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In Vitro Activities of Voriconazole (UK-109,496) and Four Other Antifungal Agents against 394 Clinical Isolates of *Candida* spp.

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Voriconazole (formerly UK-109,496) is a new monotriazole antifungal agent which has potent activity against *Candida*, *Cryptococcus*, and *Aspergillus* species. We investigated the in vitro activity of voriconazole compared to those of fluconazole, itraconazole, amphotericin B, and flucytosine (5FC) against 394 bloodstream isolates of *Candida* (five species) obtained from more than 30 different medical centers. MICs of all antifungal drugs were determined by the method recommended by the National Committee for Clinical Laboratory Standards using RPMI 1640 test medium. Overall, voriconazole was quite active against all the yeast isolates (MIC at which 90% of the isolates are inhibited [MIC_{90}], ≤0.5 μg/ml). *Candida albicans* was the most susceptible species (MIC_{90}, 0.06 μg/ml) and *Candida glabrata* and *Candida krusei* were the least (MIC_{90}, 1 μg/ml). Voriconazole was more active than amphotericin B and 5FC against all species except *C. glabrata* and was also more active than itraconazole and fluconazole. For isolates of *Candida* spp. with decreased susceptibility to fluconazole and itraconazole MICs of voriconazole were also higher. Based on these results, voriconazole has promising antifungal activity and further in vitro and in vivo investigations are warranted.

Newer azoles such as fluconazole and itraconazole are frequently used in the treatment of fungal infections due to *Candida* spp. They offer potential advantages over amphotericin B, including reduced toxicity and versatility of oral or intravenous (fluconazole only) administration. However, acquired or intrinsic resistance to these compounds is well known, and failure of azole therapy has been reported (11, 12). There is, therefore, a clear need for new drugs to improve the treatment of fungal infections.

Voriconazole (UK-109,496) is a new monotriazole antifungal agent obtained by modification of the structure of fluconazole (14). It exhibits dose-dependent pharmacokinetics and is usually well tolerated after oral or intravenous administration (10). Early clinical studies have suggested that voriconazole may be effective in the treatment of oropharyngeal candidiasis and of acute or chronic pulmonary aspergillosis (3, 4, 17). The efficacy of voriconazole in experimental models of invasive aspergillosis and in the treatment of *Aspergillus fumigatus* endocarditis has also been documented (5, 7, 8). Previous in vitro investigations have shown activity against several fungal pathogens, including *Candida* spp., *Cryptococcus neoformans*, and *Aspergillus* spp. (1, 6, 16). Barry and Brown (2) found that voriconazole had better in vitro activity than fluconazole against six *Candida* species. Ruhnke et al. (15) also reported good activity of voriconazole against *Candida albicans* strains isolated from patients with human immunodeficiency virus infection. However, the number of clinical isolates of *Candida* spp. included in these studies is limited, and there is a lack of comparative data for other antifungal agents.

In this study, we evaluated the in vitro activities of voriconazole and four other antifungal agents against 394 clinical isolates of *Candida* spp. The comparison agents tested were fluconazole, itraconazole, amphotericin B, and flucytosine (5FC). The in vitro susceptibility testing method employed was a microdilution adaptation of the guidelines set forth by the National Committee for Clinical Laboratory Standards (NCCLS) (9).

MATERIALS AND METHODS

Yeast isolates. A total of 394 recent bloodstream isolates of *Candida* spp. obtained from 31 different medical centers were selected for this study. The isolates included were *C. albicans* (206 strains), *Candida glabrata* (77 strains), *Candida tropicalis* (54 strains), *Candida parapsilosis* (40 strains), and *Candida krusei* (17 strains). All isolates were stored as suspensions in sterile distilled water at room temperature until the study was performed. Prior to testing, each isolate was subcultured at least twice on potato dextrose agar plates (Remel, Lenexa, Kans.) to ensure purity and optimal growth.

Antifungal agents. Standard antifungal powders of voriconazole and fluconazole were supplied by Pfizer Inc., Central Research Division (Groton, Conn.). Amphotericin B, 5FC, and itraconazole were obtained from their respective manufacturers. Stock solutions were prepared in water (fluconazole and 5FC), dimethyl sulfoxide (amphotericin B), and polyethylene glycol (voriconazole and itraconazole). Antifungal agents were diluted with RPMI 1640 medium (Sigma Chemical Co., St. Louis, Mo.) which had been buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS) buffer (Sigma), and the mixtures were dispensed into 96-well microdilution trays. Trays containing an aliquot of 0.1 ml in each well were sealed and frozen at −70°C until they were used in the study. The NCCLS recommendations (9) were followed for the dilution of each antifungal agent.

Antifungal susceptibility testing. Broth microdilution MICs were determined by the NCCLS method (9). The final concentrations of the antifungal agents ranged from 0.015 to 16 μg/ml for voriconazole, 0.125 to 128 μg/ml for fluconazole, 0.007 to 8 μg/ml for itraconazole, 0.015 to 8 μg/ml for amphotericin B, and 0.06 to 128 μg/ml for 5FC. The yeast inoculum was adjusted to a concentration of 0.5×10^3 to 2.5×10^3 CFU/ml in RPMI 1640 medium, and an aliquot of 0.1 ml was added to each well of the microdilution tray. In each case, the inoculum size was verified by colony counting. MIC endpoints were determined after incubation for 48 h in ambient air at 35°C. For amphotericin B this endpoint was defined as the lowest concentration that completely inhibited growth. For the azole compounds and 5FC the MIC was defined as the lowest concentration that produced an 80% reduction of growth compared with that of the drug-free growth control.

