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In Vitro Studies of Activity of Voriconazole (UK-109,496), a New Triazole Antifungal Agent, against Emerging and Less-Common Mold Pathogens

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The in vitro activity of voriconazole was compared with that of itraconazole. Eighty-six isolates of pathogenic molds belonging to 23 species were tested by an agar dilution method in High Resolution medium. Voriconazole was more active than itraconazole against a number of hyaline molds, including several *Fusarium* spp. and *Scedosporium prolificans*. Voriconazole and itraconazole showed comparable good activity against several hyaline molds, including *Penicillium marneffei* and *Scedosporium apiospermum*, and a number of dematiaceous molds, including *Bipolaris australiensis*, *Cladophialophora bantiana*, several *Exophiala* spp., and several *Fonsecaea* spp. Our results suggest that voriconazole could be effective against a wide range of mold infections in humans.

Voriconazole (UK-109,496) is a new broad-spectrum triazole antifungal agent which is suitable for both oral and parenteral administration. It has been reported to have potent in vitro and in vivo activities against isolates of *Aspergillus* species, *Candida* species, and *Cryptococcus neoformans* (6–9, 16). It has also been shown to be effective and well tolerated in the treatment of immunocompromised patients with acute invasive aspergillosis (1), nonneutropenic patients with chronic invasive aspergillosis (2), and human immunodeficiency virus (HIV)-positive patients with oropharyngeal candidosis (16). To evaluate the potential usefulness of voriconazole against other infections, we compared its activity in vitro against 23 species of pathogenic molds with that of the broad-spectrum triazole compound itraconazole.

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Eight-six mold isolates (Table 1) were obtained from the United Kingdom National Collection of Pathogenic Fungi, Mycology Reference Laboratory, Public Health Laboratory Service, Bristol, and were subcultured at 28°C on slopes of Oxoid Sabouraud dextrose agar (Unipath Ltd., Basingstoke, England) with 0.5% (wt/vol) chloramphenicol until good growth was obtained. Spores were harvested in 2 ml Oxoid High Resolution (HR) medium. The suspensions were vortexed for 10 s to break up clumps of cells, and the number of spores was counted by using a modified Fuchs Rosenthal hemocytometer. The concentration of each suspension was adjusted to 10^6 conidia/ml with HR medium. In the case of five nonsporulating molds (*Cladophialophora bantiana*, *Lasiodiplodia theobromae*, *Leptosphaeria senegalensis*, *Madurella mycetomatis*, and *Ramichloridium mackenziei*), the inoculum consisted of small blocks of agar (2 by 2 mm) cut from growing cultures on Sabouraud dextrose agar.

Voriconazole was obtained from Pfizer Central Research, Sandwich, England, and itraconazole was obtained from Janssen Research Foundation, Beerse, Belgium. Voriconazole was dissolved at a concentration of 10,000 μg/ml in 1 ml of dimethyl sulfoxide. Itraconazole was dissolved at a concentration of 10,000 μg/ml in a 50:50 solution of 20% (vol/vol) acetone in water and 20% (vol/vol) hydrochloric acid (specific gravity, 1.18) in water. The drug solutions were diluted to 1,000 μg/ml with sterile water. Doubling drug dilutions from 640 to 0.3 μg/ml were then prepared in 2-ml volumes of sterile water. Volumes (18 ml) of molten, cooled HR agar were added, and the contents were mixed, poured into petri dishes, and allowed to set before drying. Three control plates were included in each test run. Two control plates contained the highest concentration of each solvent, and the third contained sterile water, in addition to the agar.

The drug-containing plates were inoculated with each spore suspension in duplicate by using a multipoint inoculator. In the case of the five nonsporulating molds, duplicate blocks of agar were placed on the surface of each drug-containing plate. The plates were incubated at 28°C, and the MICs were determined once visible growth had developed on the drug-free and solvent-containing control plates. The MIC was defined as the lowest drug concentration at which there was no visible growth on the agar. For the five nonsporulating molds, the MIC was taken as the lowest drug concentration at which there was no visible spread of growth from the agar block inoculum to the drug-containing agar.

The in vitro activities of voriconazole and itraconazole against 86 mold isolates are presented in Table 1. Voriconazole was more active in vitro than itraconazole against *Acremonium kiliense*, *Fusarium oxysporum*, *Fusarium solani*, *Lasiodiplodia theobromae*, *Scedosporium prolificans*, and *Scopulariopsis brevicaulis*. Both compounds were active against *Sporothrix schenckii* in vitro, although voriconazole appeared to be less active than itraconazole. Voriconazole was more active than itraconazole against *Paecilomyces lilacinus*, but itraconazole was more active against *Paecilomyces variotii*. Both compounds were highly active in vitro against the other species tested, including the hyaline molds *Penicillium marneffei* and *Scedosporium apiospermum* (the anamorphic form of *Pseudallescheria boydii*). Voriconazole was active against a wide range of dematiaceous molds, including *Bipolaris australiensis*, *Cladophialophora bantiana*, and several *Exophiala* and *Fonsecaea* spp. It also had useful activity against several molds that cause eumycetoma, including *Leptosphaeria senegalensis* and *Madurella mycetomatis.*

