MICs and minimum fungicidal concentrations of posaconazole, voriconazole and fluconazole for Cryptococcus neoformans and Cryptococcus gattii

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Sir,

The major aetiological species of cryptococcosis is Cryptococcus neoformans, which is distributed especially in association with pigeon droppings, and the most common infection is in the CNS of immunocompromised patients. Cryptococcus gattii, previously considered a biovariety of C. neoformans, is the second agent of cryptococcosis; four basic serotypes have been described: A and D for C. neoformans and B and C for C. gattii. Although its geographical distribution is restricted, C. gattii is being reported in new areas and has produced epidemic outbreaks in humans and animals. Unlike C. neoformans, C. gattii can infect immunocompetent subjects.

The majority of the isolates from both species are susceptible to azoles in vitro, although most reports do not discriminate between Cryptococcus species and serotypes. The main goal of this study was to determine the MICs and minimum fungicidal concentrations (MFCs) of the new antifungal drug posaconazole in comparison with those of voriconazole and fluconazole for C. neoformans and C. gattii isolates from various sources.

A total of 80 isolates of Cryptococcus from the collection of the Research Unit on Infectious Diseases and Mycology (Barcelona, Spain) were studied. Seventy-five were isolated from the CSF of patients infected with HIV, and five isolates were cultured from environmental samples. Fifty strains were C. neoformans: 25 serotype A (variety grubii) and 25 serotype D (variety neoformans). The remaining 30 isolates were C. gattii strains: 25 serotype B and 5 serotype C. Candida parapsilosis ATCC 22019 and Candida krusei ATCC 6258 were used for quality control.

Posaconazole was provided by Schering-Plough (Kenilworth, NJ, USA), and voriconazole and fluconazole were provided by Pfizer Pharmaceuticals (Gorton, CT, USA).

Stock solutions of azoles and microplates were prepared and processed, as described in CLSI (formerly NCCLS) document M27-A2. Yeast inocula were diluted to a final concentration of 0.5–2.5 × 10^6 cfu/mL.

From optically clear wells, 10 µL was withdrawn and plated on Sabouraud dextrose agar for the determination of MFC. Plates were incubated at 35°C for 72 h. MFC was defined as the lowest drug concentration that yielded less than three colonies, a killing activity of ~99%.

For statistical analysis, the Wilcoxon rank-sum test was used using SPSS 14.0 for Windows (SPSS Inc., Chicago, IL, USA), with significance being set at P < 0.05.

Results of the study confirmed that MICs for the two quality control Candida isolates were within the limits described in the M27-A2 document. Table 1 shows MIC50 and MFC50 values, geometric means and ranges of the three azoles.

Statistical analysis revealed no significant differences between MICs of posaconazole and voriconazole. However, highly significant differences were found between fluconazole MICs and those of the other two azoles (P < 0.001). The MICs for the two Cryptococcus species were compared; they were found to be significantly higher for C. gattii than for C. neoformans (P = 0.007), especially the MICs of fluconazole. The MFCs of fluconazole and voriconazole were higher for C. gattii serotype B. In contrast, the MFCs of posaconazole were lower, only 2 mg/L for one C. neoformans serotype A isolate and one C. gattii serotype B isolate. The MFCs of voriconazole were higher: ≥1 mg/L for 4 C. neoformans isolates and ≥2 mg/L for 11 C. gattii serotype B isolates (44%). The highest MFCs were 2 mg/L for posaconazole, 4 mg/L for voriconazole and 16 mg/L for fluconazole.

Several cases of C. neoformans isolates exhibiting marked reduction in susceptibility to fluconazole have been reported in AIDS patients. Diverse authors attributed the increase in resistance to the widespread use of maintenance therapy with fluconazole. Other studies have reported the opposite trend. The antifungal susceptibility of 70 Spanish C. neoformans clinical isolates did not change significantly between 1994–96 and 1997–2005. The fluconazole MIC50 values remained stable, and the authors concluded that the in vitro resistance to fluconazole decreased over the 11 years. Pfaffer et al. examined a large series of strains from 100 medical institutions and reported an accumulative percentage of 99% for isolates inhibited by voriconazole or posaconazole: 99% of the isolates being susceptible at MIC ≤1 mg/L. Although the authors did not discriminate between C. neoformans and C. gattii, presumably the majority of isolates were C. neoformans.

