

In Vitro Evaluation of Voriconazole against Some Clinically Important Fungi

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Voriconazole was compared to amphotericin B, fluconazole, and itraconazole by using an in vitro macrobroth dilution test based upon current National Committee for Clinical Laboratory Standards tentative standards against the dimorphic fungi and several opportunistic molds and yeasts. In all instances, the voriconazole MICs were lower than those of fluconazole. In most instances, the MICs were lower than the recorded MICs of amphotericin B and itraconazole.

Voriconazole (UK-109,496) is a new triazole antifungal agent that shows promise for the treatment of mycoses caused by a broad spectrum of fungal pathogens (1, 2, 8, 9, 14, 20, 24, 26). Voriconazole is a derivative of fluconazole having the replacement of one triazole moiety by a fluoropyrimidine group and the addition of a methyl group to the propanol backbone (22, 25). This results in its excellent pharmacokinetics (11, 12, 18, 19) and selective action (10, 23) against the fungal ergosterol biosynthetic pathway.

Of special interest is the observation that voriconazole is effective both in vitro against *Aspergillus* spp. and in vivo against aspergillosis in humans and animal models (3, 4, 7, 8, 13, 16). Its fungicidal activity against *Aspergillus* spp. is significant in light of the fact that *Aspergillus* spp. are serious opportunistic pathogens in immunocompromised patients.

Study isolates (Table 1) were taken from the culture collections at the University of Texas Health Science Center at San Antonio and the University of Texas Medical Branch at Galveston. The 239 test isolates and the quality control fungi *Candida albicans* (ATCC 90028) and *Paecilomyces variotii* (ATCC 36257) were maintained on Sabouraud glucose agar (Emmons modification, pH 7) by incubation at 27 to 30°C prior to testing with a broth macrodilution procedure based on National Committee for Clinical Laboratory Standards (NCCLS) guidelines (17).

Voriconazole (Pfizer Central Research, Sandwich, United Kingdom), amphotericin B (U.S.P.C., Rockville, Md.), fluconazole (U.S.P.C.), and itraconazole (U.S.P.C.) were dissolved in the appropriate solvents (Table 2). Each was prepared at 10-fold concentrations in RPMI 1640 (American Biorganics, Inc., Niagara Falls, N.Y.), except for amphotericin B, which was diluted in Bacto antibiotic medium 3 (Difco Laboratories, Detroit, Mich.). One-tenth milliliter of the twofold serial dilution was dispensed into a sterile plastic snap-cap test tube (12 by 75 mm) that was then maintained at -70°C until needed.

The drugs in the test tubes (10-fold concentration) were additionally diluted to the final test concentrations by addition of 0.9 ml of inoculum. The inoculum was prepared from 24- to 48-h-old yeast cells, 48- to 72-h mold cells, and cells of slow-

growing molds like *Paracoccidioides brasiliensis* grown for 7 days on Sabouraud glucose agar by incubation at 25 to 30°C. Fungal cells in sterile saline were spectrophotometrically adjusted to give a concentration equal to a McFarland 0.5 turbidity standard (10³ CFU/ml for yeast cells, 10⁴ CFU/ml for mold cells). Positive and negative growth controls and purity plate counts to verify the inoculum concentration were run along with the quality control isolates for each set of tests. An assay was considered valid when the MICs of the drugs for the quality control isolates fell within the expected ranges previously established in our laboratory for *P. variotii* or by the NCCLS (17) method for *C. albicans*.

Tests were read at 24 to 48 h for most fungi. Slow-growing fungi could require up to 96 h. All tests were incubated at 35°C. The test was read when the growth control showed adequate growth. MICs of amphotericin B were the lowest concentrations at which the drug dilutions were considered optically clear. MICs of fluconazole, itraconazole, and voriconazole were the lowest concentrations at which the drug dilutions caused a prominent (80% or more) reduction in turbidity compared with that of the drug-free growth control.

