In vitro activity of amphotericin B, itraconazole, voriconazole, posaconazole, caspofungin and terbinafine against Scytalidium dimidiatum and Scytalidium hyalinum clinical isolates

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Objectives: The objective of this work was to evaluate the in vitro activity of amphotericin B, itraconazole, voriconazole, posaconazole, caspofungin and terbinafine against clinical isolates of Scytalidium dimidiatum and Scytalidium hyalinum.

Methods: Microdilution testing was performed in accordance with the CLSI M38-A method on 17 S. dimidiatum and 15 S. hyalinum clinical isolates.

Results: The MIC ranges of voriconazole, posaconazole, itraconazole, terbinafine, caspofungin and amphotericin B were <0.03 to 0.5 mg/L, 0.06–2 mg/L, <0.03 to >16 mg/L, 0.06–2 mg/L, 0.06–8 mg/L and 0.06–1 mg/L, respectively.

Conclusions: Amphotericin B and voriconazole exhibit the lowest MICs for Scytalidium spp. Voriconazole could be a promising agent for the treatment of these refractory infections.

Keywords: scytalidiosis, dermatomycosis, onychomycosis, antifungal agents, triazoles

Introduction

Scytalidium dimidiatum, a synanamorph of Natrassia mangiferae, and Scytalidium hyalinum are two non-dermatophyte moulds responsible for dermatomycosis with clinical manifestations similar to those caused by Trichophyton rubrum. They are rarely, and mainly in immunosuppressed patients, responsible for deep infections. Scytalidioses are endemic in tropical and subtropical areas and represent 35% to 45% of dermatomycosis of feet in these countries.1 However, many cases are now reported from temperate countries among immigrants from these tropical areas.2 At present, there is no orally or topically effective treatment for skin and nail infections due to Scytalidium,3 and amphotericin B, used to treat deep infections, cannot be easily used in dermatology practice with only a lotion formulation. Recently, the new triazole agents voriconazole and posaconazole were shown to have clinical activity in phaeohyphomycete infections.3,4 It suggests that they could be active against S. dimidiatum, a black fungus, and S. hyalinum, which is considered as a closely related mutant of S. dimidiatum.3 As no study has yet evaluated the in vitro susceptibility of Scytalidium, we chose to test antifungal drugs available in dermatology practice along with the newer drugs, voriconazole, posaconazole and caspofungin.

The objective of the present study was to describe the in vitro activities of voriconazole, posaconazole, caspofungin and terbinafine as compared with those of amphotericin B and itraconazole against 32 isolates of Scytalidium [17 S. dimidiatum (Sd) and 15 S. hyalinum (Sh)] from our institution.

Materials and methods

Isolates were recovered from toenail (10 S. dimidiatum, 6 S. hyalinum), fingernail (2 S. dimidiatum, 1 S. hyalinum) and sole or toeweb (5 S. dimidiatum, 8 S. hyalinum) cultures on Sabouraud agar slants supplemented with antibiotics (SA) (Bio-Rad, Marnes la Coquette, France). Species were identified according to usual macroscopic and microscopic examination criteria. Isolates were subcultured on SA agar slants at 27°C for 14 days before testing. Caspofungin (Merck Research Laboratories, Rahway, NJ, USA), terbinafine (Chemolberica, Madrid, Spain), voriconazole (Pfizer Pharmaceutical Group, New York, NY, USA), posaconazole (Schering-Plough, Kenilworth, NJ, USA), itraconazole and

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amphotericin B (Sigma, France) were dissolved in dimethyl sulphoxide (Sigma, France) and diluted in RPMI 1640 medium buffered with MOPS (Sigma, France), pH 7.0, to a final concentration range of 16–0.03 mg/L. Microdilution testing was performed using the CLSI M38-A microdilution method. Inocula were prepared by scraping colonies with a loop in sterile 0.85% saline and vigorously vortexed. Fungal suspensions were settled for ~5 min. The upper concentration was adjusted with a haemocytometer to a final concentration of 5 × 10³–5 × 10⁴ cfu/mL. Inoculum concentration was retrospectively controlled by subculture on SA. A 0.1 mL fungal inoculum was added to each well, except negative controls and the 96-well round-bottomed microtitre plates were incubated at 35°C. The MIC endpoints were read at 96 h and were defined as the lowest concentration to inhibit 100% of fungal growth compared with the growth control for amphotericin B and to inhibit 80% for azoles, terbinafine and caspofungin. MIC₅₀ and MIC₉₀ are reported as MICs at concentration to inhibit 50% or 90% of isolates are inhibited. Quality control was ensured by testing the Candida krusei ATCC 6528 strain.

