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In Vitro Activities of Voriconazole, Fluconazole, and Itraconazole against 566 Clinical Isolates of *Cryptococcus neoformans* from the United States and Africa

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We investigated the in vitro activity of voriconazole compared to those of fluconazole and itraconazole against 566 clinical isolates of *Cryptococcus neoformans* from Africa (164) and the United States (402). Isolates were obtained from cerebrospinal fluid (362), blood (139), and miscellaneous sites (65). Voriconazole (MIC at which 90% of the isolates are inhibited [MIC_{90}], 0.12 to 0.25 μg/ml) was more active than either itraconazole (MIC_{90}, 0.5 μg/ml) or fluconazole (MIC_{90}, 8.0 to 16 μg/ml) against both African and U.S. isolates. Isolates inhibited by ≥16 μg of fluconazole per ml were almost all (99%) inhibited by ≤1 μg of voriconazole per ml. These results suggest that voriconazole may be useful in the treatment of cryptococcosis.

Among the community-acquired opportunistic fungal pathogens, perhaps the most important and certainly the single most common agent of serious infection is *Cryptococcus neoformans* (8). A rare disease prior to the onset of the AIDS epidemic, cryptococcosis is a leading mycological cause of morbidity and mortality among AIDS patients (8, 13). Although precise estimates of the incidence of cryptococcal disease are not available, it is thought to affect 6 to 10% of patients with AIDS in the United States and 15 to 30% in sub-Saharan Africa (13, 17). Recent data from the Centers for Disease Control and Prevention (CDC) suggested that, in metropolitan areas with a high concentration of human immunodeficiency virus-infected persons, the incidence may be as high as five cases per 100,000 population (8). *C. neoformans* var. *neoformans* is now the most common cause of meningitis at many large hospitals caring for AIDS patients (6, 8). A recent prospective study in Zimbabwe found that *C. neoformans* var. *neoformans* accounted for 45% of all laboratory-proven cases of meningitis in adults (9).

Current treatment regimens for cryptococcal meningitis have remained focused on amphotericin B, with or without flucytosine (6, 13, 17, 18, 22, 24). The toxicity of this regimen is well known. Although fluconazole is better tolerated, it is used primarily as maintenance therapy in AIDS patients, and concerns regarding the development of fluconazole-resistant strains of *C. neoformans* have been raised (3, 5, 13, 16, 18, 24). Among the available alternative therapeutic agents, itraconazole has been found to be less effective than either amphotericin B or fluconazole in the treatment of cryptococcal meningitis in HIV-infected patients (22). Given these limitations, investigation of the activity of newer antifungal agents against *C. neoformans* is indicated.

Voriconazole is a new monotriazole antifungal agent with potent in vitro activity against several fungal pathogens including *Candida* spp., *C. neoformans*, and *Aspergillus* spp. (1, 2, 7, 11, 12, 15, 20). Although the activity of voriconazole against *C. neoformans* looks promising (7, 15), the number of clinical isolates included in the previous studies is limited and there is a lack of comparative data for isolates from countries other than the United States.

In this study, we evaluated the in vitro activities of voriconazole, fluconazole, and itraconazole against 566 clinical isolates of *C. neoformans* including 402 isolates from the United States and 164 isolates from Africa. The in vitro susceptibility testing method employed was a microdilution method performed according to the guidelines set forth by the National Committee for Clinical Laboratory Standards (NCCLS) (14, 23).

A total of 566 recent clinical isolates of *C. neoformans* var. *neoformans* from the United States (402 isolates) and from Africa (164 isolates) were selected for this study. The collection included 362 isolates from cerebrospinal fluid cultures, 139 from blood cultures and 65 isolates from miscellaneous clinical sources (pleural fluid, tissue, urine, etc.). The U.S. isolates were obtained from AIDS patients located in California, Iowa, Texas, and Georgia. Approximately 300 of these isolates were collected as part of a population-based survey of cryptococcal disease conducted by the CDC, and the epidemiologic characteristics of some of these isolates have been described previously (4, 5). The African isolates were obtained from AIDS patients with cryptococcal meningitis who were seen in a clinic in Kampala, Uganda. Identification was confirmed by standard methods (4, 10). All isolates were of the *neoformans* variety, as determined by growing cells on canavanine-glycine-bromthymol blue agar (10). Isolates were stored frozen at −20°C in 20% glycerol 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.

Standard powders of voriconazole and fluconazole were supplied by Pfizer Pharmaceuticals Group, Central Research Division (Groton, Conn.). Itraconazole was obtained from the Janssen Research Foundation (Beerse, Belgium). Stock solutions were prepared in water (fluconazole) or dimethyl sulfox-

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TABLE 1. In vitro susceptibilities of 566 clinical isolates of *C. neoformans* to voriconazole and itraconazole stratified by fluconazole susceptibility category

Fluconazole susceptibility category (μg/ml)[a]	Isolate source	No. of isolates tested	MIC (μg/ml)					
			Itraconazole			Voriconazole		
			Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
≤8.0	Africa	154	0.06–0.5	0.25	0.5	0.015–0.25	0.12	0.12
	United States	321	≤0.007–0.5	0.25	0.5	≤0.007–0.12	0.06	0.06
	All	475	≤0.007–0.5	0.25	0.5	≤0.007–0.25	0.06	0.12
16–32	Africa	10	0.25–1.0	0.5	1.0	0.25–0.5	0.25	0.25
	United States	78	0.25–1.0	0.5	1.0	0.03–0.5	0.12	0.25
	All	88	0.25–1.0	0.5	1.0	0.03–0.5	0.12	0.25
≥64	Africa							
	United States	3	0.5–1.0	0.5		0.25–2.0	1.0	
	All	3	0.5–1.0	0.5		0.25–2.0	1.0	
Total	Africa	164	0.06–1.0	0.25	0.5	0.015–0.5	0.12	0.25
	United States	402	≤0.007–1.0	0.25	0.5	≤0.007–2.0	0.06	0.12
	All	566	≤0.007–1.0	0.25	0.5	≤0.007–2.0	0.06	0.12

[a] Fluconazole MICs ranged from 0.12 to >128 μg/ml with a MIC_{50} of 8.0 μg/ml (both United States and Africa) and a MIC_{90} of 16 μg/ml (United States, 16 μg/ml; Africa, 8.0 μg/ml).

ide (voriconazole and itraconazole). Antifungal agents were diluted as described in NCCLS document M27-A (14) with RPMI 1640 medium (Sigma, 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.