We have found a very low level of resistance of Cryptococcus species to azoles. The highest MICs were obtained for C. gattii serotype B; this species appeared to be less susceptible to the azoles than both serotypes (A and D) of C. neoformans (P = 0.007 for MIC and P = 0.020 for MFC). MFCs could be better predictors of clinical response to antifungal therapy; however, standard methods have not been developed. Most investigators follow the proposals of Espinel-Ingroff et al.

Greater differences in MFCs than MICs were seen; 2 isolates of C. gattii had MFCs of fluconazole ≥16 mg/L and 13 isolates had MFCs of voriconazole ≥2 mg/L.
The possibility of change for in vitro susceptibility of Cryptococcus spp. in the future justifies the need for further systematic studies using standardized techniques. This is particularly valid for azoles, as their period of clinical and therapeutic use is still very short.

Acknowledgements

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Transparency declarations

None to declare.

References


Table 1. Susceptibilities (mg/L) of 80 Cryptococcus isolates belonging to four serotypes to fluconazole (FLC), voriconazole (VRC) and posaconazole (POS)

<table>
<thead>
<tr>
<th>Antifungal agent</th>
<th>Serotype</th>
<th>MIC range</th>
<th>MFC range</th>
<th>MIC50</th>
<th>MFC50</th>
<th>GM MIC</th>
<th>GM MFC</th>
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<tbody>
<tr>
<td>FLC</td>
<td>A</td>
<td>&lt;0.125–1.0</td>
<td>&lt;0.125–2</td>
<td>0.5</td>
<td>0.5</td>
<td>0.4</td>
<td>0.5</td>
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<td></td>
<td>D</td>
<td>&lt;0.125–1</td>
<td>&lt;0.125–1</td>
<td>0.5</td>
<td>1.0</td>
<td>0.4</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.25–2</td>
<td>0.25–16</td>
<td>1</td>
<td>2.0</td>
<td>1</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.5–1.0</td>
<td>1–2</td>
<td>0.5</td>
<td>2.0</td>
<td>0.7</td>
<td>1.5</td>
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<tr>
<td>VRC</td>
<td>A</td>
<td>&lt;0.03–0.25</td>
<td>&lt;0.03–2</td>
<td>0.12</td>
<td>0.12</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>0.06–0.25</td>
<td>0.06–&gt;1</td>
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<td>0.5</td>
<td>0.1</td>
<td>0.1</td>
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<td></td>
<td>B</td>
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<td>1.0</td>
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<tr>
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<td>C</td>
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<td>0.12–0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.1</td>
<td>0.2</td>
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<tr>
<td>POS</td>
<td>A</td>
<td>&lt;0.03–0.25</td>
<td>&lt;0.03–2</td>
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<td>0.25</td>
<td>0.1</td>
<td>0.1</td>
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<tr>
<td></td>
<td>D</td>
<td>&lt;0.03–0.25</td>
<td>&lt;0.03–0.5</td>
<td>0.12</td>
<td>0.12</td>
<td>0.1</td>
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<tr>
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<tr>
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<td>0.25</td>
<td>0.25</td>
<td>0.2</td>
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</table>

GM, geometric mean. Isolates tested were 25 C. neoformans serotype A (variety grubii), 25 C. neoformans serotype D (variety neoformans), 25 C. gattii serotype B and 5 C. gattii serotype C.

In vitro activity of ceftobiprole against clinical isolates of Pseudomonas aeruginosa obtained from Canadian intensive care unit (ICU) patients as part of the CAN-ICU Study

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Sir,

In recent years, Pseudomonas aeruginosa isolates resistant to multiple classes of antimicrobial agents have become