TABLE 1. In vitro susceptibility to four antifungal drugs

Fungus	MIC range (μg/ml), no. of isolates tested			
	Voriconazole	Amphotericin B	Fluconazole	Itraconazole
<i>Blastomyces dermatitidis</i>	≤0.03-1, 37	≤0.03-1, 28	1-64, 30	≤0.03-1, 33
<i>Coccidioides immitis</i>	≤0.03-0.125, 38	0.125-1, 27	2-64, 29	0.125-2, 27
<i>Histoplasma capsulatum</i>	≤0.03-1, 39	≤0.03-2, 31	≤0.125-64, 29	≤0.03-8, 31
<i>Paracoccidioides brasiliensis</i>	≤0.03-2, 19	0.125-4, 14	≤0.125-64, 14	≤0.03-1, 14
<i>Penicillium mameffei</i>	≤0.03, 27	≤0.03-8, 25	1-8, 25	≤0.03-2, 25
<i>Pseudallescheria boydii</i>	0.06-1, 23	2-≥16, 23	4-64, 23	1-4, 23
<i>Sporothrix schenckii</i>	0.50-6, 32	0.025-2, 23	32-≥128, 23	0.25-4, 26
<i>Trichosporon beigelii</i> group	≤0.03-1, 24	0.125-≥16, 24	0.5-≥128, 24	0.125-2, 24

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TABLE 2. Parameters tested in this study

Drug	Solvent	Range of twofold serial drug dilutions ($\mu\text{g/ml}$)	Growth medium	Concn (CFU/ml) of:	
				Yeast inoculum	Mold inoculum
Voriconazole	DMF ^a	0.03–32	RPMI 1640	10 ³	10 ⁴
Amphotericin B	DMSO ^b	0.03–16	M-3	10 ³	10 ⁴
Fluconazole	DMF	0.125–128	RPMI 1640	10 ³	10 ⁴
Itraconazole	PEG ^c	0.03–32	RPMI 1640	10 ³	10 ⁴

^a DMF, dimethylformamide.

^b DMSO, dimethyl sulfoxide.

^c PEG, polyethylene glycol.

A major goal of standardized in vitro antifungal drug susceptibility testing is to provide correlated data for the management of mycoses. Rex et al. (21), who studied candidiasis and isolates of *Candida* spp. by using NCCLS M27-T (17) via macrodilution testing for fluconazole and itraconazole, recommended that in vitro MICs of fluconazole be interpreted as follows: MICs of $\leq 8 \mu\text{g/ml}$, susceptible; MICs of 16 to 32 $\mu\text{g/ml}$, susceptible, depending on the dose; MICs of $\geq 64 \mu\text{g/ml}$, resistant. Itraconazole was interpreted as follows: MICs of $\leq 0.125 \mu\text{g/ml}$, susceptible; MICs of 0.25 to 0.5 $\mu\text{g/ml}$, susceptible, depending on the dose; MICs of $\geq 1 \mu\text{g/ml}$, resistant. The breakpoints for itraconazole apply only to mucosal infections, whereas those for fluconazole apply to all *Candida* infections. These breakpoints are tentative and need further scrutiny by the scientific community. In our judgement, a breakpoint beyond 1 $\mu\text{g/ml}$ for amphotericin B probably indicates resistance. Unfortunately, similar breakpoints have not been developed for dimorphic fungi and other opportunistic fungi.

The MICs (Table 1) of voriconazole were substantially lower than those of fluconazole. The MICs of voriconazole for *Pseudallescheria boydii* were extremely low in comparison with those of the other three antifungal agents. For the dimorphic fungi *Blastomyces dermatitidis*, *Histoplasma capsulatum*, *P. brasiliensis*, and *Penicillium marneffei*, the MICs of voriconazole, amphotericin B, and itraconazole were similar to each other and lower than those of fluconazole. For *Coccidioides immitis*, the MIC of voriconazole was lower than those of fluconazole, amphotericin B, and itraconazole. This opportunistic pathogen causes serious mycotic infections in immunocompromised patients. It can be extremely refractile to antifungal chemotherapy (15). The low MICs of voriconazole are encouraging and indicate that this antifungal agent has potential for use against this fungus.

For the dimorphic fungi, the voriconazole MICs were lower than those of fluconazole but essentially the same as those of amphotericin B and itraconazole. Voriconazole may have a role in the prophylactic management of *P. marneffei* infections that is similar to the current use of itraconazole and ketoconazole owing to the fact that the MICs of voriconazole and itraconazole are nearly identical. It is surprising to see MICs of voriconazole that are higher than those of amphotericin B and itraconazole for *Sporothrix schenckii*. If we consider *B. dermatitidis*, *C. immitis*, *H. capsulatum*, *P. brasiliensis*, and *P. marneffei* to be more closely phylogenetically related to each other than to *S. schenckii* as an unrelated taxon, the differences seen in the MICs are not unexpected.

