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In Vitro Interaction of Posaconazole and Caspofungin against Clinical Isolates of *Candida glabrata*

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Combinations of caspofungin and posaconazole were evaluated by fractional inhibitory concentration index against 119 *Candida glabrata* isolates. Synergy was seen in 18% of all isolates and in 4% of fluconazole-resistant isolates at 48 h without evidence of antagonism. This antifungal combination may have utility against this organism.

Candida glabrata has recently emerged as a significant systemic pathogen, and oral and vaginal infections are being reported. This epidemiological change may be due to the extensive use of fluconazole in severely immunocompromised patients (4, 5). However, elderly patients, without prior exposure to fluconazole, have shown increased rates of infections with this organism as well (3).

Combination therapy is a promising approach in the treatment of strains of *Candida* resistant to conventional antifungal agents (12). Posaconazole, a triazole with broad spectrum antifungal activity, inhibits ergosterol synthesis, affecting the integrity of the fungal cell membrane (11). Caspofungin acetate, an echinocandin, inhibits fungal cell wall synthesis (9). With different mechanisms of action, these two drugs could be effective in combination. Thus, we compared the antifungal susceptibility patterns of *C. glabrata* against combinations of posaconazole and caspofungin to evaluate whether this drug combination may be a suitable alternative in treating candidiasis in those patients whose infections are due to *C. glabrata*.

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One-hundred nineteen isolates were obtained from the oral cavity of 22 patients with oropharyngeal candidiasis or oral colonization with *C. glabrata*. Twenty-six of these were resistant to fluconazole (MIC $\geq$ 64 μg/ml). All strains evaluated were clinical isolates from patients with either human immunodeficiency virus infection or receiving radiation therapy for head and neck cancer. The isolates were submitted to the Infectious Disease Mycology Laboratory, University of Texas Health Science Center at San Antonio. The isolates were presumptively identified as *C. glabrata* by plating them on CHROMagar *Candida* (CHROMagar Company, Paris, France) and confirmed by utilizing API-20C carbohydrate assimilation testing (BioMeriux, Marcy-L'Etoile, France). CHROMagar *Candida* is not recommended for *C. glabrata* identification, but it was utilized as a screening tool and to identify possible colonies of *C. albicans*, *C. tropicalis*, or *C. krusei* in the sample (10). Isolates were considered unique if they were from different patients, if they were from different patient visits, or if they showed unique DNA karyotypes in the same patient visit. Isolates were stored in sterile deionized water at room temperature until they were used in the study. Isolates were then submitted to the Fungus Testing Laboratory for susceptibility testing. Posaconazole (Schering-Plough) and caspofungin (Merck, Rahway, N.J.) were obtained in reagent-grade powder form from their respective manufacturers. Stock solutions were prepared in water (caspofungin) and polyethylene glycol 400 (posaconazole). Serial twofold dilutions of each antifungal agent were prepared as outlined in NCCLS document M27-A2 (6). Final dilutions were made in RPMI 1640. The final concentrations of the antifungal agents ranged from 0.015 to 4 μg/ml for posaconazole and 0.03 to 4 μg/ml for caspofungin. Trays were incubated at 35°C. MICs were read at 24 and 48 h using a plate reading mirror without mixing. The MIC_{50} was determined to be the lowest concentration where a 50% or greater reduction in turbidity was noted (6).

Drug interactions were assessed by a checkerboard microdilution method that also included the determination of the MIC of each drug alone. Parameters recommended in NCCLS document M27-A2 (6) were utilized with appropriate modifications to permit checkerboard testing. The mean MICs and MIC ranges were analyzed to evaluate the in vitro activities of both drugs, alone and in combination among fluconazole-resistant isolates, isolates that showed dose-dependent resistance, and fluconazole-susceptible isolates. MIC_{50} and MIC_{90} were also determined for both drugs. Drug interactions were classified as synergistic, indifferent, or antagonistic on the basis of the fractional inhibitory concentration (FIC) index (FICI). The FICI is defined as the sum of the MIC of each drug when used in combination divided by the drug alone, i.e., FICI = (MIC drug A in combination/MIC drug A alone) + (MIC drug B in combination/MIC drug B alone). The drug interactions were defined as synergistic if the FICI was $\leq$0.5, indifferent if the FICI was $>$0.5 and $\leq$4.0, and antagonistic if the FICI was $>$4.0 (2).

The median MIC for posaconazole alone was 0.5 μg/ml at

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TABLE 1. Antifungal susceptibility of fluconazole, posaconazole, caspofungin, and a posaconazole-caspofungin combination against clinical isolates of *C. glabrata* at 48 h[a]

Isolate type	Fluconazole susceptibilty, *n* (%)	MIC_{50}/MIC_{90} (μg/ml) for:		FICIcat, *n* (%)		
		CSP	POS	Antagonism	Indifference	Synergy
R	26 (22)	1/2	2/4	0	25 (96)	1 (4)
S-DD	33 (28)	1/2	0.5/2	0	29 (88)	4 (12)
S	60 (50)	1/2	0.5/2	0	44 (73)	16 (27)
Total	119 (100)			0 (0)	98 (82)	21 (18)

[a] CSP, caspofungin; POS, posaconazole; FICIcat, fractional inhibitory concentration index interpretive category; R, isolates resistant to fluconazole (MIC ≥ 64 μg/ml); S-DD, isolates that showed dose-dependent susceptibility to fluconazole; S, isolates susceptible to fluconazole.

48 h. The median MIC for caspofungin alone was 1.0 μg/ml at 48 h. Eighteen percent of the drug combinations were synergistic against all the isolates ($n = 119$) compared to 4% among fluconazole-resistant isolates ($n = 26$) at 48 h. Indifference was seen in 82% of all isolates and in 96% of the fluconazole-resistant isolates. No antagonism was seen (Table 1).

Data on the effectiveness of combining posaconazole and caspofungin against *C. glabrata* have not been previously reported. Since echinocandins are highly active against most *Candida* spp., including *C. glabrata*, it may be difficult to improve upon. However, clinical and in vitro resistance of *C. glabrata* to the echinocandins can occur. Similarly, the in vitro activity of the newer azoles including posaconazole is better than fluconazole or itraconazole, but resistance to those agents is usually associated with higher MICs for the newer azoles as well (8). Most physicians and/or institutions have a therapy of choice for invasive candidiasis which usually involves a single agent (7). Combinations, however, might be considered in difficult cases such as hepatosplenic candidiasis, endocarditis, and relapsing infections and for those isolates with higher MICs. Furthermore, alternative treatment options, such as combination therapy, should be available as *Candida* resistance to caspofungin is now being reported (1).

Combination therapy with these two drugs may be advantageous against *C. glabrata*, since synergy was seen with some of the isolates (up to 18%) and frank antagonism was not seen. This also appears to be true in isolates resistant to fluconazole, which may have high posaconazole MICs as well. Animal studies are warranted to elucidate the potential utility of this combination therapy. If animal studies show similar positive results, then human clinical studies would be warranted.

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