Determination of MICs of Aminocandin for Candida spp. and Filamentous Fungi

N. Isham and M. A. Ghannoum*

Center for Medical Mycology, University Hospitals of Cleveland/Case Western Reserve University, Cleveland, Ohio

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Candida and Aspergillus spp., as well as other filamentous molds, have increasingly been reported as the causes of severe invasive fungal infections. We evaluated the new echinocandin aminocandin (AMN) for its antifungal activities against a range of fungal pathogens by determination of the MICs for the organisms. The MICs of the comparator drugs amphotericin B, caspofungin, micafungin, and voriconazole were also determined. The MICs of AMN for 25 strains each of non-Candida albicans Candida spp. (including Candida parapsilosis, Candida krusei, Candida guilliermondii, and Candida tropicalis), Aspergillus fumigatus, Scedosporium spp., Fusarium spp., and zygomycetes (including Absidia, Mucor, and Rhizopus spp.) were determined by using the Clinical and Laboratory Standards Institute M27-A2 and M38-A methodologies for yeasts and filamentous molds, respectively. The MIC ranges of AMN for all yeasts were similar (0.03 to 4.0 μg/ml), while the MIC ranges of AMN for filamentous fungi were species specific. AMN demonstrated potent antifungal activity against A. fumigatus, limited activity against Scedosporium spp., and no activity against zygomycetes or Fusarium spp. Our data showed that AMN demonstrated potent antifungal activities against all of the yeasts and Aspergillus isolates tested, suggesting that AMN could be an important addition to our arsenal of antifungals for the treatment of invasive fungal disease.

Invasive candidiasis and aspergillosis remain the most common invasive fungal infections, with bloodstream infections with Candida spp. (yeasts) representing the fourth most common bloodstream infection in the United States (19). Aspergillus infections are becoming more frequent, resulting in significant morbidity and mortality in developing countries (15). The risk of infection is especially high among the immunocompromised population and in nosocomial settings. Furthermore, other filamentous molds, such as Fusarium, Scedosporium, and zygomycete species, have increasingly been reported as the causes of severe invasive fungal infections in these patient populations (9, 18).

Recently developed therapeutic options include the new triazole voriconazole (VOR) and a new class of antifungal agents, the echinocandins, which inhibit the synthesis of the fungal cell wall component 1,3-beta-D-glucan. Despite these advances, the rate of cure of invasive mycoses is still not optimal, hovering at about 50%. Additionally, the treatment of Candida infections has led to a rise in the number of intrinsically resistant species and the development ofazole resistance in previously susceptible species (1, 2, 11). Furthermore, the echinocandins have demonstrated less activity against strains of Candida parapsilosis and Candida guilliermondii (7). The frequent failure of monotherapy for invasive aspergillosis has led to the use of combination therapy with echinocandins and newer azoles (13); and the current therapeutic approaches for invasive fungal infections caused by Fusarium, Scedosporium, and zygomycete species are suboptimal, resulting in exceedingly high mortality rates (10).

Thus, there is a need for new potent and safe antifungals. Aminocandin (AMN) is a new drug that belongs to the echinocandin class of compounds undergoing early clinical development. Establishing the in vitro antifungal activities of AMN against non-C. albicans spp. and opportunistic filamentous molds is essential. In this study, we evaluated the susceptibilities of non-C. albicans Candida species and filamentous fungi to AMN.

MATERIALS AND METHODS

Test organisms. Test isolates were taken from the culture collection at the Center for Medical Mycology and included Candida parapsilosis, C. guilliermondii, Candida krusei, Candida tropicalis, Aspergillus fumigatus, Fusarium, Scedosporium, and zygomycete species (Absidia, Mucor, and Rhizopus spp.). Candida isolates were identified by using the API 20C system (BioMerieux, Durham, NC), while filamentous fungi were identified by their colonial and microscopic morphologies. The test isolates were subcultured from frozen stocks (−80°C) onto potato dextrose agar (Fisher Scientific, Hampton, NH) and incubated at 35°C for 24 h for the Candida spp. and approximately 1 week for the filamentous fungi. Twenty-five strains of each were tested.