The *Trichosporon* species that we tested consisted of numerous type isolates. We elected to present our data under the heading of the *Trichosporon beigeli* group. Even though the number of strains of each taxon tested was limited, there is an obvious consistency among the MICs of specific antifungal agents for the species. Overall, the voriconazole MICs were

lower than those of the other antifungal agents. These data also suggest that separation of these species may not be necessary for therapy.

In conclusion, our in vitro data indicates that voriconazole is a potent antifungal agent that is active against a number of opportunistic fungal pathogens, as well as the dimorphic fungi. Initial animal model and human studies, coupled with our results and the initial evidence that azole in vitro susceptibility testing data can be correlated to clinical response, suggest that voriconazole will have clinical importance in the management of a broad spectrum of mycoses.

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REFERENCES

- Barchiesi, F., M. Restrepo, D. A. McGough, and M. G. Rinaldi. 1995. In vitro activity of a new antifungal triazole: UK-109,496, abstr. F71, p. 125. In Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
- Barry, A. L., and S. D. Brown. 1996. In vitro studies of two triazole antifungal agents (voriconazole [UK-109,496] and fluconazole) against *Candida* species. Antimicrob. Agents Chemother. 40:1948–1949.
- Denning, D., A. del Favero, E. Gluckman, D. Norfolk, M. Ruhnke, S. Yonren, P. Troke, and N. Sarantis. 1995. UK-109,496, a novel, wide-spectrum triazole derivative for the treatment of fungal infections: clinical efficacy in acute invasive aspergillosis, abstr. F80, p. 126. In Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
- Dupont, B., D. Denning, H. Lode, S. Yonren, P. Troke, and N. Sarantis. 1995. UK-109,496, a novel, wide-spectrum triazole derivative for the treatment of fungal infections: clinical efficacy in chronic invasive aspergillosis, abstr. F81, p. 127. In Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
- Espinel-Ingroff, A. 1996. Evaluation of the in vitro activity of the new triazole voriconazole against opportunistic filamentous fungi, abstr. F84, p. 114. In Abstracts of the 36th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
- Espinel-Ingroff, A., and T. Flynn. 1996. Antifungal activity of the new triazole voriconazole against established, new and emerging yeast pathogens: a comparative study, abstr. F83, p. 114. In Abstracts of the 36th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
- George, D., P. Minitier, and V. T. Andriole. 1996. Efficacy of UK-109496, a new azole antifungal agent, in an experimental model of invasive aspergillosis. Antimicrob. Agents Chemother. 40:86–91.
- Hitchcock, C. A., R. J. Andrews, B. G. H. Lewis, and P. F. Troke. 1995. UK-109,496, a novel, wide-spectrum triazole derivative for the treatment of fungal infections: antifungal activity in experimental infections with *Aspergillus*, abstr. F74, p. 125. In Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
- Hitchcock, C. A., R. J. Andrews, B. G. H. Lewis, and P. F. Troke. 1995. UK-109,496, a novel, wide-spectrum triazole derivative for the treatment of fungal infections: antifungal activity in experimental infections with *Cryptococcus*, abstr. F75, p. 126. In Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
- Hitchcock, C. A., G. W. Pye, G. P. Oliver, and P. F. Troke. 1995. UK-109,496,

