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# In Vitro Activity of Micafungin against Planktonic and Sessile *Candida albicans* Isolates▽

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**Planktonic and sessile susceptibilities to micafungin were determined for 30 clinical isolates of *Candida albicans* obtained from blood or other sterile sites. Planktonic and sessile $MIC_{90}$s for micafungin were 0.125 and 1.0 μg/ml, respectively.**

*Candida albicans* device-related infections are associated with growth of organisms in a biofilm state (3, 6). Device removal is often considered necessary for cure (10), since antimicrobial agents have been considered to have poor activity against microbial biofilms. If, however, antimicrobial agents were active against microbial biofilms, device removal might be avoidable.

Cell walls are integral to *C. albicans* biofilms; therefore, antifungal agents that target cell wall synthesis may be active against fungal biofilms (1). We previously showed that caspofungin and anidulafungin had $MIC_{90}$s of 2 and ≤0.03 μg/ml, respectively, against 30 *C. albicans* isolates in biofilms (7, 12). We also demonstrated that caspofungin was active in vivo in an experimental intravascular catheter infection model (13).

Herein, we evaluated the activity of micafungin against planktonic and sessile forms of the 30 clinical isolates of *C. albicans* against which we had previously studied caspofungin, anidulafungin, amphotericin B deoxycholate, and voriconazole (7, 12). One isolate per patient was included; isolates were included only if ≤3 types of organisms were cultured from the specimen from which *C. albicans* was isolated. Isolates were from blood cultures ($n$ = 10), peritoneal fluid ($n$ = 6), abscess fluid ($n$ = 5), soft tissue ($n$ = 5), bone ($n$ = 2), pleural fluid ($n$ = 1), and urine ($n$ = 1). *C. albicans* GDH 2346 was used as a positive control.

Planktonic MICs were determined using broth microdilution (5). Isolates were grown on Sabouraud dextrose agar for 24 h at 37°C. *C. albicans* was titrated to 76.6% transmittance at 530 nm in sterile saline and then diluted 1/1,000 in RPMI. Serial twofold micafungin dilutions ranging from 16 to 0.03 μg/ml were assayed. Drug dilution and titrated organism (100 μl each) were placed into corresponding wells of a 96-well, round-bottomed microtiter plate and incubated at 37°C. Forty-eight hours later, MICs were read using a reading mirror and scored according to CLSI guidelines. The lowest concentration associated with a ≥50% reduction in turbidity compared with that for the positive-control well was reported as the MIC. Planktonic MICs for micafungin ranged from ≤0.03 to 0.25 μg/ml (Table 1). The $MIC_{50}$ and $MIC_{90}$ were 0.125 μg/ml. The GDH 2346 MIC was 0.06 μg/ml.

Sessile MICs (SMICs) were determined with biofilms formed in 96-well, flat-bottomed microtiter plates, as previously described (12). Organisms were inoculated into 7 ml of yeast nitrogen base medium. After 24 h, they were centrifuged and rinsed twice with phosphate-buffered saline (PBS). After being standardized to $1 \times 10^7$ CFU/ml in RPMI, 100 μl of each suspension was placed in the wells of a 96-well, flat-bottomed microtiter plate and incubated at 37°C. Approximately 24 h later, the suspensions were discarded, and the wells were rinsed three times with sterile PBS and filled with 100 μl of micafungin in RPMI. Serial twofold micafungin dilutions ranging from 16 to 0.03 μg/ml were studied. Negative-control wells received 100 μl RPMI alone. Microtiter plates were incubated at 37°C for an additional 48 h. Then, media were discarded and wells rinsed three times with sterile PBS. A mixture (100 μl) of 1:10 menadione (1 mM solution in acetone; Sigma, St. Louis, MO) and 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide inner salt (1 mg/ml in phosphate-buffer saline; sigma) was then placed into each well. Plates were incubated at 37°C for 2 h. A microtiter plate reader was used to measure each well's absorbance at 492 nm. The lowest concentration associated with a 50% reduction in absorption compared with the level for the control well was reported as the SMIC. SMICs for micafungin were ≤0.03 to 1.0 μg/ml for the 30 clinical isolates (Table 1). The $SMIC_{50}$ and $SMIC_{90}$ were 0.5 to 1.0 μg/ml, respectively. The GDH 2346 SMIC was 0.5 μg/ml.

