In Vitro Susceptibility of Madurella mycetomatis to Posaconazole and Terbinafine

Alex van Belkum, Ahmed H. Fahal, and Wendy W. J. van de Sande

Erasmus MC, University Medical Center Rotterdam, Department of Medical Microbiology and Infectious Diseases, s-Gravendijkwal 230, 3015 GD Rotterdam, Netherlands, and Mycetoma Research Centre, University of Khartoum, Khartoum, Sudan

Presently, therapy of eumycetoma in Sudan is still based on surgery combined with prolonged ketoconazole therapy. This usually results in a poor clinical outcome. To determine if posaconazole and terbinafine could offer better therapeutic alternatives, the in vitro susceptibilities of 34 Madurella mycetomatis strains were determined. It appeared that posaconazole was highly active against M. mycetomatis but terbinafine was only moderately active. Since posaconazole has an excellent safety profile, it might provide an important alternative in mycetoma therapy.

The fungus Madurella mycetomatis is one of the most common causative agents of black-grain eumycetoma. For this chronic infectious and inflammatory disease, characterized by a large subcutaneous mass and the excretion of fungal grains, no adequate therapy is available. To date, eumycetoma therapy in Sudan still consists of surgery combined with prolonged antifungal therapy with ketoconazole. Unfortunately, for 25% of the patients treated in dedicated mycetoma clinics, a lack of successful therapy still leads to amputation of the infected limb (A. Fahal, personal communication). More-effective antifungal follow-up after surgery might improve the clinical outcome. In order to find better alternatives to ketoconazole in the therapy of eumycetoma, the activities of additional antifungal agents toward mycetoma need to be determined. For M. mycetomatis, antifungal susceptibilities have been determined toward amphotericin B, various azoles, 5-flucytosine, and the echinocandins (2, 13–14). From these studies it appeared that M. mycetomatis seemed to be most susceptible to the azoles; no activity was seen with 5-flucytosine or the echinocandins (2, 13–14). Apparently, inhibition of ergosterol synthesis in M. mycetomatis offers the best way to inhibit M. mycetomatis growth. Next to the azoles, the allylamine terbinafine is able to inhibit ergosterol synthesis in fungi but at an earlier step in the ergosterol synthesis pathway than the azoles do. The allylamines inhibit the conversion from squalene to squalene epoxide, while the azoles inhibit the conversion from lanosterol into 4,4-di-methylcholesta-8,14,24-trienol (10). Most filamentous fungi are highly susceptible to terbinafine, but for M. mycetomatis, no in vitro susceptibility data are yet available (5). Also, for a new azole (posaconazole), no in vitro susceptibility data for M. mycetomatis are available. Therefore, we set out to determine the in vitro susceptibilities of 34 clinical isolates of M. mycetomatis (32 strains were isolated from 31 patients in 1999 and 2000 in the Mycetoma Research Centre, University of Khartoum, Sudan, 2 strains were obtained from 2 different patients in 2001 and 2002, originating from Mali, and all strains were obtained before the start of antifungal therapy) for ketoconazole (Janssen Pharmaceutical products, Belgium), itraconazole (Janssen Pharmaceutical products, Belgium), posaconazole (Schering-Plough, Kenilworth, NJ), and terbinafine (Novartis Pharma AG, Basel, Switzerland). The strains were isolated from biopsy specimens and maintained in culture on Sabouraud dextrose agar (Difco Laboratories, Paris, France). The strains were previously identified on the basis of morphology and PCR-restriction fragment length polymorphism (RFLP) and internal transcribed spacer (ITS) sequencing (1, 12). MICs were determined independently in triplicate using the previously reported 2,3-bis(2-methoxy-4-nitro-5-sulfo-phenyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide (XTT) assay (2). Briefly, in this assay a fungal inoculum was prepared from cultures grown in RPMI 1640 with l-glutamine (0.3 g/liter) and 20 mM morpholinepropanesulfonic acid, and mycelia were harvested by 5 min of centrifugation at 2,158 × g and washed once with sterile saline. After sonication (20 s at 28-μm maximum power; Soniprep, Beun de Ronde, Netherlands. Phone: 31-10-4631975. Fax: 31-10-4633875. E-mail: w.vandesande@erasmusmc.nl.

* Corresponding author. Mailing address: Erasmus MC, University Medical Center Rotterdam, Department of Medical Microbiology and Infectious Diseases, s-Gravendijkwal 230, 3015 GD Rotterdam, Netherlands. Phone: 31-10-4631975. Fax: 31-10-4633875. E-mail: w.vandesande@erasmusmc.nl.