- a novel, wide-spectrum triazole derivative for the treatment of fungal infections: antifungal activity and selectivity in vitro, abstr. F72, p. 125. *In Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy*. American Society for Microbiology, Washington, D.C.
11. **Jezequel, S. G., M. Clark, S. Cole, K. E. Evans, and P. Wastall.** 1995. UK-109,496, a novel, wide-spectrum triazole derivative for the treatment of fungal infections: pre-clinical pharmacokinetics, abstr. F76, p. 126. *In Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy*. American Society for Microbiology, Washington, D.C.
 12. **Jezequel, S. G., M. Clark, K. Evans, S. Roffey, P. Wastall, and R. Webster.** 1995. UK-109,496, a novel, wide-spectrum triazole derivative for the treatment of fungal infections: disposition in animals, abstr. F77, p. 126. *In Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy*. American Society for Microbiology, Washington, D.C.
 13. **Martin, M. V., J. Yeats, and C. A. Hitchcock.** 1997. Comparison of voriconazole (UK-109,496) and itraconazole in prevention and treatment of *Aspergillus fumigatus* endocarditis in guinea pigs. *Antimicrob. Agents Chemother.* **41**:13–16.
 14. **McGinnis, M. R., L. Pasarell, and C. R. Cooper, Jr.** 1995. *In vitro* susceptibility of clinical mould isolates to UK-109,496, amphotericin B, fluconazole, and itraconazole, abstr. E76, p. 99. *In Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy*. American Society for Microbiology, Washington, D.C.
 15. **McGinnis, M. R., and M. G. Rinaldi.** 1996. Antifungal drugs: mechanisms of action, drug resistance, susceptibility testing, and assays of activity in biologic fluids, p. 176–211. *In V. Lorian (ed.), Antibiotics in laboratory medicine*, 4th ed. The Williams & Wilkins, Co., Baltimore, Md.
 16. **Murphy, M., E. M. Bernard, T. Ishimaru, and D. Armstrong.** 1997. Activity of voriconazole (UK-109,496) against clinical isolates of *Aspergillus* species and its effectiveness in an experimental model of invasive pulmonary aspergillosis. *Antimicrob. Agents Chemother.* **41**:696–698.
 17. **National Committee for Clinical Laboratory Standards.** 1995. Reference method for broth dilution antifungal susceptibility testing of yeasts. Tentative standard M27-T. National Committee for Clinical Laboratory Standards, Villanova, Pa.
 18. **Patterson, B. E., and P. E. Coates.** 1995. UK-109,496, a novel, wide-spectrum triazole derivative for the treatment of fungal infections: pharmacokinetics in man, abstr. F78, p. 126. *In Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy*. American Society for Microbiology, Washington, D.C.
 19. **Patterson, B. E., S. Roffey, S. G. Jezequel, and B. Jones.** 1995. UK-109,496, a novel, wide-spectrum triazole derivative for the treatment of fungal infections: disposition in man, abstr. F79, p. 126. *In Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy*. American Society for Microbiology, Washington, D.C.
 20. **Radford, S. A., E. M. Johnson, and D. W. Warnock.** 1997. *In vitro* studies of activity of voriconazole (UK-109,496), a new triazole antifungal agent, against emerging and less-common mold pathogens. *Antimicrob. Agents Chemother.* **41**:841–843.
 21. **Rex, J. H., M. A. Pfaller, J. N. Galgiani, M. S. Bartlett, A. Espinel-Ingroff, M. A. Ghannoum, M. Lancaster, F. C. Odds, M. G. Rinaldi, T. J. Walsh, and A. L. Barry.** 1997. Development of interpretive breakpoints for antifungal susceptibility testing: conceptual framework and analysis of *in vitro-in vivo* correlation data for fluconazole, itraconazole, and *Candida* infections. *Clin. Infect. Dis.* **24**:235–247.
 22. **Richardson, K., A. S. Bell, R. P. Dickinson, S. Narayanaswami, and S. J. Ray.** 1995. UK-109,496, a novel, wide-spectrum triazole derivative for the treatment of fungal infections: synthesis and SAR, abstr. F69, p. 125. *In Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy*. American Society for Microbiology, Washington, D.C.
 23. **Sanati, H., P. H. Belanger, R. A. Fratti, and M. A. Ghannoum.** 1996. Effect of voriconazole (UK-109,496) on the sterol biosynthesis of *Candida albicans* and *Candida krusei*, abstr. C65, p. 46. *In Abstracts of the 36th Interscience Conference on Antimicrobial Agents and Chemotherapy*. American Society for Microbiology, Washington, D.C.
 24. **Sutton, D. A., A. W. Fothergill, F. J. Barchiesi, and M. G. Rinaldi.** 1996. *In vitro* activity of voriconazole against dimorphic fungi, abstr. F85, p. 114. *In Abstracts of the 36th Interscience Conference on Antimicrobial Agents and Chemotherapy*. American Society for Microbiology, Washington, D.C.
 25. **Troke, P. F., A. S. Bell, R. P. Dickinson, C. A. Hitchcock, S. Jezequel, S. Narayanaswami, S. J. Ray, and K. Richardson.** 1995. UK-109,496, a novel, wide-spectrum triazole derivative for the treatment of fungal infections: discovery and antifungal properties, abstr. F70, p. 125. *In Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy*. American Society for Microbiology, Washington, D.C.
 26. **Troke, P. F., K. W. Brammer, C. A. Hitchcock, S. Youren, and N. Sarantis.** 1995. UK-109,496, a novel, wide-spectrum triazole derivative for the treatment of fungal infections: activity in systemic candidiasis models and early clinical efficacy in oropharyngeal candidiasis (OPC), abstr. F73, p. 125. *In Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy*. American Society for Microbiology, Washington, D.C.