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Comparison of In Vitro Activities of Voriconazole and Five Established Antifungal Agents against Different Species of Dermatophytes Using a Broth Macrodilution Method

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The in vitro activities of voriconazole against 19 different species of dermatophytes were compared with those of terbinafine, itraconazole, ketoconazole, griseofulvin, and fluconazole. MICs were determined according to a National Committee for Clinical Laboratory Standards broth macrodilution method. Voriconazole appeared more active than ketoconazole, griseofulvin, and fluconazole and less active than itraconazole and terbinafine. Based on these results, voriconazole merits further investigation as a potentially useful agent for the treatment of dermatophytosis.

The dermatophytes are a group of closely related fungal species that have the capacity to invade keratinized tissue of humans and other animals and produce dermatophytosis. The organisms belong to three genera, *Trichophyton*, *Epidermophyton*, and *Microsporum* (7, 17). The treatment of these cutaneous infections is based on the use of topical and systemic antifungal agents. While topical application of an antifungal is usually sufficient to eradicate the organism and to cure the majority of these afflictions, the most severe and chronic dermathophytosis, which includes tinea capitis and tinea unguium, often requires the administration of systemic treatments. Antifungal drugs, such as the allylamines (terbinafine) and the orally active triazoles (itraconazole), have been reported to have substantial activity in these diseases and are currently used in the treatment of dermatophytosis (6, 15, 16).

Voriconazole (UK-109,496) is a novel broad-spectrum triazole antifungal agent similar in structure and spectrum of action to fluconazole and itraconazole, respectively (1). This agent has demonstrated substantial preclinical activity, in both in vitro and in vivo models against a variety of fungi, such as dimorphic fungi, yeasts, and opportunistic filamentous fungi (including dermatophytes) (2, 3, 9, 14, 18; A. Espinel-Ingroff, A. del Palacio, and M. Moore, Abstr. 38th Intersci. Conf. Antimicrob. Agents Chemother., abstr. J-7, p. 452, 1998).

The present study compares the in vitro activities of voriconazole and several of the established agents used for the treatment of dermatophytosis, including griseofulvin, itraconazole, terbinafine, ketoconazole, and fluconazole against isolates of 19 species of dermatophytes using a broth macrodilution method.

A total of 100 strains of dermatophytes were evaluated, consisting of *Trichophyton rubrum* ($n = 27$), *Trichophyton mentagrophytes* ($n = 23$), *Epidermophyton floccosum* ($n = 10$), *Trichophyton tonsurans* ($n = 7$), *Microsporum canis* and *Trichophyton gypseum* (6 each), *Trichophyton verrucosum* ($n = 4$), *Trichophyton equinum* ($n = 3$), *Microsporum nanum*, *Microsporum audouinnii*, and *Trichophyton soudanense* (2 each), and *Trichophyton terrestre*, *Trichophyton megninii*, *Trichophyton raubitschekii*, *Microsporum cookel*, *Microsporum persicolor*, *Microsporum ferrugineum*, *Trichophyton erinacei*, and *Microsporum distortum* (1 each). The identification of the different organisms was based on the macroscopic and microscopic characteristics of the strains when they were grown in culture (7, 17). Further classification was based on additional tests, including the production of red pigment when grown on potato-glucose agar, urease activity, growth in different vitamin and amino acid test agars (*Trichophyton* agars), and a hair perforation test. The isolates, maintained frozen in the Fungus Testing Laboratory, University of Texas Health Science Center (UTHSC) collection, were revived and subcultured onto potato flake agar tubes.

Voriconazole (Pfizer Pharmaceutical Group, New York, N.Y.), itraconazole (Janssen Pharmaceutica, Beerse, Belgium), terbinafine (Novartis Pharmaceuticals Inc., Basel, Switzerland), ketoconazole (Janssen Pharmaceutica), and griseofulvin (Novartis Pharmaceuticals Inc.) were provided as standard powders by the manufacturers. Fluconazole was provided as a liquid formulation (Diflucan; Pfizer Pharmaceutical Group). Voriconazole (2,000 μg/ml), itraconazole (5,000 μg/ml), and terbinafine (1,000 μg/ml) stock solutions were prepared in 100% polyethylene glycol (PEG). Fluconazole (2,000 μg/ml) and ketoconazole (1,600 μg/ml) stock solutions were prepared in sterile distilled water while griseofulvin (3,200 μg/ml) was prepared in ethyl alcohol. Final drug concentrations were 0.015 to 8 μg/ml for itraconazole, 0.03 to 16 μg/ml for ketoconazole, 0.125 to 64 μg/ml for fluconazole and voriconazole, 0.03 to 8 μg/ml for griseofulvin, and 0.004 to 2 μg/ml for terbinafine. Tenfold drug concentrations prepared in twofold serial dilutions (0.1-ml drug volume) were maintained at −70°C until needed.

