In Vitro Activities of Ravuconazole (BMS-207147) against 541 Clinical Isolates of Cryptococcus neoformans

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Cryptococcus neoformans has a worldwide distribution and is one of the most important agents of life-threatening infection among the community-acquired opportunistic fungal pathogens (4). Since the 1980s the incidence of Cryptococcus infections in some countries has increased dramatically as a result of AIDS (4, 7). In the United States, the majority of studies report a prevalence of C. neoformans infection among human immunodeficiency virus (HIV)-infected patients in the 5 to 10% range (4, 11). For the treatment of cryptococcal meningitis, fluconazole has been primarily used for maintenance therapy or prophylaxis (15). However, concerns regarding fluconazole-resistant strains of C. neoformans have been expressed by several investigators (2, 3). Itraconazole has been found to be less effective than fluconazole in the treatment of cryptococcal meningitis in HIV-infected patients (17). For these reasons, investigations of the activities of newer antifungal agents against C. neoformans is desired.

Ravuconazole (BMS-207147) is a novel triazole antifungal agent (1, 18) with a broad antifungal spectrum and potent activities against major pathogenic fungal species such as Aspergillus fumigatus, C. neoformans, Candida spp., and dermatophytes (5, 6, 8, 9). Although its activity against C. neoformans is promising, earlier investigations included limited numbers of clinical isolates, and there is a lack of comparative data with other azole agents. This study provides comparative intravertebral results for three triazole antifungal agents against a large number of clinical isolates of C. neoformans.

A total of 541 clinical isolates of C. neoformans from geographically diverse locations were selected for this study. The collection included 396 isolates from cerebrospinal fluid cultures, 116 from blood cultures, and 29 from miscellaneous clinical specimens (pleural fluid, urine, etc.). All isolates were stored as suspensions in sterile distilled water at room temperature 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 antifungal powders of ravuconazole (Bristol-Myers Squibb), fluconazole (Pfizer), and itraconazole (Janssen) were obtained from their respective manufacturers. Stock solutions were prepared in water (fluconazole) or dimethyl sulfoxide (ravuconazole and itraconazole). Antifungal agents were diluted with RPMI 1640 medium (Sigma, St. Louis, Mo.), buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS) buffer (Sigma), and dispensed into 96-well microtiter trays. Trays containing an aliquot of 0.1 ml in each well were sealed and frozen at −70°C until needed.

Broth microdilution MICs were determined by the NCCLS method (12, 17). The yeast inoculum was adjusted spectrophotometrically to a concentration of 0.5 × 10⁸ to 2.5 × 10⁹ cells/ml in RPMI 1640 medium, and an aliquot of 0.1 ml was added to each well of the microdilution tray. The final concentrations of the antifungal agents ranged from 0.007 to 8 µg/ml for ravuconazole and itraconazole and from 0.125 to 128 µg/ml for fluconazole. 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 MIC results read at 72 h are reported herein. The MIC of each triazole was defined as the lowest concentration that produced an 80% reduction in growth (prominent decrease in turbidity) compared with that of drug-free growth control (3, 5, 12, 14, 17). Candida parapsilosis ATCC 22019 and C. krusei ATCC 6258 were used as quality control organisms and were included each time that a set of isolates was tested (5, 12, 14). Since interpretive breakpoints for antifungal susceptibility testing of C. neoformans have not yet been established, for simplicity we adopted the fluconazole breakpoint values proposed by the NCCLS for Candida spp. (12, 16) to Cryptococcus. Ravuconazole and itraconazole were compared with respect to the fluconazole susceptibility category (Table 1; also see Fig. 2).

