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In Vitro Activities of Voriconazole, Itraconazole, and Amphotericin B against *Blastomyces dermatitidis*, *Coccidioides immitis*, and *Histoplasma capsulatum*

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The in vitro activity of voriconazole was compared to those of itraconazole and amphotericin B against the mold forms of 304 isolates of three dimorphic fungi, *Blastomyces dermatitidis*, *Coccidioides immitis*, and *Histoplasma capsulatum*. MICs were determined by a broth microdilution adaptation of the National Committee for Clinical Laboratory Standards M27-A procedure. RPMI 1640 medium was used for tests with voriconazole and itraconazole, whereas Antibiotic Medium 3 with 2% glucose was used for amphotericin B. Minimum fungicidal concentrations (MFCs) were also determined. Amphotericin B was active against all three dimorphic fungi, with MICs at which 90% of the isolates tested are inhibited (MIC_{90}s) of 0.5 to 1 μg/ml. Itraconazole had MIC_{90}s of 0.06 μg/ml for *H. capsulatum*, 0.125 μg/ml for *B. dermatitidis*, and 1 μg/ml for *C. immitis*. The MIC_{90}s of voriconazole were 0.25 μg/ml for all three fungi. Amphotericin B was fungicidal for *B. dermatitidis* and *H. capsulatum* with MFCs at which 90% of strains tested are killed (MFC_{90}s) of 0.5 and 2 μg/ml, respectively. It was less active against *C. immitis*, with MFCs ranging from 0.5 to >16 μg/ml. Voriconazole and itraconazole were lethal for most isolates of *B. dermatitidis*, with MFC_{50}s and MFC_{90}s of 0.125 and 4 μg/ml, respectively. Both azoles were fungicidal for some isolates of *H. capsulatum*, with MFC_{50}s of 2 and 8 μg/ml for itraconazole and voriconazole, respectively; neither had a lethal effect upon *C. immitis*. Our results suggest that voriconazole possesses promising activity against these important human pathogens.

Until recently, amphotericin B has been the principal agent for the treatment of most systemic fungal infections, despite the fact that its use is limited by a range of serious side effects, particularly renal toxicity (5). Lipid-based preparations have reduced the toxicity but have not significantly increased its efficacy (19). Although the imidazole agent ketoconazole proved efficacious for some chronic forms of blastomycosis, histoplasmosis, and coccidioidomycosis, it was not regarded as a first-line treatment for life-threatening or severe infections (7). More recently, the triazole itraconazole has become the treatment of choice for mild to moderate forms of histoplasmosis and blastomycosis. Coccidioidomycosis can be treated with either itraconazole or fluconazole, with the latter agent being the drug of choice for coccidioidal meningitis (6). Because not all cases respond to treatment with amphotericin B, itraconazole, or fluconazole, there is a continuing need for new antifungal agents.

Voriconazole (UK-109,496) is a new triazole antifungal agent that shows promise for the treatment of a broad spectrum of fungal pathogens, including *Aspergillus* species, *Candida* species, *Cryptococcus neoformans*, *Penicillium marneffei*, *Scedosporium apiospermum*, and others (1, 2, 3, 6, 8, 12–15, 17). Voriconazole has been reported to have fungistatic activity against *Blastomyces dermatitidis*, *Coccidioides immitis*, and *Histoplasma capsulatum* (3, 9). However, the number of isolates that have been studied is limited, and there are no data regarding its possible fungicidal activity against the dimorphic fungi. In this study, we evaluated the in vitro fungistatic and fungicidal activities of voriconazole, itraconazole, and amphotericin B against *B. dermatitidis*, *C. immitis*, and *H. capsulatum*.

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Test isolates. A total of 304 isolates were tested. These comprised 100 clinical isolates each of *B. dermatitidis* and *C. immitis* and 104 clinical isolates of *H. capsulatum*. Isolates of *C. immitis* were retrieved from storage in 50% glycerol at −70°C and then subcultured onto slants of Sabouraud glucose agar (SGA) incubated at 25°C. Isolates of *B. dermatitidis* and *H. capsulatum* were retrieved from storage at −70°C and subcultured onto slants of brain heart infusion agar incubated at 30°C. Prior to testing, each isolate was recultured on the same medium to verify purity and induce arthroconidium or conidium formation. All procedures were performed within a class II biological safety cabinet in a biosafety level 3 laboratory.