Stock inocula of the molds were prepared from 7- to 14-day cultures grown on potato flake agar at 30 to 35°C. Mature colonies were covered with approximately 2 ml of sterile water,

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and suspensions were made by gently probing the colony with the tip of a sterile Pasteur pipette. The resulting suspended mixture was withdrawn and transferred to a sterile tube. Heavy particles of the suspension (when they were present) were allowed to settle for 3 to 5 min, and the upper homogeneous suspension was used for further testing. The suspensions were mixed for 15 s with a vortex mixer, and their densities were read at 530 nm and adjusted to 95% transmittance (T). The suspensions containing conidia and hyphal fragments were diluted 1:10 with RPMI 1640 medium (pH 7.0, with 0.165 M morpholinepropanesulfonic acid [MOPS]) to obtain the final desired inoculum size of approximately 0.5×10^4 to 5×10^4 CFU/ml.

MICs were determined according to a National Committee for Clinical Laboratory Standards (NCCLS) broth macrodilution method for yeasts, which was modified for mold testing (NCCLS M-27A) (4, 10). On the day of the test, the 10× drug dilutions were thawed, and then each tube was inoculated by adding 0.9 ml of the corresponding well-mixed, diluted conidial suspension (final volume of each tube was 10 ml). Growth and sterility control tubes were included for each isolate tested. The growth control contained a 0.9-ml volume of inoculum suspension and a 0.1-ml volume of drug-free medium. A sterility control was run in parallel by including a 1-ml volume of uninoculated, drug-free medium. A quality control isolate of *T. rubrum* (UTHSC 91-661) was tested each time a set of isolates was evaluated. Tubes were incubated at 35°C (H. A. Plavan, B. E. Elewski, and M. A. Ghannoum, Abstr. 37th Intersci. Conf. Antimicrob. Agents Chemother., abstr. D-143, p. 108, 1997). Growth control tubes were observed for the presence or absence of visible growth. When growth was visible, each tube was vortexed for 10 s immediately prior to being scored, which allowed the detection of a small amount of growth. The growth in each tube was compared with that of the growth control tube. Each tube was given a numerical score as follows: 0, optically clear or the absence of growth; 1, dramatic reduction in turbidity compared to that of the drug-free control tube; 2, clear reduction in turbidity as compared to that of the drug-free control tube (>80% reduction); 3, slight reduction in turbidity as compared to that of the drug-free control tube; and 4, no reduction in turbidity as compared to that of the drug-free control tube. The MICs of azoles and terbinafine were determined to have a score of 2, and the MIC of griseofulvin was given a score of 0. MIC ranges were obtained for each species-drug combination tested. Geometric mean MICs were determined to facilitate comparisons of the activities of the drugs, as well as readings of the MIC at which 50% of the isolates are inhibited (MIC_{50}) and MIC_{90}.

All isolates of dermatophytes tested produced detectable growth at time points ranging from 6 to 10 days (*M. canis*, *M. gypseum*, *M. cookei*, *T. mentagrophytes*, *T. megninii*, *T. terrestre*, *E. floccosum*) and up to 12 to 21 days (*T. rubrum*, *T. tonsurans*, *M. ferrugineum*, *T. soudanense*, *Trichophyton schoenleini*, *T. verrucosum*, *Trichophyton violaceum*, *M. persicolor*, *T. equinum*, *T. erinacei*, *M. audouinii*). The range of the inoculum size obtained at 95% T (530-nm wavelength) was 0.5×10^4 to 5×10^4 CFU/ml. The ranges of MICs for the macrodilution test of the six drugs are summarized in Tables 1 and 2. The MIC readings were taken 48 h after sufficient growth in the no-drug control tubes had occurred. The growths of the dermatophytes in those cases that PEG was used to dissolve the drug were slower compared to those in which distilled water was used, necessitating longer periods of incubation (48 to 72 h versus 10 to 14 days), with all genera being equally affected. This phenomenon made it critical that MIC tubes containing PEG were read against PEG growth control tubes. While PEG clearly affected the times of the readings, it did not affect drug activities, read as MICs in this test system.