Antifungals. AMN was supplied by Indevus Pharmaceuticals, Inc. (Lexington, MA). Caspofungin (CAS), micafungin (MFN), and VOR were supplied by Merck & Co., Inc. (Whitehouse Station, NJ), Astellas Pharma US, Inc. (Bever Hills, PA), and Pfizer, Inc. (New York, NY), respectively. Amphotericin B (AMB) was obtained from Sigma Chemicals (St. Louis, MO). Antifungal stock solutions were prepared in dimethyl sulfoxide (AMB and VOR) or sterile water (AMN, CAS, MFN) and were stored at −80°C until the day of testing. Drug dilutions were prepared in accordance with the Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) M27-A2 and M38-A susceptibility standards for the susceptibility testing of the Candida spp. and the filamentous fungi, respectively (4, 5).

MICs. The MICs of AMN and the comparator agents for each isolate were determined according to the CLSI standards. Cell counts were standardized by using a hemacytometer, and the suspensions were adjusted in RPMI 1640 buffered with 3-(N-morpholino)propanesulfonic acid (Hardy Diagnostics, Santa Maria, CA) to 0.5 × 10^8 to 2.5 × 10^8 CFU/ml and 0.4 × 10^6 to 5 × 10^6 CFU/ml for the Candida spp. and the filamentous fungi, respectively. Microdilution plates
were incubated at 35°C for 24 h for Candida and zygomycetes, 48 h for Aspergillus and Fusarium, and 72 h for Scedosporium isolates.

The echinocandin MIC endpoint was defined as the lowest concentration that inhibited 50% of fungal growth compared to the growth of the growth control. VOR inhibition endpoints were 50% for the inhibited 50% of fungal growth compared to the growth control.

RESULTS

The MIC data for the Candida spp. are summarized in Table 1. The range of MICs of AMN for all yeasts was 0.03 to 4.0 μg/ml, with each species showing a similar range. The MIC<sub>50</sub> of AMN for C. parapsilosis, C. krusei, C. guilliermondii, and C. tropicalis were 1.0, 0.12, 0.5, and 0.25 μg/ml, respectively, while the MIC<sub>90</sub> for these strains were 2.0, 0.5, 1.0, and 1.0 μg/ml, respectively.

VOR demonstrated the most potent activity against the yeasts tested, with an MIC range, MIC<sub>50</sub>, and MIC<sub>90</sub> of ≤0.016 to 32, 0.03, and 0.12 μg/ml, respectively. One C. tropicalis isolate was resistant to VOR (MIC = 32 μg/ml). AMB and CAS had identical MIC<sub>50</sub> and MIC<sub>90</sub> of 0.5 and 1.0 μg/ml, respectively. The MICs of MFN for all yeasts tested were generally higher than those of AMN, AMB, CAS, and VOR, with an MIC range, MIC<sub>50</sub>, and MIC<sub>90</sub> of 0.001 to 64, 0.5, and 8.0 μg/ml, respectively.

The MIC ranges of AMN for the filamentous fungi were species specific (Table 2). The MIC range, MIC<sub>50</sub>, and MIC<sub>90</sub> of AMN for A. fumigatus were 0.12 to 0.5, 0.25, and 0.5 μg/ml, respectively. The MIC range of AMN for Scedosporium was 4.0 to 8.0, while the MIC<sub>50</sub> and the MIC<sub>90</sub> were both equal to 8.0 μg/ml. The MIC range, MIC<sub>50</sub>, and MIC<sub>90</sub> of AMN for the zygomycetes were 4.0 to >16, 16, and >16 μg/ml, respectively. AMN showed no activity against the Fusarium isolates tested (MIC range = 128 to >256 μg/ml).

MFN demonstrated the most potent activity against A. fumigatus, with an MIC range, MIC<sub>50</sub>, and MIC<sub>90</sub> of 0.016 to 0.06, 0.03, and 0.06 μg/ml, respectively. VOR had an MIC range, MIC<sub>50</sub>, and MIC<sub>90</sub> of 0.06 to 0.5, 0.12, and 0.25 μg/ml, respectively, for A. fumigatus, while the MIC<sub>90</sub> of AMB and CAS were 1.0 and 0.5 μg/ml, respectively, for this organism. AMB and VOR demonstrated similar activities (MIC<sub>90</sub> = 4.0 μg/ml) against the Fusarium and Scedosporium isolates tested, while neither CAS nor MFN showed activity against isolates of either of these genera. Finally, AMB demonstrated the most potent activity against the zygomycetes, with an MIC range, MIC<sub>50</sub>, and MIC<sub>90</sub> of 0.06 to 1.0, 0.25, and 0.5 μg/ml, respectively. VOR had an MIC<sub>90</sub> of 4.0 μg/ml, while neither CAS nor MFN showed activity against the zygomycetes tested.