Antifungal agents. The three antifungal agents were obtained from their respective manufacturers: voriconazole from Pfizer Inc., Central Research Division, Groton, Conn.; itraconazole from Janssen Pharmaceutica, Titusville, N.J.; and amphotericin B from Bristol-Myers Squibb, Princeton, N.J. Stock solutions were prepared in dimethylformamide (voriconazole) or dimethyl sulfoxide (amphotericin B and itraconazole). Further dilutions of each antifungal agent were prepared as outlined in National Committee for Clinical Laboratory Standards (NCCLS) document M27-A (10). Voriconazole and itraconazole were tested in RPMI 1640 medium (with L-glutamine and without sodium bicarbonate) (Sigma Chemical Co., St. Louis, Mo.), buffered to pH 7.0 with 0.165 M morpholinopropanesulfonic acid (MOPS; Sigma). Amphotericin B was tested

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TABLE 1. Quality control MIC ranges of antifungal agents

Organism	Antifungal agent	MIC range (μg/ml)	
		Present study	Reference range[b]
C. krusei (ATCC 6258)	Voriconazole	0.25–0.5	NA
	Itraconazole	0.25–0.5	0.125–0.5
	Amphotericin B	0.25–0.5[a]	0.5–2
P. variotii (ATCC 22319)	Voriconazole	2–8	NA
	Itraconazole	0.06–0.5	0.03–1
	Amphotericin B	0.125–0.05[a]	0.25–1

[a] For amphotericin B, Antibiotic Medium 3 was used rather than RPMI 1640. This medium is expected to result in lower MICs.
[b] The reference MIC ranges for *C. krusei* were obtained from NCCLS document M27-A (10). MIC ranges for *P. variotii* were obtained from a published collaborative study (4). NA, not available.

in Bacto Antibiotic Medium 3 (Difco Laboratories, Detroit, Mich.) supplemented with 2% glucose. The drug dilutions were dispensed in 0.1-ml amounts in sterile plastic snap-cap tubes (12 by 75 mm) that were then stored at −70°C until needed.

Antifungal susceptibility testing. Broth macrodilution testing was performed in accordance with the guidelines in NCCLS document M27-A (10). Conidial suspensions of each isolate were prepared in RPMI 1640 medium (for voriconazole and itraconazole) or Antibiotic Medium 3 with 2% glucose (for amphotericin B) and adjusted spectrophotometrically to 0.5×10^3 to 2.5×10^4 CFU/ml as demonstrated by quantitative colony counts on SGA plates. Aliquots of 0.9 ml of inoculum suspension were added to each of the previously prepared tubes containing serial dilutions of antifungal agents. Final drug concentrations were 0.03 to 32 μg/ml for voriconazole and itraconazole and 0.03 to 16 μg/ml for amphotericin B. Control tubes without drug contained medium with 1% solvent.

MIC endpoints were determined after 48 h of incubation at 35°C or after the control tubes showed appropriate growth. For amphotericin B, the MIC was defined as the lowest concentration of drug that completely inhibited growth. For the azoles, the MIC was defined as the lowest concentration resulting in 80% inhibition of growth compared to that for untreated controls (10).

The minimum fungicidal concentration (MFC) was determined by removing 10 μl of the contents from all tubes showing no visible growth and from the last tube to show growth. The samples were spread onto brain heart infusion agar (*B. dermatitidis* and *H. capsulatum*) or SGA in plates (*C. immitis*). These were incubated at 30°C (25°C for *C. immitis*) until the inoculum from the tube containing the lowest drug concentration showed good growth. The MFC was defined as the lowest concentration that allowed the growth of three or fewer colonies. This represents killing of >97% of the original inoculum.

Quality control. *Candida krusei* ATCC 6258 and *Paecilomyces variotii* ATCC 22319 were included in each batch of tests.

Results. All *C. immitis* isolates produced sufficient growth to determine MICs after 48 or 72 h of incubation. For *B. dermatitidis* and *H. capsulatum*, the MICs were determined after 5 and 7 days, respectively. Likewise, the MFCs for these organisms were determined after 7 days of incubation. The test conditions used did not result in conversion of *B. dermatitidis* or *H. capsulatum* isolates to the yeast form. The MIC ranges for the two quality control strains are listed in Table 1. The results for these organisms were within the expected ranges.

Table 2 summarizes the in vitro susceptibilities of the 304 isolates tested to amphotericin B, itraconazole, and voriconazole. The data are presented as MIC and MFC ranges and as the drug concentrations required to inhibit or kill 50 and 90% of the isolates of each species (MIC_{50}, MIC_{90}, MFC_{50}, and MFC_{90}). Amphotericin B was active against *B. dermatitidis*, *C. immitis*, and *H. capsulatum*, with MIC_{90}s of 0.5 or 1 μg/ml. With one exception, the MIC_{90}s of itraconazole and voriconazole were lower than those for amphotericin B, ranging from 0.06 to 0.25 μg/ml. The exception was *C. immitis*, for which the MIC_{90} of itraconazole was 1 μg/ml. In addition, for a few isolates of *B. dermatitidis*, the itraconazole and/or voriconazole MICs were 16 μg/ml or higher. Nonetheless, the MIC_{90}s of voriconazole were 0.25 μg/ml for all three dimorphic fungi. The corresponding values for itraconazole ranged from 0.125 μg/ml for *B. dermatitidis* to 1 μg/ml for *C. immitis*.