Figure 1 shows the cumulative distribution of MICs for each of the three antifungal agents. The isolates were generally susceptible to all three azole agents. Fluconazole had MICs of ≤8 µg/ml for 90.6% of the C. neoformans isolates tested, 16 to 32 µg/ml for 8.1%, and ≥64 µg/ml for 1.3% of these isolates. Overall, ravuconazole was the most active agent (MIC at which 90% of the isolates are inhibited [MIC₉₀], 0.25 µg/ml). Itra-
CONAZOLE AND FLUCONAZOLE SHOWED MIC 90 AT 0.5 AND 8 MG/ML, RESPECTIVELY (TABLE 1 AND FIG. 1). THE WILCOXON SIGNED-RANK TEST WAS USED TO COMPARE RAVUCONAZOLE AND ITRACONAZOLE MICS. THE DIFFERENCE IN MICs WAS STATISTICALLY SIGNIFICANT (P < 0.001), WITH THE RAVUCONAZOLE MIC BEING LOWER THAN THAT OF ITRACONAZOLE FOR 432 OF THE 541 ISOLATES.

FIGURE 2 SHOWS THE CUMULATIVE DISTRIBUTION OF RAVUCONAZOLE AND ITRACONAZOLE MICs FOR THE ISOLATES CATEGORIZED AS FLUCONAZOLE SUSCEPTIBLE (FLUCONAZOLE MIC, ≤8 MG/ML) AND FLUCONAZOLE SUSCEPTIBLE–DOSE-DEPENDENT (FLUCONAZOLE MIC, 16 TO 32 MG/ML). AMONG THE ISOLATES INHIBITED BY ≤8 MG OF FLUCONAZOLE/ML, RAVUCONAZOLE WAS MORE POTENT THAN ITRACONAZOLE (MIC50 AND MIC90, 0.12 AND 0.25 MG/ML VERSUS 0.25 AND 0.5 MG/ML, RESPECTIVELY). OUT OF 490 STRAINS IN THIS CATEGORY, 96.3% (472 STRAINS) WERE INHIBITED BY ≤0.25 MG OF RAVUCONAZOLE/ML, AND 72.9% (357 STRAINS) WERE INHIBITED BY THE SAME CONCENTRATION OF ITRACONAZOLE (P < 0.001).

AMONG THE ISOLATES INHIBITED BY 16 TO 32 MG OF FLUCONAZOLE/ML, RAVUCONAZOLE REMAINED SLIGHTLY MORE ACTIVE THAN ITRACONAZOLE (P < 0.001). SINCE A SIGNIFICANT SHIFT TOWARD HIGHER RAVUCONAZOLE AND ITRACONAZOLE MICs WAS OBSERVED IN THIS CATEGORY (FIG. 2), THE INDEPENDENT SAMPLE T TEST WAS USED TO COMPARE MEAN ITRACONAZOLE AND RAVUCONAZOLE MICs BETWEEN FLUCONAZOLE-SUSCEPTIBLE AND FLUCONAZOLE-SUSCEPTIBLE–DOSE-DEPENDENT ISOLATES. IN EACH CASE, THE MEAN MIC WAS LOWER FOR FLUCONAZOLE-SUSCEPTIBLE ISOLATES THAN FOR FLUCONAZOLE-SUSCEPTIBLE–DOSE-DEPENDENT ISOLATES (MEAN ITRACONAZOLE MICs, 0.28 versus 0.65 MG/ML [P < 0.01]; MEAN RAVUCONAZOLE MICs, 0.125 versus 0.45 MG/ML [P < 0.001]).

SEVEN ISOLATES REQUIRED ≥64 MG OF FLUCONAZOLE/ML TO INHIBIT GROWTH IN VITRO. FOR THESE ISOLATES, MICs RANGED FROM 0.12 TO 4.0 MG/ML FOR RAVUCONAZOLE AND 0.12 TO 2.0 MG/ML FOR ITRACONAZOLE. THREE ISOLATES WERE INHIBITED BY ≤0.5 MG OF EITHER RAVUCONAZOLE OR ITRACONAZOLE/ML.