TABLE 1. MICS of the six drugs against the three genera of dermatophytes

Genus (no. of isolates)	Antifungal agent	MIC (μg/ml)			
		Range	Geometric mean	MIC_{50}	MIC_{90}
Microsporum spp. (20)	Voriconazole	<0.125–2	0.55	0.5	2
	Itraconazole	0.03–1	0.09	0.06	0.5
	Terbinafine	<0.004–>2	0.03	0.01	0.25
	Fluconazole	2–>64	13.93	32	64
	Ketoconazole	0.5–4	1.36	1	2
	Griseofulvin	0.125–8	0.75	0.5	2
Trichophyton spp. (70)	Voriconazole	<0.125–>64	0.50	0.5	1
	Itraconazole	<0.015–>8	0.09	0.06	0.5
	Terbinafine	<0.004–>2	0.02	0.01	0.06
	Fluconazole	0.5–>64	12.39	8	64
	Ketoconazole	0.25–>16	1.30	1	8
	Griseofulvin	0.125–>8	2.06	2	8
E. floccosum (10)	Voriconazole	0.25–1	0.50	0.5	1
	Itraconazole	<0.015–0.06	0.11	0.125	0.5
	Terbinafine	0.01–0.25	0.02	0.01	0.06
	Fluconazole	0.5–4	2.46	2	4
	Ketoconazole	0.25–1	0.61	0.5	1
	Griseofulvin	0.25–1	0.47	0.5	1
Total (100)	Voriconazole	<0.125–>64	0.51	0.5	1
	Itraconazole	<0.015–>8	0.09	0.125	0.5
	Terbinafine	<0.004–>2	0.02	0.01	0.125
	Fluconazole	0.5–>16	10.71	16	64
	Ketoconazole	0.25–>16	1.21	2	8
	Griseofulvin	0.125–>8	1.43	2	8

The observed MICs of all the drugs tested showed a broad range of variability against the different species of *Microsporum* and *Trichophyton*. The calculated MICs of the controls were within an acceptable range for the six drugs tested. The genus *Epidermophyton* was the most susceptible to voriconazole, with *Microsporum* spp. and *Trichophyton* spp. being less susceptible. Differences in the susceptibilities of the various species of *Microsporum* and *Trichophyton* are depicted in the tables. *M. nanum* and *M. gypseum* were the most susceptible and *M. audouinii* was the least susceptible. In the case of *Trichophyton* spp., the most susceptible species were *T. erinacei* and *T. raubistchekii*, with *T. verrucosum*, *T. terrestre*, and *T. megninii* being the least susceptible. The comparison of the in vitro susceptibilities to voriconazole and the other agents showed that voriconazole was more active than ketoconazole, griseofulvin, and fluconazole against all species and was less active than itraconazole and terbinafine.

These results support and extend findings of previous reports which evaluated the activity of voriconazole against dermatophytes using various in vitro susceptibility test methods (broth macrodilution technique not following NCCLS methodology and broth microdilution technique using NCCLS reference method). In agreement with previous reports, we found