The MFC ranges of the three compounds showed marked differences. Amphotericin B was fungicidal for *B. dermatitidis*, with an MFC_{90} of 0.5 μg/ml. It was less active against *C. immitis* and *H. capsulatum*, having MFC_{90}s of >16 and 2 μg/ml, respectively. Voriconazole and itraconazole were fungi-

TABLE 2. In vitro susceptibilities of 304 isolates to voriconazole, itraconazole, and amphotericin B

Sp. (no. of isolates)	Antifungal agent	MIC (μg/ml)			MFC (μg/ml)		
		Range	50%	90%	Range	50%	90%
B. dermatitidis (100)	Voriconazole	≤0.03–16	≤0.03	0.25	≤0.03–32	0.125	4
	Itraconazole	≤0.03–>16	≤0.03	0.125	≤0.03–>16	0.125	4
	Amphotericin B	≤0.03–1	0.06	0.5	≤0.03–4	0.125	0.5
C. immitis (104)	Voriconazole	≤0.03–0.5	0.125	0.25	>32	>32	>32
	Itraconazole	0.125–1	0.5	1	16–>16	>16	>16
	Amphotericin B	0.25–2	0.5	1	0.5–>16	4	>16
H. capsulatum (100)	Voriconazole	≤0.03–2	0.06	0.25	≤0.03–>32	8	>32
	Itraconazole	≤0.03–0.5	≤0.03	0.06	≤0.03–>16	2	16
	Amphotericin B	≤0.03–2	0.25	1	≤0.03–>16	0.5	2

cidal for many isolates of *B. dermatitidis*, with MFC_{50}s and MFC_{90}s of 0.125 and 4 μg/ml, respectively. Both azoles were fungicidal for some isolates of *H. capsulatum*, with MFC_{50}s of 2 and 8 μg/ml for itraconazole and voriconazole, respectively. Neither had a fungicidal effect on *C. immitis*.

Discussion. Voriconazole is a new triazole antifungal agent with a broad spectrum of fungistatic action in vitro, including action against *Aspergillus* species, *Candida* species, *C. neoformans*, *P. marneffei*, and *S. apiospermum* (1, 2, 3, 6, 8, 12–15, 17). Voriconazole has also been reported to be a promising agent for the treatment of invasive aspergillosis and human immunodeficiency virus-associated oral candidiasis (D. W. Denning, A. del Favero, E. Gluckman, D. Norfolk, M. Ruhnke, S. Yonren, P. Troke, and N. Sarantis, Abstr. 35th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F80, p. 126, 1995; B. Dupont, D. Denning, H. Lode, S. Yonren, P. F. Troke, and N. Sarantis, Abstr. 35th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F81, p. 126, 1995; P. F. Troke, K. W. Brammer, C. A. Hitchcock, S. Yonren, and N. Sarantis, Abstr. 35th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F73, p. 125, 1995). To date, however, there have been no published reports of its clinical use in patients with blastomycosis, coccidioidomycosis, or histoplasmosis.

Our findings extend those of several recent studies which showed that voriconazole is more active in vitro than amphotericin B against the mold forms of the dimorphic fungi *B. dermatitidis*, *C. immitis*, and *H. capsulatum* (3, 9). Our results indicate that the fungistatic effect of voriconazole is similar to, or better than, that of itraconazole against these pathogens. In addition, both of these triazole agents are fungicidal in vitro for some isolates of *B. dermatitidis* and *H. capsulatum*, although neither had a lethal effect on *C. immitis*. These MFC data complement recent reports which indicate that voriconazole has a fungicidal effect upon *Aspergillus* spp. (1, 6, 13), as well as on a number of dematiaceous molds, including *Cladophialophora bantiana*, *Fonsecaea pedrosoi*, *Phialophora parasitica*, and *Wangiella dermatitidis* (6).

The relative importance of the fungicidal rather than the fungistatic effects of antifungal agents in vitro is unclear. In the case of amphotericin B, the MFC has been found to be a better predictor of clinical outcome in patients with candidemia (11) and trichosporonosis (18). Morever, there is presently no consensus as to how the MFCs of antifungal agents should be determined or defined. In this study, the MFC was defined as the lowest concentration of antifungal agent that resulted in the growth of three or fewer colonies, which corresponds to a 97% kill rate. Other investigators, who have transferred 100 μl from each MIC tube, have defined the MFC as the lowest concentration of agent that permits the growth of five or fewer colonies. This corresponds to a 99.5% kill rate (16).

It remains to be seen to what extent the low MICs and MFCs seen in this and other studies will be predictive of clinical outcome in patients with endemic fungal infections. The tests were done with the saprotrophic mold forms of *B. dermatitidis*, *C. immitis*, and *H. capsulatum*, rather than the pathogenic forms. Nonetheless, our results indicate that voriconazole is a potent antifungal agent and suggest that its further clinical evaluation for patients with different forms of blastomycosis, histoplasmosis, and coccidioidomycosis is justified.

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