We showed that micafungin is active against *C. albicans* biofilms; its activity cannot necessarily be predicted based on the activity of other echinocandins (Table 1). The seven isolates with caspofungin SMIC values of ≥2 μg/ml had anidulafungin SMIC values of <0.03 μg/ml and micafungin SMIC values of <0.5 μg/ml (7). Overall, anidulafungin was the most potent agent against *C. albicans* biofilms; the anidulafungin SMIC was previously determined to be ≤0.03 μg/ml for 28/30 isolates (7). However, the remaining two isolates had anidulafungin SMIC values of >16 μg/ml, one having the highest planktonic anidulafungin MIC (2 μg/ml) observed (7). The two isolates with anidulafungin SMICs of >16 μg/ml had caspo-

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TABLE 1. Comparison of planktonic and sessile susceptibilities of 30 *C. albicans* isolates[a]

| Antimicrobial susceptibility | No. of isolates with MIC (μg/ml) of: | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ≤0.03 | 0.06 | 0.13 | 0.25 | ≤0.5 | 0.5 | 1 | 2 | 4 | 8 | 16 | >16 | 32 | 64 | >256 |
| Planktonic | | | | | | | | | | | | | | | |
| Anidulafungin ($n$ = 30) | 17 | 8 | 3 | | | 1 | | 1 | | | | | | | |
| Caspofungin ($n$ = 30) | | | 1 | 16 | | 13 | | | | | | | | | |
| Micafungin ($n$ = 30) | 2 | 9 | 16 | 3 | | | | | | | | | | | |
| Voriconazole ($n$ = 30) | 26 | 4 | | | | | | | | | | | | | |
| Amphotericin B ($n$ = 30) | | 1 | 17 | 12 | | | | | | | | | | | |
| Sessile | | | | | | | | | | | | | | | |
| Anidulafungin ($n$ = 30) | 28 | | | | | | | | | | | 2 | | | |
| Caspofungin ($n$ = 29) | 5 | 4 | 2 | 6 | | 5 | | 4 | 1 | 2 | | | | | |
| Micafungin ($n$ = 30) | 1 | 2 | 4 | 6 | | 13 | 4 | | | | | | | | |
| Voriconazole ($n$ = 28) | | | | | 11 | | 3 | | 3 | 1 | 1 | | 1 | 2 | 6 |
| Amphotericin B ($n$ = 29) | | | | | 14 | | 7 | 7 | | 1 | | | | | |

[a] Susceptibilities of anidulafungin are from reference 7; susceptibilities of caspofungin, voriconazole, and amphotericin B are from reference 12; and susceptibilities of micafungin were determined in this study.

fungin SMICs of ≤0.25 μg/ml and micafungin SMICs of 0.25 μg/ml (7, 12). Together, these data suggest that there may be a need to determine individual echinocandin SMIC values if results are to be translated to the clinical setting.

Choi et al. reported micafungin SMIC values of ≤0.5 μg/ml for 12 *C. albicans* isolates (4). Cateau et al. recently published a study comparing echinocandin treatments of two strains of *C. albicans* in biofilms on sections of silicone catheters in microtiter plates (2). Exposure to 2 μg/ml of caspofungin or 5 μg/ml of micafungin for 12 h significantly reduced the metabolic activity of 12-h- and 5-day-old *C. albicans* biofilms, an effect that was maintained, even 48 h later (2). Finally, Kuhn et al. studied the activity of micafungin against two isolates of *C. albicans* (including GDH 2346, studied herein) (8). Planktonic MICs were 0.001 μg/ml for both isolates; the SMIC for GDH 2346 was identical to ours, and the SMIC of the second isolate was 0.25 μg/ml (8).

Our planktonic MIC findings for micafungin are in accordance with previously published results. A recently published study of 2,869 *C. albicans* isolates showed that the $MIC_{90}$s were 0.06, 0.06, and 0.03 μg/ml for anidulafungin, caspofungin, and micafungin, respectively (11). In the same study, the highest MICs for anidulafungin, caspofungin, and micafungin were 2, 0.5, and 1 μg/ml, respectively (11). There were 12 isolates with anidulafungin MICs of 2 μg/ml, which, although considered susceptible based on CLSI breakpoints, is high, given that the modal MIC for this species is 0.3 μg/ml; the 12 isolates had micafungin MICs of 0.5 to 1 μg/ml (the modal MIC of micafungin was 0.015 μg/ml) and caspofungin MICs of 0.12 to 0.25 μg/ml (the modal MIC of caspofungin was 0.03 μg/ml) (11). Isolates with such high echinocandin MICs have been associated with echinocandin treatment failure (9). The highest micafungin MIC noted in our study, however, was only 0.25 μg/ml ($n$ = 3); these three isolates had anidulafungin MICs of ≤0.03 ($n$ = 2) or 0.06 μg/ml and caspofungin MICs of 0.25 ($n$ = 2) or 0.5 μg/ml.

Our in vitro studies show that micafungin is active against *C. albicans* in biofilms.

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