In Vitro Activities of Voriconazole, Fluconazole, and Itraconazole against 566 Clinical Isolates of Cryptococcus neoformans from the United States and Africa

M. A. PFALLER,1* J. ZHANG,1 S. A. MESSER,1 M. E. BRANDT,2 R. A. HAJJEH,2 C. J. JESSUP,3 M. TUMBERLAND,4 E. K. MBIDDE,5 AND M. A. GHANNOUM1

Department of Pathology, University of Iowa College of Medicine, Iowa City, Iowa; Mycotic Diseases Branch, Centers for Disease Control and Prevention, Atlanta, Georgia; Mycology Reference Laboratory, Center for Medical Mycology, Department of Dermatology, Case Western Reserve University and University Hospitals of Cleveland, Cleveland, Ohio; University of California San Francisco, San Francisco, California; and Uganda Cancer Institute, Kampala, Uganda

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We investigated the in vitro activity of voriconazole compared to those of fluconazole and itraconazole against 566 clinical isolates of Cryptococcus neoformans from Africa (164) and the United States (402). Isolates were obtained from cerebrospinal fluid (362), blood (139), and miscellaneous sites (65). Voriconazole (MIC<sub>90</sub>, 0.12 to 0.25 μg/ml) was more active than either itraconazole (MIC<sub>90</sub> 0.5 μg/ml) or fluconazole (MIC<sub>90</sub> 8.0 to 16 μg/ml) against both African and U.S. isolates. Isolates inhibited by ≥16 μg of fluconazole per ml were almost all (99%) inhibited by ≤1 μg of voriconazole per ml. These results suggest that voriconazole may be useful in the treatment of cryptococcosis.

Among the community-acquired opportunistic fungal pathogens, perhaps the most important and certainly the single most common agent of serious infection is Cryptococcus neoformans (8). A rare disease prior to the onset of the AIDS epidemic, cryptococcosis is a leading mycological cause of morbidity and mortality among AIDS patients (8, 13). Although precise estimates of the incidence of cryptococcal disease are not available, it is thought to affect 6 to 10% of patients with AIDS in the United States and 15 to 30% in sub-Saharan Africa (13, 17). Recent data from the Centers for Disease Control and Prevention (CDC) suggested that, in metropolitan areas with a high concentration of human immunodeficiency virus-infected persons, the incidence may be as high as five cases per 100,000 population (8). C. neoformans var. neoformans is now the most common cause of meningitis in many large hospitals caring for AIDS patients (6, 8). A recent prospective study in Zimbabwe found that C. neoformans var. neoformans accounted for 45% of all laboratory-proven cases of meningitis in adults (9).

Current treatment regimens for cryptococcal meningitis have remained focused on amphotericin B, with or without flucytosine (6, 13, 17, 18, 22, 24). The toxicity of this regimen included 362 isolates from cerebrospinal fluid cultures, 139 from blood cultures and 65 isolates from miscellaneous clinical sources (pleural fluid, tissue, urine, etc.). The U.S. isolates were obtained from AIDS patients located in California, Iowa, Texas, and Georgia. Approximately 300 of these isolates were collected as part of a population-based survey of cryptococcal disease conducted by the CDC, and the epidemiologic characteristics of some of these isolates have been described previously (4, 5). The African isolates were obtained from AIDS patients with cryptococcal meningitis who were seen in a clinic in Kampala, Uganda. Identification was confirmed by standard methods (4, 10). All isolates were of the neoformans variety, as determined by growing cells on canavamine-glycine-bromthymol blue agar (10). Isolates were stored frozen at −20°C in 20% glycerol until the study was performed. Prior to testing, each isolate was subcultured at least twice on potato dextrose agar plates (Remel, Lenexa, Kans.) to ensure purity and optimal growth.

Standard powders of voriconazole and fluconazole were supplied by Pfizer Pharmaceuticals Group, Central Research Division (Groton, Conn.). Itraconazole was obtained from the Janssen Research Foundation (Beerse, Belgium). Stock solutions were prepared in water (fluconazole) or dimethyl sulfox-
ide (voriconazole and itraconazole). Antifungal agents were diluted as described in NCCLS document M27-A (14) with RPMI 1640 medium (Sigma, St. Louis, Mo.) which had been buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS) buffer (Sigma), and the mixtures were dispensed into 96-well microdilution trays. Trays containing an aliquot of 0.1 ml in each well were sealed and frozen at −70°C until they were used in the study.

Broth microdilution MICs were determined by the NCCLS method (14, 23). The final concentrations of the antifungal agents ranged from 0.007 to 8 µg/ml for voriconazole and itraconazole and 0.125 to 128 µg/ml for fluconazole. The yeast inoculum was adjusted to a concentration of 0.5 \times 10^3 to 2.5 \times 10^3 CFU/ml in RPMI 1640 medium, and an aliquot of 0.1 ml was added to each well of the microdilution tray. In each case, the inoculum size was verified by colony counting. The microdilution trays were incubated at 35°C. The MIC endpoints were read visually following 48 and 72 h of incubation and were defined for the three azoles as the lowest concentration that produced an 80% reduction in growth (prominent decrease in turbidity) compared with that of the drug-free growth control (5, 11, 14, 23). All isolates grew in the test system, and MIC results read at 48 and 72 h were in complete agreement. Thus, the 48-h MIC data is reported herein.

