In Vitro Interaction of Currently Used Azoles with Terbinafine against Madurella mycetomatis

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Eumycetoma is a chronic fungal infection of the subcutaneous tissues predominantly caused by Madurella mycetomatis. Treatment usually involves a combination of surgery and prolonged antifungal therapy with either ketoconazole or itraconazole (1). However, clinical outcomes after treatment are often disappointing, amputation is common, and extensive continuation of antifungal therapy is needed.

In vitro, M. mycetomatis is known to be more susceptible to azoles than to terbinafine (2, 3). To our knowledge, there are no reports on the in vitro interaction of these antifungal agents in treatment of eumycetoma. In this study, the in vitro activities of ketoconazole and itraconazole in combination with terbinafine against M. mycetomatis isolates (n = 8) were investigated. All experiments were performed in duplicates as described previously (2). To evaluate the in vitro activity of individual antifungal agents and combinations, a checkerboard microdilution assay was used. Ketoconazole and itraconazole were used in concentrations ranging from 0.001 to 1 μg/ml, while terbinafine concentrations ranged from 0.25 to 16 μg/ml.

Drug interactions were analyzed using a nonparametric approach based on the fractional inhibitory concentration (FIC) (4) and the interaction ratio (IR) (5, 6). The FIC values were calculated as follows:

\[
FIC = \frac{C_A}{MIC_A} + \frac{C_B}{MIC_B}
\]

where \( C_A \) and \( C_B \) are the concentrations of the drugs A and B in the combinations and MICA \(_{\text{alone}}\) and MICB \(_{\text{alone}}\) are the concentrations of the drugs A and B when acting individually. A FIC index (FIC) value of ≤ 0.5 is considered synergistic, a value of >0.5 to ≤ 4 is considered indifferent, and a value of >4 is considered antagonistic (4).

Interaction ratios (IR) were calculated according to the formula \( IR = 10 / I_e \), in which I0 and Ie are the observed and expected percentage of inhibition for a given interaction, respectively. Ie is calculated as follows: \( I_e = A + B - (AB/100) \), where A and B are the inhibition observed for each compound alone. The interaction was considered synergistic when IR was >1.5, indifferent when IR was between 0.5 and 1.5, and antagonistic when IR was < 0.5 (5, 6).

In agreement with previous studies, M. mycetomatis isolates showed low MICs for both ketoconazole and itraconazole and high MICs for terbinafine ranging from 0.008 to 0.125, 0.016 to 0.0125, and 4 to >16 μg/ml, respectively (Table 1).

We demonstrate that the in vitro combination of ketoconazole and itraconazole with terbinafine did not result in synergy for M. mycetomatis. Of note, according to FIC calculations, syn-

### Table 1 MICs, FIC, and IR of itraconazole, ketoconazole, and terbinafine alone and in combination against M. mycetomatis strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>MIC (μg/ml)</th>
<th>FIC</th>
<th>IR</th>
<th>MIC (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBS 132260/MM14</td>
<td>16/0.06</td>
<td>2/0.008</td>
<td>0.30 (S)</td>
<td>0.008</td>
</tr>
<tr>
<td>CBS 132268/MM31</td>
<td>8/0.03</td>
<td>4/0.003</td>
<td>0.96 (I)</td>
<td>0.10 (I)</td>
</tr>
<tr>
<td>CBS 132270/MM36</td>
<td>16/0.125</td>
<td>8/0.03</td>
<td>1.11 (I)</td>
<td>0.15 (I)</td>
</tr>
<tr>
<td>CBS 132271/MM41</td>
<td>16/0.06</td>
<td>8/0.08</td>
<td>2.60 (I)</td>
<td>0.87 (I)</td>
</tr>
<tr>
<td>CBS 131320/MM55</td>
<td>16/0.125</td>
<td>8/0.06</td>
<td>1.77 (I)</td>
<td>0.96 (I)</td>
</tr>
<tr>
<td>CBS 132284/MM71</td>
<td>8/0.016</td>
<td>8/0.016</td>
<td>1.73 (I)</td>
<td>0.94 (I)</td>
</tr>
<tr>
<td>CBS 132286/MM73</td>
<td>8/0.016</td>
<td>8/0.008</td>
<td>1.92 (I)</td>
<td>0.88 (I)</td>
</tr>
</tbody>
</table>

*a Abbreviations: ITZ, itraconazole; KTZ, ketoconazole; TRB, terbinafine.
*b The Centraalbureau voor Schimmelcultures (CBS) and Erasmus (MM) collection numbers are given for the strains.
*c For the drugs given in combination, the TRB MIC is shown before the slash, and the ITZ or KTZ MIC is shown after the slash.
*d Abbreviations: S, synergistic; I, indifferent.
nergy was found for one isolate with the combination of terbinafine and itraconazole (Table 1). Although we did not find synergy, we did not find antagonism either. This may imply that there is still room for therapeutic improvement in the clinical setting.

Although limited, some information is available on the clinical performance of azoles and terbinafine in the treatment of mycetoma (7, 8, 9). There was only one case reported in which mycetoma was treated with a combination of terbinafine and itraconazole, and the treatment resulted in a complete cure (10). The application of this combination requires further evaluation both in animal models and in the clinical settings.

REFERENCES