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

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Received 9 May 2000/Returned for modification 29 May 2000/Accepted 20 July 2000

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 fungi 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 otherazole agents. This study provides comparative in vitro susceptibility data 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 × 10^3 to 2.5 × 10^3 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 itracon-
azole for 432 of the 541 isolates.

Figure 2 shows the cumulative distribution of ravuconazole 
and itraconazole MICs for the isolates categorized as flucon-
azole susceptible (fluconazole MIC, ≤8 mg/ml) and flucon-
azole susceptible-dose dependent (fluconazole MIC, 16 to 32 
mg/ml). Among the isolates inhibited by ≤8 μg of flucon-
azole/ml, ravuconazole was more potent than itraconazole 
(MIC50 and MIC90, 0.12 and 0.25 μg/ml versus 0.25 and 0.5 
μg/ml, respectively). Out of 490 strains in this category, 96.3% 
(472 strains) were inhibited by the same concentration of itraconazole 
(P<0.001).

Among the isolates inhibited by 16 to 32 μg of flucon-
azole/ml, ravuconazole remained slightly more active than itra-
conazole (P<0.001). Since a significant shift toward higher 
ravuconazole and itraconazole MICs was observed in this cat-
egory (Fig. 2), the independent sample t test was used to 
compare mean itraconazole and ravuconazole MICs between 
fluconazole-susceptible and fluconazole-susceptible–dose-de-
pendent isolates. In each case, the mean MIC was lower for 
ravuconazole-susceptible isolates than for fluconazole-suscepti-
ble–dose-dependent isolates (mean itraconazole MICs, 0.28 versus 0.65 μg/ml [P<0.01]; mean ravuconazole MICs, 0.125 versus 0.45 μg/ml [P<0.001]).

Seven isolates required ≥64 μg of fluconazole/ml to inhibit 
growth in vitro. For these isolates, MICs ranged from 0.12 to 
4.0 μg/ml for ravuconazole and 0.12 to 2.0 μg/ml for itracon-
azole. Three isolates were inhibited by ≤0.5 μg of either ra-
vuconazole or itraconazole/ml.

These results support and extend findings reported previ-
ously (6, 8). We found that ravuconazole was more active than 
either itraconazole or fluconazole against clinical isolates of C. neoformans. Both ravuconazole and itraconazole appeared 
most active against isolates exhibiting the greatest susceptibil-
ity to fluconazole (MIC of fluconazole, ≤8 μg/ml). Although 
other investigators have evaluated the activity of ravuconazole 
against fluconazole-susceptible isolates (6, 8), we also exam-
inied the activity of ravuconazole 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 ravuconazole 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>Ravuconazole</th>
<th>IMC (μg/ml)</th>
<th>IMC50</th>
<th>IMC90</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤8</td>
<td>490</td>
<td>≤0.007–1.0</td>
<td>0.25</td>
<td>≤0.007–1.0</td>
<td>0.12</td>
<td>0.25</td>
</tr>
<tr>
<td>16–32</td>
<td>44</td>
<td>0.12–2.0</td>
<td>0.5</td>
<td>0.03–4.0</td>
<td>0.25</td>
<td>1.0</td>
</tr>
<tr>
<td>≥64</td>
<td>7</td>
<td>≤0.03–2.0</td>
<td>0.25</td>
<td>≤0.007–4.0</td>
<td>0.12</td>
<td>0.25</td>
</tr>
</tbody>
</table>

*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.

![FIG. 1. Cumulative distribution of MICs for three azole agents against clinical isolates of Cryptococcus neoformans. Shown are the MICs of ravuconazole (■), itraconazole (●), and fluconazole (∗).](image-url)
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 of fluconazole/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 16 to 32 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 these promising in vitro results are warranted.

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.

We thank Daniel Diekema for help in statistical analysis. Toshiaki Yamazumi is partly supported by a grant from the Japan Clinical Pathology Foundation for International Exchanges. This study was supported by a grant from Bristol-Myers Squibb Company.

REFERENCES

FIG. 2. Comparison of ravuconazole and itraconazole MIC distributions according to different categories of fluconazole susceptibility. Shown are the MICs of ravuconazole (●) and itraconazole (○). Solid lines indicate the MICs against the isolates for which fluconazole MICs are ≤8 μg/ml. Broken lines indicate the MICs against the isolates for which fluconazole MICs are 16 to 32 μg/ml.