Quality control. *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were used as quality control organisms and were included each time a set of isolates was tested.

RESULTS AND DISCUSSION

Table 1 summarizes the MICs of voriconazole, itraconazole, fluconazole, amphotericin B, and 5FC for the 394 clinical iso-

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TABLE 1. In vitro susceptibilities of 394 clinical yeast isolates to voriconazole and other antifungal agents

Organism (no. of isolates)	Antifungal agent	MIC (μg/ml)		
		Range	50%	90%
C. albicans (206)	Voriconazole	≤0.015–>16	0.03	0.06
	Itraconazole	0.03–>8	0.125	0.25
	Fluconazole	≤0.125–>128	0.25	2
	Amphotericin B	0.5–2	1	1
	5FC	≤0.06–>128	0.25	4
C. glabrata (77)	Voriconazole	0.03–8	0.5	1
	Itraconazole	0.06–>8	1	4
	Fluconazole	0.25–>128	16	64
	Amphotericin B	1–2	1	2
	5FC	≤0.06–16	0.125	0.25
C. tropicalis (54)	Voriconazole	≤0.015–>16	0.06	0.125
	Itraconazole	0.03–>8	0.25	0.5
	Fluconazole	0.25–>128	0.5	2
	Amphotericin B	0.5–2	1	2
	5FC	≤0.06–>128	0.25	1
C. parapsilosis (40)	Voriconazole	≤0.015–1	0.06	0.25
	Itraconazole	0.125–2	0.25	0.5
	Fluconazole	0.25–16	1	8
	Amphotericin B	0.5–2	1	2
	5FC	≤0.06–>128	0.125	1
C. krusei (17)	Voriconazole	0.25–1	0.5	1
	Itraconazole	0.5–2	1	2
	Fluconazole	32–128	64	128
	Amphotericin B	1–2	2	2
	5FC	16–64	32	64
All organisms (394)	Voriconazole	≤0.015–>16	0.06	0.5
	Itraconazole	0.03–>8	0.25	2
	Fluconazole	≤0.125–>128	0.5	16
	Amphotericin B	0.5–2	1	2
	5FC	≤0.06–>128	0.25	8

lates of *Candida* spp. Overall, voriconazole was highly active (MIC at which 90% of the isolates are inhibited [MIC_{90}], ≤0.5 μg/ml) against all isolates, and *C. albicans* was the most susceptible species (MIC_{90}, 0.06 μg/ml). *C. glabrata* and *C. krusei* were the least susceptible to voriconazole (MIC_{90}, 1 μg/ml); the most highly resistant strains were *C. albicans* and *C. tropicalis* strains (MIC, >16 μg/ml). Voriconazole was more active than amphotericin B and 5FC against all species except *C. glabrata* and was also more active than itraconazole and fluconazole.

Among the 394 isolates studied, a total of 18 strains (7 *C. albicans*, 8 *C. glabrata*, and 3 *C. tropicalis* strains) from eight different medical centers were resistant to both fluconazole (MIC, ≥64 μg/ml) and itraconazole (MIC, ≥1 μg/ml) (13). MICs of voriconazole for these strains were >16 μg/ml (seven *C. albicans* and two *C. tropicalis* strains), 8 μg/ml (three *C. glabrata* strains), 4 μg/ml (three *C. glabrata* and one *C. tropicalis* strain), and 2 μg/ml (two *C. glabrata* strains).

These results support and extend findings reported previously (1, 2, 12). Like Barry and Brown (2), we found that voriconazole was more active than fluconazole against all *Candida* isolates tested. In addition, the spectrum of activity was better than that of itraconazole. This enhanced in vitro activity against two species frequently considered refractory to azoles, *C. krusei* and *C. glabrata* (MIC_{90}, 1 μg/ml), is remarkable. Although pharmacokinetic studies with animal models have found that levels of voriconazole in serum could range from 1 to 5 μg/ml (5, 7, 8) and similar levels are expected to be achieved in humans, clinical trials are clearly required to prove the utility of voriconazole in infections due to these two species.

Ruhnke et al. (15) used a broth microdilution test following the NCCLS guidelines (9) to determine the in vitro activities of voriconazole and fluconazole against 105 isolates of *C. albicans* recovered from the oral cavities of human immunodeficiency virus-infected patients. They also observed that voriconazole was more potent in vitro than fluconazole.

The data from standardized and reference in vitro susceptibility testing indicate that voriconazole is more potent than either itraconazole or fluconazole against all clinical isolates tested. Although others have reported that voriconazole could be active against fluconazole-resistant *C. albicans* isolates (2, 15), we were unable to demonstrate this finding, and in our study the MICs of voriconazole (>16 μg/ml) for fluconazole-resistant *C. albicans* isolates tested were high. Even though greater voriconazole activity was observed with eight *C. glabrata* isolates and one *C. tropicalis* isolate that were resistant to fluconazole, MICs for these isolates were ≥2 μg/ml. These data suggest a possible cross-resistance mechanism among highly azole-resistant strains.

The translation of this in vitro activity into clinical efficacy still needs to be established; however, Troke et al. (17) have shown in a guinea pig model of systemic candidiasis that voriconazole efficacy was similar to that of fluconazole or itraconazole in *C. albicans* infections but that voriconazole was more active when the animal was infected with *C. krusei*, *C. glabrata*, or azole-resistant strains of *C. albicans*. Preliminary clinical data also suggest that voriconazole is efficacious in the treatment of oropharyngeal candidiasis, even that caused by fluconazole-resistant strains (15, 17). In view of the potent in vitro activity demonstrated here as well as the promising early in vivo information, voriconazole warrants further investigation.

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