TABLE 2. MICs of the six drugs against the 19 different species of dermatophytes

Species (no. of isolates)	Antifungal agent	MIC (μg/ml)			
		Range	Geometric mean	MIC_{50}	MIC_{90}
M. canis (6)	Voriconazole	0.25–0.5	0.40	0.5	0.5
	Itraconazole	0.03–1	0.10	0.06	0.125
	Terbinafine	0.03–2	0.06	0.01	0.06
	Fluconazole	2–64	10.07	8	16
	Ketoconazole	0.5–4	1.122	1	1
	Griseofulvin	0.25–1	0.561	0.5	1
M. gypseum (6)	Voriconazole	0.25–2	1	1	2
	Itraconazole	0.03–0.06	0.04	0.03	0.06
	Terbinafine	<0.04–0.06	0.01	0.007	0.03
	Fluconazole	16–64	28.51	32	32
	Ketoconazole	1–2	1.78	2	2
	Griseofulvin	0.5–1	0.63	0.5	1
M. cookei (1)	Voriconazole	0.5			
	Itraconazole	0.5			
	Terbinafine	<0.04			
	Fluconazole	0.5			
	Ketoconazole	1			
	Griseofulvin	0.5			
M. persicolor (1)	Voriconazole	0.5			
	Itraconazole	0.5			
	Terbinafine	0.25			
	Fluconazole	16			
	Ketoconazole	2			
	Griseofulvin	2			
M. ferrugineum (1)	Voriconazole	0.5			
	Itraconazole	0.5			
	Terbinafine	1			
	Fluconazole	0.125			
	Ketoconazole	2			
	Griseofulvin	2			
M. distortum (1)	Voriconazole	0.5			
	Itraconazole	0.06			
	Terbinafine	0.01			
	Fluconazole	32			
	Ketoconazole	2			
	Griseofulvin	1			
M. audouinii (2)	Voriconazole	0.5–2	1		
	Itraconazole	0.25	0.25		
	Terbinafine	0.007–0.06	0.02		
	Fluconazole	64–>64	64		
	Ketoconazole	1–2	1.41		
	Griseofulvin	0.5–1	0.71		
M. nanum (2)	Voriconazole	<0.125–0.25	0.18		
	Itraconazole	0.03	0.03		
	Terbinafine	0.125–0.01	0.03		
	Fluconazole	4	4		
	Ketoconazole	0.5–1	0.71		
	Griseofulvin	4–8	5.66		
T. mentagrophytes (23)	Voriconazole	<0.125–1	0.46	0.5	1
	Itraconazole	<0.015–2	0.04	0.03	0.125
	Terbinafine	<0.004–0.125	0.02	0.01	0.06
	Fluconazole	1–>64	19.39	16	64
	Ketoconazole	0.5–16	1.55	1	4
	Griseofulvin	0.125–8	1.16	1	4
T. rubrum (27)	Voriconazole	<0.125–1	0.38	0.5	0.5
	Itraconazole	0.03–1	0.08	0.06	0.25
	Terbinafine	<0.04–0.25	0.01	0.007	0.01
	Fluconazole	2–8	3.31	4	8
	Ketoconazole	0.125–2	0.47	0.5	1
	Griseofulvin	0.5–8	1.95	2	4
T. erinacei (1)	Voriconazole	0.25			
	Itraconazole	0.25			
	Terbinafine	0.01			
	Fluconazole	64			
	Ketoconazole	16			
	Griseofulvin	2			
T. raubistchekii (1)	Voriconazole	0.25			
	Itraconazole	0.5			
	Terbinafine	0.007			
	Fluconazole	2			
	Ketoconazole	1			
	Griseofulvin	2			
T. verrucosum (4)	Voriconazole	0.5–>64	2.83	1	64
	Itraconazole	0.125–>8	0.35	0.125	8
	Terbinafine	0.06–>2	0.25	0.125	2
	Fluconazole	>64	64	64	64
	Ketoconazole	2–16	6.72	4	16
	Griseofulvin	2–8	5.66	8	8
T. terrestre (1)	Voriconazole	2			
	Itraconazole	0.5			
	Terbinafine	0.01			
	Fluconazole	64			
	Ketoconazole	2			
	Griseofulvin	>8			
T. megninii (1)	Voriconazole	2			
	Itraconazole	0.5			
	Terbinafine	0.01			
	Fluconazole	0.5			
	Ketoconazole	0.25			
	Griseofulvin	1			
T. soudanense (2)	Voriconazole	0.25–0.5	0.35		
	Itraconazole	0.06–0.125	0.09		
	Terbinafine	0.01–0.06	0.02		
	Fluconazole	64–>64	64		
	Ketoconazole	16–>16	16		
	Griseofulvin	8–>8	8		
T. tonsurans (7)	Voriconazole	0.25–1	0.67	0.5	1
	Itraconazole	0.03–1	0.15	0.06	0.5
	Terbinafine	0.007–0.25	0.04	0.01	0.125
	Fluconazole	64–>64	64	64	64
	Ketoconazole	1–8	2.97	2	8
	Griseofulvin	1–8	2.44	2	4
T. equinum (3)	Voriconazole	0.25–0.5	0.40		
	Itraconazole	0.03–0.06	0.04		
	Terbinafine	0.01–0.03	0.02		
	Fluconazole	64–>64	64		
	Ketoconazole	4–8	6.35		
	Griseofulvin	8	8		
E. floccosum (10)	Voriconazole	0.25–1	0.5	0.5	1
	Itraconazole	<0.015–0.06	0.11	0.125	0.5
	Terbinafine	0.01–0.25	0.02	0.01	0.06
	Fluconazole	0.5–4	2.46	2	4
	Ketoconazole	0.25–1	0.61	0.5	1
	Griseofulvin	0.25–1	0.47	0.5	1

that the in vitro activity of voriconazole was superior to those of ketoconazole, griseofulvin, and fluconazole. There were, however, some discrepancies in the cases of terbinafine and itraconazole. In one of the previous reports, voriconazole appeared to be less active than itraconazole, which was in agreement with our findings, whereas in another study voriconazole

showed activity greater than those of itraconazole and terbinafine (18; Espinel-Ingroff et al., 38th ICAAC). This could be attributed, at least partially, to the different methodology employed and the lack of standardized protocols. To date, there is only a proposed reference method for determining broth dilution antifungal susceptibility of filamentous fungi (11). As has been demonstrated in previous studies, variations in critical technical factors, such as inoculum size (variability in the proportion of different fungal structures, such as hyphae, macroconidia, and microconidia), type of medium, incubation temperature, and time of reading, are potential factors that may explain the different results in antifungal susceptibility testing obtained by various investigators and laboratories (5, 8, 12, 13; A. Espinol-Ingroff, Abstr. 37th Intersci. Conf. Antimicrob. Agents Chemother., abstr. J-7, p. 452, 1997; J. Martin, A. W. Fothergill, and M. G. Rinaldi, Abstr. 31st Intersci. Conf. Antimicrob. Agents Chemother., abstr. 479, p. 179, 1991). Nevertheless, and despite technical difficulties and constraints, these results demonstrate that voriconazole displays substantial activity against the majority of the dermatophytes, compares favorably with other widely used antifungal agents, and supports the clinical evaluation of voriconazole in this setting.

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