† Published ahead of print on 24 January 2011.
Netherlands), the transmissions were adjusted to 70% at 660 nm (Novaspec II; Pharmacia Biotech). After 7 days of incubation at 37°C, XTT was administered and MICs were determined. The MIC endpoints for each antifungal agent were defined as the first concentration at which spectrophotometrically the growth of all strains was strongly inhibited by ketoconazole, the drug of choice for treatment of eumycetoma 

<table>
<thead>
<tr>
<th>Antifungal agent</th>
<th>MIC (µg/ml)</th>
<th>50%</th>
<th>90%</th>
</tr>
</thead>
<tbody>
<tr>
<td>KTZ</td>
<td>0.03-1</td>
<td>0.06</td>
<td>0.25</td>
</tr>
<tr>
<td>ITZ</td>
<td>0.03-0.5</td>
<td>0.03</td>
<td>0.25</td>
</tr>
<tr>
<td>PCZ</td>
<td>0.03-0.125</td>
<td>≤0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>TBF</td>
<td>1–16</td>
<td>8</td>
<td>16</td>
</tr>
</tbody>
</table>

* KTZ, ketoconazole; ITZ, itraconazole; PCZ, posaconazole; TBF, terbinafine.

In concordance with previously published MICs for *M. mycetomatis*, growth of all strains was strongly inhibited by ketoconazole and itraconazole were evenly distributed, with concentrations ranging from <0.03 µg/ml to 1 µg/ml for ketoconazole and from <0.03 µg/ml to 0.5 µg/ml for itraconazole (Fig. 1). A concentration of 0.25 µg/ml was needed to inhibit the growth of 90% of the isolates for both azoles (Table 1). Significantly lower MICs were obtained for posaconazole (Mann-Whitney, \( P < 0.001 \)) (Fig. 1); only 0.06 µg/ml was needed to inhibit 90% of the isolates (Table 1). The MICs reported here for posaconazole are comparable to those found for other black-grain mycetoma agents, such as *Exophiala jeaneselmei* (3). Interestingly, white-grain mycetoma agents had slightly higher MICs for posaconazole. For *Pseudallescheria boydii*, 1 µg/ml was needed to inhibit 90% of the isolates (4). In contrast to the azoles, high concentrations of terbinafine were needed to inhibit *M. mycetomatis* growth (Fig. 1). A concentration of 16 µg/ml was needed to inhibit 90% of the isolates (Table 1). These susceptibilities are comparable to those reported for other mycetoma causative agents, including *P. boydii* and *Madurella pseudomykometalis* (4, 15).

Although this study is the first to describe the in vitro susceptibility of *M. mycetomatis* to posaconazole and terbinafine, the efficacy and safety of both posaconazole and terbinafine in the treatment of eumycetoma have been studied before (8–9). Twenty eumycetoma patients (10 patients infected with *M. mycetomatis*, 3 with *Leptosphaeria senegalensis*, and 7 with un-known causative agents) were treated with terbinafine in an open-label study. Clinical improvement was seen in 16 patients, but in at least 12 patients the fungus could still be cultured from the lesion, and in 7 patients grains were still present (8).

Since in neither of the clinical studies were the in vitro susceptibilities determined, it remains impossible to determine if the clinical outcome was related to the initial antifungal susceptibility of the isolate. We have shown that all our clinical isolates had low MICs for posaconazole and that none of the isolates had an MIC above 0.5 µg/ml. All MICs were below the maximum concentration of drug in serum (\( C_{\text{max}} \)) of 2.6 µg/ml of posaconazole as measured in serum of healthy volunteers (11). The posaconazole concentrations in plasma vary more in patients suffering from fungal infections (range, 0.3 to 4.3 µg/ml); most of the MICs found for *M. mycetomatis* are still below the concentrations measured in these patients (7). *M. mycetomatis* was less susceptible to terbinafine; for 32 out of 34 isolates, MICs were higher than the 1.7-µg/ml terbinafine concentration which can be reached in serum (6). Indeed, in vitro antifungal susceptibility data are only a small part of all relevant factors related to the success or failure of therapy in mycetoma. Mycetoma patients tend to seek medical attention only in the advanced stages of disease, when they present large subcutaneous areas of infection with dense fibrous tissue containing cavities full of exudates and fungal grains and sometimes even with already-severe bone involvement. Consequently, it is easy to understand that besides antifungal susceptibility data, host factors, clinical presentation, and the pharmacokinetic-pharmacodynamic (PK-PD) parameters of the antifungal drugs used are all factors that should be considered before defining the best therapeutic approach for each patient.

In conclusion, *M. mycetomatis* has in vitro low MICs for posaconazole and high MICs for terbinafine. A clinical study with posaconazole is needed to determine if this drug can be successfully used in the treatment of black-grain mycetoma caused by *M. mycetomatis*.

**REFERENCES**

10. Onyewu, C., J. R. Blankenship, M. Del Poeta, and J. Heitman. 2003. Ergosterol biosynthesis inhibitors become fungicidal when combined with cal-

**TABLE 1. Susceptibilities of *M. mycetomatis* to ketoconazole, itraconazole, posaconazole, and terbinafine*"


