In Vitro Activities of Caspofungin Compared with Those of Fluconazole and Itraconazole against 3,959 Clinical Isolates of Candida spp., Including 157 Fluconazole-Resistant Isolates

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Caspofungin is an echinocandin antifungal agent with broad-spectrum activity against Candida and Aspergillus spp. The in vitro activities of caspofungin against 3,959 isolates of Candida spp. obtained from over 95 different medical centers worldwide were compared with those of fluconazole and itraconazole. The MICs of the antifungal drugs were determined by broth microdilution tests performed according to the NCCLS method using RPMI 1640 as the test medium. Caspofungin was very active against Candida spp. (MIC at which 90% of the isolates were inhibited [MIC<sub>90</sub>], 1 μg/ml; 96% of MICs were ≤ 2 μg/ml). Candida albicans, C. dubliniensis, C. tropicalis, and C. glabrata were the most susceptible species of Candida (MIC<sub>90</sub> 0.25 to 0.5 μg/ml), and C. guilliermondii was the least susceptible (MIC<sub>90</sub> >8 μg/ml). Caspofungin was very active against Candida spp., exhibiting high-level resistance to fluconazole and itraconazole (99% of MICs were ≤ 1 μg/ml). These results provide further evidence for the spectrum and potency of caspofungin activity against a large and geographically diverse collection of clinically important isolates of Candida spp.

Presently we are witnessing the development and introduction into clinical practice of several new systemic antifungal agents, including both extended-spectrum triazoles and echinocandin antifungal agents (2, 3, 6, 15, 19–21, 23, 26). Both the new triazoles and the echinocandins exhibit a spectrum of activity that includes Candida and Aspergillus (6, 10, 15, 20, 21, 23). Whereas the new triazoles have the same mechanism of action (inhibition of ergosterol synthesis) as the licensed antifungal agents, fluconazole and itraconazole, the echinocandins exhibit a novel mechanism of action based on the inhibition of cell wall glucan synthesis (6, 23). In contrast to the triazoles, which are fungistatic for Candida spp., the echinocandins exhibit concentration-dependent fungicidal activity against Candida spp. but not against Aspergillus spp. (6–11, 19).

Caspofungin is an echinocandin antifungal agent which has recently been approved for treatment of aspergillosis in patients refractory to or intolerant of other therapies (6, 11). Caspofungin also has demonstrated potent in vitro and in vivo activity against Candida spp. and has approved indications for treatment of candidemia, intra-abdominal abscesses, peritonitis, pleural space infections, and esophageal candidiasis (1, 15, 16a, 26). Although numerous studies documenting the in vitro activity of caspofungin against Candida spp. have been published, these studies are limited in the number of isolates of the various species of Candida tested and also are restricted in the geographic distribution of the tested strains (5, 10, 13, 15, 20, 21, 25). In the present study we determined the in vitro activity of caspofungin against an international collection of 3,959 clinical isolates of Candida spp. representing predominantly bloodstream infection and other invasive forms of candidiasis. We compare the activity of caspofungin against those of the licensed agents, fluconazole and itraconazole, and provide an evaluation of the activity of caspofungin against 157 isolates demonstrating high-level resistance (MIC, ≥64 μg/ml) to fluconazole.

MATERIALS AND METHODS

Organisms. A total of 3,959 clinical isolates of Candida spp. obtained from more than 95 different medical centers internationally were tested. The collection included the following numbers of isolates: C. albicans, 2,453; C. glabrata, 512; C. parapsilosis, 420; C. tropicalis, 285; C. dubliniensis, 88; C. guilliermondii, 75; C. krusei, 72; C. lusitaniae, 26; C. famata, 9; C. kefyr, 4; C. rugosa, 6; C. pelliculosa, 3; C. ligmata, 2; C. lipolytica, 1; C. humicola, 1; C. zeylanoides, 1. The isolates were all resistant clinical isolates, and the majority (>80%) were from blood or normally sterile body fluid (cerebrospinal fluid, pleural fluid, or peritoneal fluid). The C. dubliniensis isolates were from mucosal sources. The isolates were identified by standard methods (27) and were stored as water suspensions until they were used in the study. Prior to testing, each isolate was passaged at least twice on potato dextrose agar (Remel, Lenexa, Kan.) to ensure purity and viability.

Antifungal agents. Standard antifungal powders of caspofungin (Merck Co., Whitehouse Station, Pa.), fluconazole (Pfizer, Inc., New York, N.Y.), and itraconazole (Janssen, Beerse, Belgium) were obtained from their respective manufacturers. Stock solutions were prepared in water (caspofungin and fluconazole) or polyethylene glycol (itraconazole). Serial twofold dilutions were prepared exactly as outlined in NCCLS document M27-A (17). Final dilutions were made in RPMI 1640 medium (Sigma, St. Louis, Mo.) buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS) buffer (Sigma). Aliquots (0.1 ml) of each antifungal agent at a 2× final concentration were dispensed into wells of plastic microdilution trays by using a Quick Spense II system (Dynatech Laboratories, Chantilly, Va.). The trays were sealed and frozen at −70°C until they were used.

