In Vitro Evaluation of Antifungal Drug Combinations against Sarocladium (Acremonium) kiliense, an Opportunistic Emergent Fungus Resistant to Antifungal Therapies

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Sarocladium kiliense, formerly known as Acremonium kiliense (1), is a ubiquitous soil saprophyte commonly found in the environment and occasionally infecting humans (2). Its pathogenicity in immunocompetent patients is low and usually is related to inoculation of the fungus via a penetrating injury that often leads to a granuloma formation. However, the presence of underlying immunological disorders can predispose to the development of a usually fatal systemic infection (3). The optimal treatment for these infections is unknown; however, amphotericin B (AMB) seems to be the most efficacious drug, although therapeutic failure has also been reported (3, 4). In addition, this drug shows important side effects that are commonly incompatible with use by patients in poor health. The therapeutic data available are based on a few clinical cases where the etiologic agent was identified only at the genus level or misidentified (5). Antifungal in vitro studies have shown that S. kiliense is resistant to almost all antifungal drugs (2, 6). In addition, recent murine studies have demonstrated that all of the therapies tested against this fungus, i.e., voriconazole (VRC), posaconazole (PSC), AMB, and anidulafungin (AFG), showed very poor efficacy (7). Regarding that, it is crucial to explore new therapeutic strategies for the treatment of severe invasive infections caused by S. kiliense. Therefore, the aim of this study was to evaluate the in vitro activity of drug combinations against a set of 12 S. kiliense strains from clinical sources previously identified by sequencing of the internal transcribed spacer region of the rRNA gene (2). We determined the individual MICs (MIC-0) of azoles, AMB, and terbinafine (TRB) and the minimal effective concentrations (MECs) of AFG by using the CLSI methodology for filamentous fungi (8). Drug interaction was evaluated in a checkerboard microdilution design based on the CLSI method (9). The combined effects were analyzed by summation of the fractional concentration indexes (FICis). Only for combinations of AFG and azoles, two criteria were used, i.e., the MEC of AFG and the MIC-0 of azoles (criterion A) and the MEC of AFG and the MIC-2 (−50% reduction in turbidity compared to the growth control) of azoles (criterion B) (10–12). Studies were performed in duplicate, and the final results were expressed as the means of these replicates. The FICi was used to classify drug interactions, which were defined as synergistic when the FICi was ≤0.5, as antagonistic when the FICi was >4.0, and absent when the FICi was >0.5 or ≤4 (12).

Table 1 shows the interactions of different combinations. In general, when using criterion A, most of the combinations showed an indiff erent effect. Synergism of PSC-TRB was observed in one strain (8.3%) and antagonism of AMB-PSC was observed in two strains (16.6%). When FICis were calculated by using criterion B, AFG-VRC synergism was detected in three strains (25%) and PSC-AFG antagonism was also detected in three strains (16.6%).

Although some of our results showed that the interactions between azoles and echinocandins depend on the endpoint used, the combination of AFG and VRC is promising; however, further experiments evaluating the in vivo efficacy of this antifungal combination are warranted in order to provide new therapeutic alternatives for the treatment of infections with this resistant pathogen.

<table>
<thead>
<tr>
<th>Antifungal combination</th>
<th>FICis a,b</th>
<th>No. (%) of strains by criterion A c</th>
<th>No. (%) of strains by criterion B d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Synergism</td>
<td>Indifference</td>
<td>Antagonism</td>
</tr>
<tr>
<td>AMB-AFG</td>
<td>1.43</td>
<td>0 (0)</td>
<td>12 (100)</td>
</tr>
<tr>
<td>AMB-PSC</td>
<td>2.08</td>
<td>0 (0)</td>
<td>10 (83.3)</td>
</tr>
<tr>
<td>AMB-TRC</td>
<td>2.00</td>
<td>0 (0)</td>
<td>12 (100)</td>
</tr>
<tr>
<td>VRC-AFG</td>
<td>1.26</td>
<td>0 (0)</td>
<td>12 (100)</td>
</tr>
<tr>
<td>VRC-TRB</td>
<td>1.43</td>
<td>0 (0)</td>
<td>12 (100)</td>
</tr>
<tr>
<td>PSC-AFG</td>
<td>3.27</td>
<td>0 (0)</td>
<td>9 (75)</td>
</tr>
<tr>
<td>PSC-TRB</td>
<td>2.29</td>
<td>1 (8.3)</td>
<td>11 (91.6)</td>
</tr>
</tbody>
</table>

a FICI of ≤0.5, synergy; FICI of >0.5 to ≤4, indifference; FICI of >4, antagonism.
b Mean FICI determined for 12 S. kiliense isolates.
c Criterion A: the MEC of AFG and the MIC-0 of azoles AMB and TRB were calculated.
d Criterion B: the MEC of AFG and the MIC-2 of azoles were calculated.
e ND, not determined.

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