Broth microdilution MICs were determined by the NCCLS method (14, 23). The final concentrations of the antifungal agents ranged from 0.007 to 8 μg/ml for voriconazole and itraconazole and 0.125 to 128 μg/ml for fluconazole. 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. The microdilution trays were incubated at 35°C. The MIC endpoints were read visually following 48 and 72 h of incubation and were defined for the three azoles as the lowest concentration that produced an 80% reduction in growth (prominent decrease in turbidity) compared with that of the drug-free growth control (5, 11, 14, 23). All isolates grew in the test system, and MIC results read at 48 and 72 h were in complete agreement. Thus, the 48-h MIC data is reported herein.

C. parapsilosis ATCC 22019 and *C. krusei* ATCC 6258 were used as quality control organisms and were included each time that a set of isolates was tested (11, 14).

The isolates were generally susceptible to all three triazoles, and only minor differences were observed between the African and the U.S. isolates (Table 1). Overall, voriconazole was the most active agent (MIC at which 90% of the isolates are inhibited [MIC_{90}], 0.12 μg/ml), followed by itraconazole (MIC_{90}, 0.5 μg/ml) and fluconazole (MIC_{90}, 16 μg/ml). Fluconazole had MICs of ≤8 μg/ml for 84% (475 of 566) of the *C. neoformans* isolates tested (94% of the African isolates and 80% of the U.S. isolates), 16 to 32 μg/ml for 15.5% (88 of 566) of these isolates (6% of the African isolates and 19% of the U.S. isolates), and ≥64 μg/ml for 0.5% (3 of 566) of these isolates (0% of the African isolates and 0.7% of the U.S. isolates).

Among the isolates inhibited by ≤8 μg of fluconazole per ml, voriconazole was more potent than itraconazole against both U.S. (MIC_{90}, 0.06 versus 0.5 μg/ml, respectively) and African (MIC_{90}, 0.12 versus 0.5 μg/ml, respectively) strains. All 475 of these isolates were inhibited by ≤0.25 μg of voriconazole per ml, and 73% (71% of African isolates and 74% of U.S. isolates) were inhibited by ≤0.25 μg of itraconazole per ml.

Voriconazole (MIC_{90}, 0.25 μg/ml) was also more active than itraconazole (MIC_{90}, 1.0 μg/ml) against the 88 isolates inhibited by 16 to 32 μg of fluconazole per ml. All of these isolates were inhibited by ≤0.5 μg of voriconazole per ml, and 75% (80% of African isolates and 74% of U.S. isolates) were inhibited by ≤0.5 μg of itraconazole per ml.

Only three isolates, all from the United States, required ≥64 μg of fluconazole per ml to inhibit growth in vitro. For these isolates, the voriconazole MICs were 0.25, 1, and 2 μg/ml and the itraconazole MICs were 0.5, 0.5, and 1 μg/ml.

These results support and extend findings reported previously (7, 15). Like Nguyen and Yu (15), we found voriconazole to be more active than either itraconazole or fluconazole against *C. neoformans* isolates. It is notable that 82% of the isolates tested were inhibited by ≤0.12 μg of voriconazole per ml and 99.6% were inhibited by ≤0.5 μg/ml. By comparison, 18% were inhibited by ≤0.12 μg and 96% were inhibited by ≤0.5 μg of itraconazole per ml. Both voriconazole and itraconazole appeared most active against isolates exhibiting the greatest susceptibility to fluconazole (MIC of fluconazole, ≤8 μg/ml). As the fluconazole MICs increased, so did the MICs of voriconazole and itraconazole; however, a greater percentage of isolates inhibited by 16 to 32 μg of fluconazole per ml remained highly susceptible (MIC, ≤0.12 μg/ml) to voriconazole (65%) than to itraconazole (0%).

In addition to providing comparative in vitro susceptibility data for three triazole antifungal agents against a large number of clinical isolates of *C. neoformans*, this study also provides for the first time a comparison of in vitro susceptibilities of U.S. versus African *C. neoformans* isolates. Importantly, isolates from both the United States and Africa appear to be quite susceptible to fluconazole and the other triazoles. There was no evidence of increased resistance to fluconazole among the African isolates, and over 99% of all isolates were inhibited by

concentrations of fluconazole (≤32 μg/ml) that are readily achieved by standard dosing regimens (21).

In summary, we have found voriconazole to be more potent than either itraconazole or fluconazole against clinical isolates of *C. neoformans* from Africa and the United States. This improved potency plus favorable pharmacokinetics suggests that voriconazole may be useful in the treatment of cryptococcosis among other invasive fungal infections. Appropriate clinical trials are encouraged. Although the majority of isolates of *C. neoformans* in this study appear to be susceptible to fluconazole and other triazoles, continued surveillance for emerging resistance is warranted on a national and international basis given the broad utilization of fluconazole as primary prophylaxis in patients with AIDS (3, 19).

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