Antifungal susceptibility studies. Broth microdilution (BMD) testing was performed in accordance with the guidelines in NCCLS document M27-A (17) by using the spectrophotometric method of inoculum preparation, an inoculum concentration of (1.5 ± 1.0) × 10<sup>5</sup> cells/ml, and RPMI 1640 medium buffered to pH 7.0 with MOPS. A 0.1-ml yeast inoculum was added to each well of the microdilution trays. The final concentrations of the antifungal agents were 0.007

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to 8 μg/ml for caspofungin and itraconazole and 0.12 to 128 μg/ml for fluconazole. The trays were incubated at 35°C, and MIC end points were read after 48 h. Drug-free and yeast-free controls were included.

Following incubation, the BMD wells were examined with the aid of a reading mirror and the growth in each well was compared to that in the growth control well. The MIC of caspofungin was defined as complete inhibition of growth, and the MICs of fluconazole and itraconazole were defined as the lowest concentrations that produced a prominent decrease in turbidity (approximately 50%) relative to that of the drug-free control well (15). The interpretative criteria for fluconazole and itraconazole were those published by Rex et al. (22) and the NCCLS (17).

**Quality control.** Quality control was performed by testing the NCCLS-recommended strains, *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 (4, 17).

**RESULTS AND DISCUSSION**

Table 1 summarizes the in vitro susceptibilities of 3,959
isolates of Candida spp. to caspofungin, fluconazole, and itraconazole. Overall, caspofungin was quite active (MIC at which 90% of the isolates were inhibited \([\text{MIC}_{90}]\), 1 \(\mu\)g/ml; 96% of isolates were inhibited by \(\leq 2 \mu\)g/ml). C. albicans, C. dublinensis, C. tropicalis, and C. glabrata were the species most susceptible to caspofungin (\([\text{MIC}_{90}]\), 0.25 to 0.5 \(\mu\)g/ml), and C. guilliermondii was the least susceptible (MIC\(_{90}\), \(>\)8 \(\mu\)g/ml). Notably, 99 to 100% of C. glabrata and C. krusei isolates were inhibited by \(\leq 2 \mu\)g of caspofungin/ml.

Among the 3,959 isolates of Candida spp. studied, a total of 157 were resistant to fluconazole, and 71% of those isolates were also resistant to itraconazole (MIC, \(\geq 1 \mu\)g/ml) (Table 2). Among these resistant isolates, 99% were susceptible to caspofungin at an MIC of \(\leq 1 \mu\)g/ml. Caspofungin was at least as active against fluconazole-resistant isolates as it was against isolates susceptible and dose-dependently susceptible to fluconazole, confirming the complete lack of cross-resistance between these two classes of antifungal agents (15, 16, 18, 25).

These findings confirm and extend those reported previously regarding the antifungal activity of caspofungin (5, 10, 13, 15, 16, 18, 20, 21, 25). Caspofungin was as active or more potent than either fluconazole or itraconazole against all Candida spp. with the exception of C. guilliermondii and C. famata. Although lower caspofungin MICs against these species may be demonstrated by testing in antibiotic medium 3 (14, 18; M. A. Pfaller, unpublished data on file), they still remain higher than those obtained for other Candida spp. It is unclear what this may mean clinically at this time, as both C. guilliermondii and C. famata are very unusual causes of fungal infection and the recommended dosing of caspofungin provides peak plasma concentrations well in excess of 8 \(\mu\)g/ml (11, 12, 19, 24).

Notably, caspofungin demonstrated excellent activity against C. glabrata and C. krusei, two species of Candida that are not covered optimally by the triazoles. In addition, caspofungin was active against isolates of Candida spp., including C. glabrata and C. krusei as well as C. albicans, exhibiting high-level resistance to both fluconazole and itraconazole. Caspofungin has been shown to be fungicidal against Candida spp. (7, 8); however, minimum-fungicidal-concentration determinations were not performed in this study.

Consistent with these in vitro results, in vivo studies have demonstrated the efficacy of treating infections due to Candida spp. with caspofungin (1, 16a, 26). Pharmacokinetic studies have demonstrated peak concentrations of caspofungin in plasma in excess of 16 \(\mu\)g/ml with dosing of 1 mg/kg of body weight daily (12, 19, 24). Pharmacodynamic studies have demonstrated concentration-dependent killing that is optimized at four or more times the MIC and a postantifungal effect of more than 12 h (7, 8). Given the MIC data presented in Tables 1 and 2 (overall MIC\(_{90}\), \(\leq 1 \mu\)g/ml), plasma caspofungin concentrations exceeding the MIC by fourfold or more should be attainable for virtually all clinical isolates of Candida spp. treated with caspofungin.

In summary, we have demonstrated that caspofungin is more potent than fluconazole and itraconazole against significant clinical isolates of Candida spp. The emerging in vivo data from animal models as well as from clinical trials appear to support the in vitro data regarding the efficacy of caspofungin in the treatment of invasive candidiasis. Caspofungin has very favorable pharmacokinetic and pharmacodynamic properties that make it a highly promising new systemic antifungal agent. Caspofungin may prove to be very useful in the treatment of serious Candida infections that are refractory to existing antifungal agents.

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**REFERENCES**