THese results support and extend findings reported previously (6, 8). We found that ravaconazole was more active than either itraconazole or fluconazole against clinical isolates of C. neoformans. Both ravaconazole and itraconazole appeared most active against isolates exhibiting the greatest susceptibility to fluconazole (MIC of fluconazole, ≤8 MG/ML). Although other investigators have evaluated the activity of ravaconazole against fluconazole-susceptible isolates (6, 8), we also examined the activity of ravaconazole against C. neoformans isolates for which the fluconazole MICs were elevated. As the flucon-

### TABLE 1. In vitro susceptibilities of 541 clinical isolates of C. neoformans to ravaconazole and itraconazole stratified by fluconazole susceptibility category

<table>
<thead>
<tr>
<th>Fluconazole susceptibility category (μg/ml)*</th>
<th>No. of isolates tested</th>
<th>Itraconazole</th>
<th>Ravaconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MIC (μg/ml)</td>
<td>MIC (μg/ml)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Range</td>
<td>MIC50</td>
</tr>
<tr>
<td>≤8</td>
<td>490</td>
<td>≤0.007–1.0</td>
<td>0.25</td>
</tr>
<tr>
<td>16–32</td>
<td>44</td>
<td>0.12–2.0</td>
<td>0.5</td>
</tr>
<tr>
<td>≥64</td>
<td>7</td>
<td>0.12–2.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

|                                            |                        | ≤0.03–2.0     | 0.25         | 0.5          | ≤0.007–4.0   | 0.12         | 0.25         |

* Fluconazole MICs ranged from 0.12 to ≥128 μg/ml, with a MIC50 of 4.0 μg/ml and a MIC90 of 8.0 μg/ml.
azole MICs increased, stepwise increases in the MICs of both ravuconazole and itraconazole were noted. However, a greater percentage of isolates inhibited by 16 to 32 μg/mL remained susceptible (MIC, ≤0.25 μg/mL) to ravuconazole (56.8%) than to itraconazole (6.8%). Out of seven isolates for which fluconazole MICs were ≥8 μg/mL, four isolates required ≥1 μg of itraconazole/mL and ≥2 μg of ravuconazole/mL to inhibit growth in vitro. These MICs for both itraconazole and ravuconazole were considerably higher than the results from other reports (6, 8, 13) and might represent itraconazole- and ravuconazole-resistant strains of C. neoformans. Nonetheless, it is noteworthy that 3 isolates in the fluconazole-resistant category were inhibited by ≤0.5 μg of either ravuconazole or itraconazole/mL.

In vivo studies with animal models have demonstrated that ravuconazole has efficacy comparable to that of fluconazole and is more effective than itraconazole against systemic cryptococcosis (8), pulmonary cryptococcosis (9), and intracranial cryptococcosis (9). In addition, pharmacokinetic studies with humans using a 400-mg/day oral multiple dosing regimen (14 days) demonstrated peak plasma concentrations of 6.02 μg/mL, an area under the plasma concentration-time curve of 119.12 μg · h/mL, and a terminal half-life of 115 h (D. M. Grasela, S. J. Olsen, V. Mummaneni, P. Rolan, L. Christopher, J. Norton, O. H. Hadjilambris, and M. R. Marino, 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr., 2000). By comparison, a 400-mg daily dose of itraconazole capsules (200 mg twice a day for 15 days) provides a maximum drug concentration of 2.28 μg/mL, an area under the concentration-time curve of 22.56 μg · h/mL, and a terminal half-life of 64 h (10). Plasma concentrations of ravuconazole have been maintained at more than four times the MIC₉₀ for C. neoformans (0.25 μg/mL) from day 4 to day 31 following administration of 400 mg daily for 14 days (D. M. Grasela et al., 40th ICAAC).

In summary, we have demonstrated ravuconazole to be more potent than fluconazole and itraconazole against clinical isolates of C. neoformans in vitro. Although ravuconazole, like itraconazole, is highly protein bound (98%), the favorable pharmacokinetic properties and greater potency suggest that ravuconazole may be useful for the treatment of infectious diseases due to C. neoformans. Further clinical trials to confirm these promising in vitro results are warranted.

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REFERENCES