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TABLE 1. In vitro activities of voriconazole and itraconazole against 86 mold isolates

Organism (no. of isolates)	Antifungal agent	MIC range (μg/ml)
Acremonium kiliense (2)	Voriconazole	1.0
	Itraconazole	>64
Alternaria alternata (2)	Voriconazole	0.5–1.0
	Itraconazole	0.5–32
Bipolaris australiensis (3)	Voriconazole	0.25–2.0
	Itraconazole	0.06–>64
Cladophialophora bantiana (7)	Voriconazole	0.12–1.0
	Itraconazole	≤0.03–1.0
Curvularia lunata (3)	Voriconazole	0.12–0.5
	Itraconazole	≤0.03–>64
Exophiala dermatitidis (4)	Voriconazole	≤0.03–0.12
	Itraconazole	0.06–0.12
Exophiala jeanselmei (9)	Voriconazole	≤0.03–8.0
	Itraconazole	≤0.03–>64
Fonsecaea compacta (3)	Voriconazole	≤0.03–0.25
	Itraconazole	0.12–>64
Fonsecaea pedrosoi (3)	Voriconazole	≤0.03
	Itraconazole	≤0.03–0.06
Fusarium oxysporum (3)	Voriconazole	0.5–2.0
	Itraconazole	0.25–>64
Fusarium solani (4)	Voriconazole	1.0–4.0
	Itraconazole	>64
Lasiodiplodia theobromae (2)	Voriconazole	1.0
	Itraconazole	>64
Lecythophora mutabilis (2)	Voriconazole	0.5
	Itraconazole	≤0.03–0.12
Leptosphaeria senegalensis (2)	Voriconazole	≤0.03–0.06
	Itraconazole	≤0.03–1.0
Madurella mycetomatis (5)	Voriconazole	0.06–0.5
	Itraconazole	0.25–16
Paecilomyces lilacinus (2)	Voriconazole	0.5–1.0
	Itraconazole	>64
Paecilomyces variotii (2)	Voriconazole	4.0–>64
	Itraconazole	0.12–0.25
Penicillium marneffei (7)	Voriconazole	≤0.03
	Itraconazole	≤0.03
Ramichloridium mackenziei (4)	Voriconazole	0.06
	Itraconazole	0.25–1.0
Scedosporium apiospermum (6)	Voriconazole	0.12–0.5
	Itraconazole	0.12–>64
Scedosporium prolificans (5)	Voriconazole	4.0
	Itraconazole	>64
Scopulariopsis brevicaulis (2)	Voriconazole	4.0–8.0
	Itraconazole	>64
Sporothrix schenckii (4)	Voriconazole	0.5–4.0
	Itraconazole	0.06–0.25

Initial pharmacokinetic data for normal human subjects indicate that voriconazole is well absorbed after oral administration, with maximum concentrations in serum being reached within 2 h (13, 14). As with itraconazole, there is a disproportionate increase in concentrations in serum with increasing dosage, suggesting saturable first-pass metabolism in the liver. Voriconazole is well distributed throughout host fluids and tissues. It has a mean serum elimination half-life of about 6 h, which is rather shorter than that of itraconazole or fluconazole. However, steady-state concentrations in serum are reached within 5 to 7 days at dosages of 200 mg twice daily. Like itraconazole, voriconazole is metabolized by the liver and the metabolites are excreted in the bile and urine.

Although aspergillosis and mucormycosis (zygomycosis) are still the commonest forms of mold infection in immunocompromised patients, a growing number of other organisms have been reported to cause lethal infection in these individuals. Among the more important of these emerging pathogens are species of *Fusarium* and *Scedosporium*, many of which appear to be resistant to amphotericin B treatment (4, 10, 11). Our results suggest that voriconazole, like itraconazole, is a broad-spectrum antifungal agent, effective in vitro against a wide range of molds. However, some caution must be exercised in making any conclusions regarding the relative potencies of the two triazole compounds. Many of the molds studied in this investigation are uncommon causes of human infection and the number of strains available for testing are limited. The differences in MICs between the compounds might have been less evident had larger numbers of strains of some molds been tested.

Standardized methods of in vitro susceptibility testing have been developed for *Candida* spp. and *C. neoformans* (12), and there is now good evidence of a correlation of MIC with clinical outcome in patients and in animal models of infection (15, 17, 18). Although standardized methods are now being developed for the testing of molds (3, 5), clear correlations with in vivo outcome have not been established. Thus, it remains to be seen to what extent the low MICs of voriconazole seen in this and other investigations will be predictive of clinical outcome.

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