*C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were used as quality control organisms and were included each time.

<table>
<thead>
<tr>
<th>Fluconazole susceptibility category (µg/ml)*</th>
<th>Isolate source</th>
<th>No. of isolates tested</th>
<th>Itraconazole</th>
<th>Voriconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Range</td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
</tr>
<tr>
<td>≤8.0</td>
<td>Africa</td>
<td>154</td>
<td>0.006–0.5</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>United States</td>
<td>321</td>
<td>≤0.007–0.5</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>475</td>
<td>≤0.007–0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>16–32</td>
<td>Africa</td>
<td>10</td>
<td>0.25–1.0</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>United States</td>
<td>78</td>
<td>0.25–1.0</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>88</td>
<td>0.25–1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>≥64</td>
<td>Africa</td>
<td>164</td>
<td>0.06–1.0</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>United States</td>
<td>402</td>
<td>≤0.007–1.0</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>566</td>
<td>≤0.007–1.0</td>
<td>0.25</td>
</tr>
</tbody>
</table>

*Fluconazole MICs ranged from 0.12 to ≥128 µg/ml with a MIC<sub>90</sub> of 8.0 µg/ml (both United States and Africa) and a MIC<sub>90</sub> of 16 µg/ml (United States, 16 µg/ml; Africa, 8.0 µg/ml).*

In addition to providing comparative in vitro susceptibility data for three triazole antifungal agents against a large number of clinical isolates of *C. neoformans*, this study also provides for the first time a comparison of in vitro susceptibilities of U.S. versus African *C. neoformans* isolates. Importantly, isolates from both the United States and Africa appear to be quite susceptible to fluconazole and the other triazoles. There was no evidence of increased resistance to fluconazole among the African isolates, and over 99% of all isolates were inhibited by both U.S. (MIC<sub>90</sub>, 0.06 versus 0.5 µg/ml, respectively) and African (MIC<sub>90</sub>, 0.12 versus 0.5 µg/ml, respectively) strains. All 475 of these isolates were inhibited by ≤0.25 µg of voriconazole per ml, and 73% (71% of African isolates and 74% of U.S. isolates) were inhibited by ≤0.25 µg of itraconazole per ml.

Voriconazole (MIC<sub>90</sub>, 0.25 µg/ml) was also more active than itraconazole (MIC<sub>90</sub>, 1.0 µg/ml) against the 88 isolates inhibited by 16 to 32 µg of fluconazole per ml. All of these isolates were inhibited by ≤0.5 µg of voriconazole per ml, and 75% (80% of African isolates and 74% of U.S. isolates) were inhibited by ≤0.5 µg of itraconazole per ml.

Only three isolates, all from the United States, required ≥64 µg of fluconazole per ml to inhibit growth in vitro. For these isolates, the voriconazole MICs were 0.25, 1, and 2 µg/ml and the itraconazole MICs were 0.5, 0.5, and 1 µg/ml.

These results support and extend findings reported previously (7, 15). Like Nguyen and Yu (15), we found voriconazole to be more active than either itraconazole or fluconazole against *C. neoformans* isolates. It is notable that 82% of the isolates tested were inhibited by ≤0.12 µg of voriconazole per ml and 99.6% were inhibited by ≤0.5 µg of MIC<sub>90</sub>. By comparison, 18% were inhibited by ≤0.12 µg and 96% were inhibited by ≤0.5 µg of itraconazole per ml. Both voriconazole and itraconazole appeared most active against isolates exhibiting the greatest susceptibility to fluconazole (MIC of fluconazole, ≤8 µg/ml). As the fluconazole MICs increased, so did the MICs of voriconazole and itraconazole; however, a greater percentage of isolates inhibited by 16 to 32 µg of fluconazole per ml remained highly susceptible (MIC, ≤0.12 µg/ml) to voriconazole (65%) than to itraconazole (0%).
concentrations of fluconazole (≤32 μg/ml) that are readily achieved by standard dosing regimens (21).

In summary, we have found voriconazole to be more potent than either itraconazole or fluconazole against clinical isolates of C. neoformans from Africa and the United States. This improved potency plus favorable pharmacokinetics suggests that voriconazole may be useful in the treatment of cryptococcosis among other invasive fungal infections. Appropriate clinical trials are encouraged. Although the majority of isolates of C. neoformans in this study appear to be susceptible to fluconazole and other triazoles, continued surveillance for emerging resistance is warranted on a national and international basis given the broad utilization of fluconazole as primary prophylaxis in patients with AIDS (3, 19).

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REFERENCES