DISCUSSION

Our data showed that AMN demonstrated potent activities against all of the yeast isolates tested, with overall MIC<sub>50</sub>s and MIC<sub>90</sub>s identical to those of AMB and CAS. These data are in agreement with published data describing the antifungal activities of other echinocandins, including CAS, MFN, and anidulafungin (3, 8, 14). Furthermore, AMN was active against the C. krusei and C. guilliermondii isolates, which are generally known to have lower susceptibilities to fluconazole. Interestingly, the MIC<sub>90</sub> of AMN for all yeasts was threefold lower than that of MFN, demonstrating that differences in the activities against non-C. albicans strains exist among members of this drug class.

Furthermore, AMN demonstrated potent activities against the A. fumigatus isolates tested, with MIC<sub>90</sub>s similar to those of AMB, VOR, and CAS. Again, the activity of AMN against A. fumigatus is similar to those of the other members of the echinocandin class (3, 6, 8).

As with the other echinocandins tested, AMN demonstrated no activity against the zygomycete or Fusarium strains, which is similar to echinocandin MIC data from earlier studies.

### Table 1. MIC ranges, MIC<sub>50</sub>s, and MIC<sub>90</sub>s of AMN and comparator agents for Candida spp.

<table>
<thead>
<tr>
<th>Candida sp.</th>
<th>AMN</th>
<th>AMB</th>
<th>VOR</th>
<th>CAS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC range (μg/ml)</td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;/MIC&lt;sub&gt;90&lt;/sub&gt;</td>
<td>MIC range (μg/ml)</td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;/MIC&lt;sub&gt;90&lt;/sub&gt;</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>0.03–2.0</td>
<td>0.25–0.5</td>
<td>0.25–0.5</td>
<td>0.12–1.0</td>
</tr>
<tr>
<td>C. krusei</td>
<td>0.03–4.0</td>
<td>0.12/0.5</td>
<td>0.25–2.0</td>
<td>0.5/1.0</td>
</tr>
<tr>
<td>C. guilliermondii</td>
<td>0.12–1.0</td>
<td>0.12/0.5</td>
<td>0.25–0.5</td>
<td>0.12–32</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>0.06–2.0</td>
<td>0.25/1.0</td>
<td>0.25–4.0</td>
<td>1.0/1.0</td>
</tr>
</tbody>
</table>

### Table 2. MIC ranges, MIC<sub>50</sub>s, and MIC<sub>90</sub>s of AMN and comparator agents against filamentous fungi

<table>
<thead>
<tr>
<th>Isolate&lt;sup&gt;a&lt;/sup&gt;</th>
<th>AMN</th>
<th>AMB</th>
<th>VOR</th>
<th>CAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. fumigatus</td>
<td>0.12–0.5</td>
<td>0.25/0.5</td>
<td>0.5–1.0</td>
<td>0.5/1.0</td>
</tr>
<tr>
<td>Fusarium</td>
<td>128–&gt;256</td>
<td>&gt;256/256</td>
<td>1.0–4.0</td>
<td>1.0/4.0</td>
</tr>
<tr>
<td>Scedosporium</td>
<td>4.0–8.0</td>
<td>8.0/8.0</td>
<td>4.0/4.0</td>
<td>4.0/4.0</td>
</tr>
<tr>
<td>Zygomycetes</td>
<td>4.0–&gt;16</td>
<td>16/16</td>
<td>0.06–1.0</td>
<td>0.25/0.5</td>
</tr>
</tbody>
</table>

<sup>a</sup> Twenty-five isolates of each species were tested.
The mechanisms underlying the lack of activity of echinocandins against zygomycetes and *Fusarium* spp. are believed to be attributable to differences in their cell wall compositions, as these organisms largely contain 1,3-alpha-glucan and glycuronomannoproteins instead of 1,3-beta-D-glucan (8). AMN did show limited activity against the *Scedosporium* isolates (MIC<sub>90</sub> = 8 μg/ml); this agrees with the limited in vitro activities of MFN against dematiaceous fungi, including *Scedosporium*, *Cladosporium*, *Exophiala*, and *Fonsecaea* spp., reported by Nakai et al. (12).

These data suggest that AMN possesses potent activities against non-*C. albicans* *Candida* spp. as well as *Aspergillus* and could be an important addition to our arsenal of antifungals for the treatment of invasive fungal disease. Further in vivo and clinical testing is warranted.

REFERENCES


