>C. kefyr 240</td>
<td>4</td>
<td>0.25</td>
<td>0.12</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>C. kefyr 270</td>
<td>4</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>C. lusitaniae 325</td>
<td>4</td>
<td>0.5</td>
<td>2</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>C. lusitaniae 447</td>
<td>4</td>
<td>0.5</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

FIG. 1. Representative time-kill plots for the following: AMB against C. guilliermondii 337 (A) and C. lusitaniae 447 (B); caspofungin against C. kefyr 270 (C); and VRC against C. lusitaniae 325 (D). Antifungals were tested at the following concentrations: 0.125 times the MIC (△), 0.25 times the MIC (▽), the MIC (●), 4 times the MIC (○), and 8 times the MIC (□). □, control.
against both range, 0.6 to 6.2 h). Caspofungin exhibited fungicidal activity lates beginning at 0.25 times the MIC (fungicidal endpoint AMB showed fungicidal activity against both C. lusitaniae trend was observed for C. kefyr lates, regardless of the concentrations tested. Composite FLC and VRC showed fungistatic activity against all test iso-

was observed for strain 240 after 8 h and 4 times the MIC. The EC90 for both AMB and caspofungin decreased to about the E...
with findings of Ernst et al. (6) concerning C. albicans and Cryptococcus neoformans.

In conclusion, our results demonstrate that AMB may be useful for the treatment of infections caused by C. kefyr, C. guilliermondii, and C. lusitaniae. Animal and clinical studies are warranted to define the clinical relevance of our data.

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REFERENCES


