In Vitro Activity of Caspofungin (MK-0991) against *Candida albicans* Clinical Isolates Displaying Different Mechanisms of Azole Resistance

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Caspofungin inhibits the synthesis of 1,3-β-D-glucan, a key step in fungal cell wall biosynthesis. Here we report on its potent in vitro activity (MIC at which 90% of the isolates tested are inhibited = 1 μg per ml of RPMI medium) against 32 *Candida albicans* fluconazole-susceptible and -resistant clinical isolates irrespective of the underlying resistance mechanism (alterations in *ERG11* and/or upregulation of *MDR* and *CDR* genes encoding efflux pumps) and provide further evidence that caspofungin is not a substrate for multidrug transporters.

The increase in occurrence of fungal infections, their changing epidemiology, the emergence of resistance, and the toxicity displayed by some of the presently used antifungal therapies have resulted in the need for an expanded arsenal of antifungal drugs. Antifungal agents at different stages in the development pipeline include new-generation azole derivatives and echinocandins. Azole agents target ergosterol biosynthesis, whereas echinocandins represent a new class of antifungal agents that act by inhibiting synthesis of 1,3-β-D-glucan, a key step in fungal cell wall biosynthesis (2). Because of their different mode of action and molecular structure, it is unlikely that cross-resistance betweenazole antifungal agents (targeting ergosterol synthesis) and echinocandins occurs. Thus, echinocandin, which are fungicidal against yeasts and have a different mode of action, may constitute effective prophylactic and therapeutic options for the management of a variety of fungal infections, including those that are refractory to azoles. Caspofungin acetate (Cancidas, formerly reported as MK-0991 and L-743,872) is a water-soluble, potent echinocandin with activity against a number of clinically important fungi (2).

Caspofungin has proven effective in treating mucosal candidiasis even in individuals with advanced immunodeficiency. However, resistance to fluconazole and other azole antifungal drugs has become an important clinical problem in the management of candidiasis. Mechanisms of azole resistance are multifactorial and include alterations in the target enzyme (lanosterol demethylase, encoded by the *ERG11* gene), including overexpression and point mutations and increased extrusion of drug mediated by two types of multidrug efflux transporters, the ABC transporters (encoded by *CDR* genes) and the major facilitators (encoded by *MDR* genes) (12). Due to the different mechanisms of action, alterations in the lanosterol demethylase are unlikely to affect susceptibility to caspofungin. However, multidrug efflux pumps display affinity for a wide variety of compounds (including different types of antifungals), and thus the possibility exists that their overexpression may affect susceptibility to caspofungin. Previous studies have demonstrated that caspofungin is highly active against *Candida* species, including some azole-resistant isolates (1, 3, 11), but the molecular mechanisms responsible for azole resistance in these isolates were not known.

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The *Candida albicans* clinical isolates included in this study have been described before (8). Briefly, they were obtained by direct swab or by oral saline rinses from 12 human immunodeficiency virus-infected patients with recurrent oropharyngeal candidiasis enrolled in a longitudinal study to assess the significance of fluconazole resistance (Table 1). The identity of the isolates as C. *albicans* was confirmed by standard biochemical and microbiological procedures (7). Initial susceptibility testing against fluconazole was performed following NCCLS broth microdilution methodology (5). For each patient, matched sets (as determined by DNA-typing methods) of fluconazole-susceptible and -resistant (MIC = 64 μg/ml) *C. albicans* isolates were included in this study. The molecular mechanisms responsible forazole resistance in this series of isolates have been previously reported (8). Briefly, a Northern blot technique was used to study levels of expression of *ERG11* (encoding lanosterol demethylase, the target enzyme for azole derivatives) and *MDR1* and *CDR* genes (encoding efflux pumps) implicated in the development of azole resistance. We also obtained the nucleotide sequences of *ERG11* genes from all isolates after PCR amplification from genomic DNA. Sequence data were compared to a published *ERG11* sequence in search of nucleotide changes resulting in amino acid substitutions affecting the affinity of the enzyme for azole derivatives. Fluconazole MICs and a summary of the molecular resistance mechanisms detected for each of the different isolates are included in Table 1.

Testing of antifungal susceptibility to caspofungin was determined following NCCLS procedures using broth microdilution method (5). Caspofungin was provided by Merck Research Laboratories (Rahway, N.J.) as a standard powder and was tested at final concentrations of 0.015 to 16 μg/ml. Both the reference RPMI 1640 and Antibiotic Medium 3 with 2% glucose (AM3) were used as test media. We used the spectro-
The reasons for this finding is still not clear. Although interpretative criteria have not yet been defined for caspofungin, the MICs obtained here were in the range of recently reported achievable levels of caspofungin in serum in humans that are approximately 1 μg/ml for a 50-mg caspofungin daily dose (J. A. Stone, J. B. McCrea, P. J. Wickersham, S. D. Holland, P. J. Deutsch, S. Bi, T. Cicero, H. Greenberg, and S. A. Waldman, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 854, p. 26, 2000) and thus should be considered susceptible.

To further assess the potential role of multidrug efflux pumps in the susceptibility to caspofungin, we also performed susceptibility testing of the parental strain C. albicans SC5314 and the following mutants with knockouts in genes encoding multidrug efflux pumps: DS 488 (Δcdr1), DS 653 (Δcdr2), DS 465 (Δmdr1), DS 654 (Δcdr1/Δcdr2), and DS 468 (Δcdr1/Δmdr1). These strains were a kind gift from D. Sanglard (9, 10). The caspofungin susceptibility profiles of all multidrug transporter
mutants tested were unchanged from that of strain SC5314. This was in contrast to their susceptibility to fluconazole, for which these mutants have been shown to be hypersusceptible (9, 10), and in our assay they demonstrated up to a three- or twofold-dilution decrease in fluconazole MICs compared to their parental strain. These strains were tested only against caspofungin in RPMI medium. At 24 h, the caspofungin MICs were between 0.5 and 1 \( \mu g/ml \), whereas at 48 h, all MICs were the same, 1 \( \mu g/ml \).

Azoles target ergosterol synthesis by blocking lanosterol demethylase, an enzyme that is encoded by the \textit{ERG11} gene. Caspofungin, on the other hand, affects fungal cell wall synthesis by inhibiting the production of 1,3-\( \beta \)-D-glucan (2). It is therefore unlikely that cross-resistance between these two classes of antifungals could be explained by an overexpression of \textit{ERG11} or a point mutation within the \textit{ERG11} gene. Our results supported this theory. It was not yet clear if overexpression of genes encoding efflux pumps, like \textit{CDR} or the fluconazole-specific \textit{MDR1}, could affect susceptibility to caspofungin. Our clinical isolates demonstrated low-stable caspofungin MICs against \textit{Candida albicans} isolates from HIV-infected children with oropharyngeal candidosis. J. Antimicrob. Chemother. 46:338–346.


