Micafungin at Physiological Serum Concentrations Shows Antifungal Activity against Candida albicans and Candida parapsilosis Biofilms

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We assessed the in vitro activity of micafungin against preformed Candida biofilms by measuring the concentration of drug causing the most fungal damage and inhibition of regrowth. We studied 37 biofilm-producing Candida spp. strains from blood cultures. We showed that micafungin was active against planktonic and sessile forms of Candida albicans strains and moderately active against Candida parapsilosis sessile cells. Concentrations of micafungin above 2 μg/ml were sufficiently high to inactivate regrowth of Candida sessile cells.

Candida spp. cause approximately 8 to 15% of bloodstream infections and are the third most common cause of these infections after coagulase-negative staphylococci and Staphylococcus aureus (1–8).

A high number of episodes of candidemia originate in central venous catheters (9, 10), leading to high rates of morbidity and mortality (11). The ability of some Candida strains to produce biofilms, especially C. albicans and C. parapsilosis, may explain the high frequency of catheter-related candidemia (CRC) (12–22).

Recent in vitro and in vivo models designed to assess the antifungal susceptibility of Candida biofilms (19, 21, 23–29) showed that the echinocandins, particularly micafungin, had the highest antifungal activities (30–34). However, the studies are limited by the inclusion of a low number of clinical isolates; furthermore, the concentration of antifungal necessary to inactivate sessile cells is unknown. Hence, it seems reasonable to determine the micafungin concentration able to inactivate the Candida sessile cells.

Our objective was to assess the in vitro concentration of micafungin causing the most fungal damage against mature biofilms involving Candida strains isolated from candidemic patients.

We collected 37 biofilm-producing Candida strains (29 C. albicans strains and 8 C. parapsilosis strains) from 37 patients with candidemia admitted to Hospital Gregorio Marañón, a tertiary hospital in Madrid, Spain. We studied the activity of micafungin against planktonic and sessile forms of the isolates.

The crystal violet binding assay was used to assess biofilm production. A cutoff optical density (OD) of ≥0.5 was used to define biofilm-forming isolates (35).

We defined catheter-related candidemia as an episode in which the same Candida species was isolated both in peripheral blood and in a catheter segment sample (36).

The MICs of planktonic isolates were determined using the EUCAST broth microdilution procedure (37). Isolates were grown on Sabouraud dextrose agar plates for 24 h at 37°C. Serial 2-fold micafungin dilutions ranging from 16 to 0.015 μg/ml were studied. The lowest concentration producing a ≥50% reduction in turbidity compared with that of the antifungal-free control well was reported as the MIC. Sessile MICs were studied on biofilms formed in 96-well, flat-bottomed microtiter plates as described by other authors (38, 39). The sessile MIC was assessed by measuring the absorbance of each well at 492 nm with XTT (2,3-bis[2-methoxy-4-nitro-5-[[sulfenylamino] carbonyl]-2H-tetrazolium-hydroxide]) to determine the lowest concentration associated with a 50% reduction in absorbance compared with the level for the control well [% fungal damage = (1 − X/C) × 100, where X is the absorbance of the experimental wells and C is the absorbance of control wells].

Candida biofilms were regrown using the same methodology as that used for the study of the sessile MICs, including 24 h of incubation (37°C) of the trays filled with 100 μl of yeast extract-peptone-dextrose (YPD) per well. We measured well absorbance at 492 nm to calculate the percentage of growth inhibition [(1 − X/C) × 100] to assess the inhibition of sessile cell regrowth. We considered Candida biofilm regrowth as any absorbance compared to the result for the negative-control well. A calibration curve was constructed to study the number of cells that regrew in each well after exposure to micafungin.

Each experiment was tested in triplicate, and the average value was used for the analysis.

<table>
<thead>
<tr>
<th>Species (no. of isolates)</th>
<th>No. of isolates with MIC (μg/ml) of:</th>
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<tr>
<td></td>
<td>and form</td>
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<tr>
<td>C. albicans (29)</td>
<td></td>
</tr>
<tr>
<td>Planktonic</td>
<td>29</td>
</tr>
<tr>
<td>Sessile</td>
<td>14</td>
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<tr>
<td></td>
<td>0.015 0.031 0.062 0.125 0.25 0.5 1 2 4 8 16 &gt;16</td>
</tr>
<tr>
<td>C. parapsilosis (8)</td>
<td></td>
</tr>
<tr>
<td>Planktonic</td>
<td>1</td>
</tr>
<tr>
<td>Sessile</td>
<td></td>
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<td>6 1</td>
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<td>2 1 3 2</td>
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TABLE 1 Distribution of micafungin MICs for the 37 planktonic and sessile Candida isolates

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Of the 37 Candida strains, 19 (51.4%) were from patients with CRC. The micafungin MICs for planktonic cells of C. albicans were always ≤0.015 μg/ml, whereas the MICs for C. parapsilosis ranged from ≤0.015 to 2 μg/ml. In contrast, the micafungin MICs for sessile cells of both C. albicans and C. parapsilosis ranged from ≤0.015 to >16 (Table 1).

Fungal damage worsened with increasing micafungin concentrations (Fig. 1a). Both C. albicans and C. parapsilosis biofilms showed a reduction in metabolic activity of ≥50% at micafungin concentrations of ≥2 μg/ml.

Inhibition of regrowth for all Candida strains was >80% after 24 h of incubation with micafungin at concentrations of ≥2 μg/ml. Inhibition of regrowth was >50% for C. albicans and C. parapsilosis at micafungin concentrations of 0.25 μg/ml and 1 μg/ml, respectively (Fig. 1b and 2a and b). The number of cells that regrew after exposure to micafungin was studied (Fig. 2a and b). Both species showed a significant decrease in the number of cells/ml after 24 h of incubation with micafungin at concentrations of ≥2 μg/ml. The number of cells/ml increased slightly when C. albicans and C. parapsilosis were incubated with micafungin at 16 μg/ml.

Our study showed that micafungin was active against both planktonic and sessile strains of C. albicans and moderately active against sessile strains of C. parapsilosis. Regrowth of strains from both species decreased by >80% when they were exposed to concentrations of micafungin up to 2 μg/ml.

As most candidemia episodes are catheter related (9, 10), our results could be applied in patients with CRC. Guidelines recommend removing the catheter in patients with suspicion of CRC, although this issue remains controversial. Nucci et al. demonstrated that nonneutropenic patients treated with echinocandins or liposomal amphotericin B did not benefit from early removal of the catheter (40). Therefore, not removing the catheter in patients with CRC may be an alternative approach (accompanied by antifungal lock therapy in combination with systemic antifungals) when the risk of replacing the catheter is greater than the benefit (41). Our data show that physiological concentrations of micafungin of 4 μg/ml (42, 43) are active against biofilm, since metabolic
activity decreased and fungal regrowth was inhibited in most of the preformed biofilms. Our in vitro observations also support the previous clinical observations reported by Nucci et al. (40).

In summary, we provide new data on the in vitro antibiofilm activity of micafungin against Candida strains from patients with candidemia. Although micafungin MICs were higher for sessile cells than for planktonic cells, regrowth of the cells forming the biofilm was reduced by more than 80% at physiological serum concentrations of 4 μg/ml. Our in vitro study supports the idea that micafungin concentrations of ≥2 μg/ml may be sufficiently high to eradicate Candida biofilms.

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