**Results and discussion**

Table 1 presents the *in vitro* activities of each antifungal drug against *Scytalidium* spp. with MIC ranges, MIC₅₀ and MIC₉₀. Itraconazole had high MICs for the majority of *S. dimidiatum* isolates (MIC₉₀ > 16 mg/L), but MIC values were lower for *S. hyalinum* (MIC₉₀ = 1 mg/L). For amphotericin B, the MIC₉₀ was 0.5 mg/L for *S. dimidiatum* and *S. hyalinum*. Posaconazole and terbinafine presented similar *in vitro* activity against *S. hyalinum* (MIC₉₀ = 1 mg/L). However, the MIC of posaconazole was higher than that of terbinafine for *S. dimidiatum* (MIC₉₀ = 2 mg/L and MIC₉₀ = 0.5 mg/L, respectively). Although a trailing effect was observed with caspofungin, MIC determination was not problematic with this echinocandin. Caspofungin appeared less active against *S. dimidiatum* (MIC₉₀ = 8 mg/L) than against *S. hyalinum* isolates (MIC₉₀ = 1 mg/L). The lowest MICs were observed with voriconazole, with MIC₉₀ of 0.25 mg/L for *S. dimidiatum* and 0.125 mg/L for *S. hyalinum*. The results of this study show that voriconazole exhibits the lowest MICs against *Scytalidium* compared with amphotericin B, the most active drug until now. The MICs of itraconazole and amphotericin B are consistent with other *in vitro* data reported by different investigators or case reports. Antifungal activities of posaconazole, caspofungin and terbinafine vary with isolates, some of them with low MIC values and others with high MIC values. Although the two species of *Scytalidium* are equally resistant to antifungals in clinical practice, *in vitro*, *S. dimidiatum* seems more resistant when compared with *S. hyalinum*. The capacity of *S. dimidiatum* to produce melanin-like pigments unlike *S. hyalinum* could explain this difference since melanization has been shown to play a role in resistance to antifungal drugs in other fungi. The clinical relevance of these *in vitro* results may be subject to debate since interpretation of mould MICs is known to be problematic. In several case reports, a poor correlation was found between *in vitro* and *in vivo* results. A poor clinical response can be observed despite MIC values within the range of achievable levels at the site of infection, as for terbinafine, known to be ineffective for treating superficial scytalidiosis. However, treatment with intravenous amphotericin B has been beneficial in deep scytalidiosis and topical amphotericin B has been used somewhat successfully to treat *Scytalidium* onychomycosis (C. Lacroix, G. Kac and M. Feuilhade de Chauvin, unpublished data). In two recent case reports with invasive *S. dimidiatum* infections, isolates were tested *in vitro* with voriconazole and showed MICs of 0.012 and 1 mg/L. Dunn et al. and Willinger et al. reported two cases of invasive *S. dimidiatum* infection in immunocompromised patients treated with voriconazole with stabilization of the disease or eradication of the mould. These reports as well as our *in vitro* data suggest that voriconazole could be a therapeutic option or an alternative to amphotericin B in the treatment of deep *Scytalidium* infections. Furthermore, as voriconazole can be orally administered unlike amphotericin B, it could be easily used to treat *Scytalidium* superficial infections.

**Table 1. In vitro activities of amphotericin B, itraconazole, voriconazole, posaconazole, terbinafine and caspofungin against *S. dimidiatum* and *S. hyalinum* isolates**

<table>
<thead>
<tr>
<th>Species (no. of isolates tested)</th>
<th>Antifungal agent</th>
<th>MIC (mg/L)</th>
<th>range</th>
<th>50%&lt;sup&gt;a&lt;/sup&gt;</th>
<th>90%&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. dimidiatum</em> (17)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>amphotericin B</td>
<td>0.06–1</td>
<td>0.5</td>
<td>0.5</td>
<td></td>
<td></td>
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<tr>
<td>itraconazole</td>
<td>&lt;0.03 to &gt;16</td>
<td>4</td>
<td>&gt;16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>voriconazole</td>
<td>0.125–2</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>posaconazole</td>
<td>0.125–0.5</td>
<td>0.25</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>terbinafine</td>
<td>0.06–8</td>
<td>0.5</td>
<td>8</td>
<td></td>
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<tr>
<td>caspofungin</td>
<td></td>
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<tr>
<td><em>S. hyalinum</em> (15)</td>
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<tr>
<td>amphotericin B</td>
<td>0.06–1</td>
<td>0.25</td>
<td>0.5</td>
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</tr>
<tr>
<td>itraconazole</td>
<td>0.125–4</td>
<td>1</td>
<td>1</td>
<td></td>
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</tr>
<tr>
<td>voriconazole</td>
<td>&lt;0.03 to 0.25</td>
<td>0.06</td>
<td>0.125</td>
<td></td>
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<tr>
<td>posaconazole</td>
<td>0.06–1</td>
<td>0.5</td>
<td>1</td>
<td></td>
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</tr>
<tr>
<td>terbinafine</td>
<td>0.06–2</td>
<td>0.5</td>
<td>1</td>
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</tr>
<tr>
<td>caspofungin</td>
<td>0.125–8</td>
<td>0.5</td>
<td>1</td>
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</tbody>
</table>

*MIC at which at least 50% of isolates are inhibited.

*bMIC at which at least 90% of isolates are inhibited.
In conclusion, voriconazole represents a promising agent for the treatment of Scytalidium infections. The clinical value of these in vitro results should be determined by animal models and clinical trials.

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Transparency declarations

None to declare.

References


In vitro susceptibility of Scytalidium spp.