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 until needed. Fungal cells in sterile saline were spectrophotometrically adjusted to give a concentration equal to a McFarland 0.5 turbidity standard (10^7 CFU/ml for yeast cells, 10^6 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

<table>
<thead>
<tr>
<th>Fungus</th>
<th>MIC range (μg/ml), no. of isolates tested</th>
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<tr>
<td>Blastomyces dermatitidis</td>
<td>≤0.03–1, 37</td>
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<tr>
<td>Coccidioides immitis</td>
<td>≤0.03–0.125, 38, 0.125–1, 27</td>
</tr>
<tr>
<td>Histoplasma capsulatum</td>
<td>≤0.03–1, 39</td>
</tr>
<tr>
<td>Paracoccidioides brasiliensis</td>
<td>≤0.03–2, 19</td>
</tr>
<tr>
<td>Penicillium marneffei</td>
<td>≤0.03, 27</td>
</tr>
<tr>
<td>Pseudallescheria boydii</td>
<td>0.06–1, 23</td>
</tr>
<tr>
<td>Sporothrix schenckii</td>
<td>0.50–6, 32</td>
</tr>
<tr>
<td>Trichosporon beigelii group</td>
<td>≤0.03–1, 24</td>
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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 \( \leq 8 \) \( \mu \)g/ml, susceptible; MICs of 16 to 32 \( \mu \)g/ml, susceptible, depending on the dose; MICs of \( \geq 64 \) \( \mu \)g/ml, resistant. Itraconazole was interpreted as follows: MICs of \( \leq 0.125 \) \( \mu \)g/ml, susceptible; MICs of 0.25 to 0.5 \( \mu \)g/ml, susceptible, depending on the dose; MICs of \( \geq 1 \) \( \mu \)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 \( \mu \)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 Coccioidoides 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.

We thank Chris Hitchcock for providing voriconazole. We thank Pfizer Inc., Roerig Division, U.S. Pharmaceuticals Group, for an educational grant.

**REFERENCES**


**TABLE 2. Parameters tested in this study**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Solvent</th>
<th>Range of twofold serial drug dilutions (µg/ml)</th>
<th>Growth medium</th>
<th>Concentration (CFU/ml)</th>
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<tr>
<td>Voriconazole</td>
<td>DMF&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.03–32</td>
<td>RPMI 1640</td>
<td>10&lt;sup&gt;3&lt;/sup&gt;</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>10&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>DMSO&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.03–16</td>
<td>M-3</td>
<td>10&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>DMF</td>
<td>0.125–128</td>
<td>RPMI 1640</td>
<td>10&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Itraconazole PEG&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.03–32</td>
<td></td>
<td>RPMI 1640</td>
<td>10&lt;sup&gt;3&lt;/sup&gt;</td>
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<td>10&lt;sup&gt;4&lt;/sup&gt;</td>
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<sup>a</sup> DMF: dimethylformamide.<br>
<sup>b</sup> DMSO, dimethyl sulfoxide.<br>
<sup>c</sup> PEG, polyethylene glycol.


