Determination of Minimum Inhibitory Concentration of Aminocandin against Yeasts and Filamentous Fungi

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Running Title: Aminocandin vs Yeast and Filamentous Fungi
ABSTRACT

OBJECTIVE. Candida and Aspergillus spp., as well as other filamentous moulds, have been increasingly reported as the cause of severe invasive fungal infections. We evaluated the new echinocandin, aminocandin (AMN), for antifungal activity against a range of fungal pathogens as determined by minimum inhibitory concentration (MIC). MICs of the comparator drugs amphotericin B (AMB), caspofungin (CAS), micafungin (MFN), and voriconazole (VOR) were also determined. METHODS. The MICs of AMN against twenty five strains each of non-albicans Candida spp., (including C. parapsilosis, C. krusei, C. guilliermondii, C. tropicalis), A. fumigatus, Scedosporium spp., Fusarium spp., and zygomycetes (including Absidia, Mucor, and Rhizopus spp.) were determined using Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) M27A2 and M38A methodologies for yeast and filamentous moulds, respectively. RESULTS. The MIC ranges of AMN against all yeasts were similar (0.03-4.0 µg/ml), while the MIC ranges of AMN against filamentous fungi were species specific. AMN demonstrated potent antifungal activity against A. fumigatus, limited activity against Scedosporium spp., and no activity against zygomycetes and Fusarium spp. CONCLUSION. Our data showed that AMN demonstrated potent antifungal activity against all of the yeast and Aspergillus isolates tested, suggesting that AMN could be an important addition to our arsenal of antifungals for the treatment of invasive fungal disease.
INTRODUCTION

Invasive candidiasis and aspergillosis remain the most common invasive fungal infections, with *Candida* spp. 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. Further, other filamentous moulds, such as *Fusarium*, *Scedosporium*, and zygomycete species, have been increasingly reported as the cause of severe invasive fungal infections in these patient populations (9,18).

Recently developed therapeutic options include the new triazole, voriconazole (VOR), and the new class of antifungal agents, the echinocandins, that inhibit the synthesis of the fungal cell wall component 1,3-beta-D-glucan. In spite of these advances, the cure rate of infections caused by invasive mycosis is still not optimal, hovering around 50%. Additionally, the treatment of *Candida* infections has led to the rise of intrinsically resistant species and the development of azole resistance in previously susceptible species. (1, 4, 11) Further, the echinocandins have demonstrated less activity against strains of *C. parapsilosis* and *C. guilliermondii.*(7) Frequent failure of monotherapy for invasive aspergillosis has led to the use of combination therapy with echinocandins and newer azoles (13), and 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 activity of AMN against non-
albicans spp. and opportunistic filamentous moulds is essential. In this study, we evaluated the susceptibility of non-albicans and filamentous fungi to AMN.

METHODS

Test Organisms

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

Antifungals

AMN was supplied by Indevus Pharmaceuticals, Inc. (Lexington, MA). CAS, MFN, and VOR were supplied by Merck & Co., Inc. (Whitehouse Station, NJ), Astellas Pharma US, Inc. (Beaver Falls, 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 DMSO (AMB and VOR) or sterile water (AMN, CAS, MFN) and stored at -80º C until the day of testing. Drug dilutions were prepared in accordance with the CLSI (formerly NCCLS) M27A2 or M38A susceptibility standard for the susceptibility testing of yeasts and filamentous fungi, respectively. (2,3)
Minimum Inhibitory Concentrations

The minimum inhibitory concentrations (MIC) of AMN and comparators against each isolate were determined according to CLSI standards. Cell counts were standardized using a hemacytometer and the suspension adjusted in RPMI-1640 buffered with MOPS [3-(N-morpholino) propanesulfonic acid] (Hardy Diagnostics, Santa Maria, CA) to 2-5 x 10^3 colony forming units (CFU)/ml and 0.4-5 x 10^4 CFU/ml for Candida and filamentous fungi, respectively. Microdilution plates were incubated at 35º C for 24 hours for Candida and zygomycetes, 48 hours for Aspergillus and Fusarium, and 72 hours for Scedosporium isolates.

The echinocandin MIC endpoint was defined as the lowest concentration that inhibited 50% of fungal growth as compared to the growth control. VOR inhibition endpoints were 50% against yeast and 100% against filamentous fungi, while the AMB endpoint was 100% inhibition against all strains.

RESULTS

The MIC data against yeasts is summarized in Table 1. The MIC range of AMN against all yeasts was 0.03-4.0 µg/ml, with each species showing a similar range. The MIC<sub>50</sub> (defined as the minimum concentration at which 50% of the isolates were inhibited) of AMN against C. parapsilosis, C. krusei, C. guilliermondii, and C. tropicalis was 1.0, 0.12, 0.5, and 0.25 µg/ml, respectively, while the MIC<sub>90</sub> (defined as the minimum concentration at which 90% of the isolates were inhibited) was 2.0, 0.5, 1.0, and 1.0 µg/ml for these strains.

VOR demonstrated the most potent activity against the yeasts tested, with an MIC range, MIC<sub>50</sub>, and MIC<sub>90</sub> of < 0.016-32, 0.03, and 0.12 µg/ml, respectively. There was
one *C. tropicalis* isolate that was resistant to VOR (MIC=32 µg/ml). AMB and CAS had identical MIC<sub>50</sub> and MIC<sub>90</sub> values of 0.5 and 1.0 µg/ml, respectively. MIC values for MFN against all yeasts tested were generally higher than AMN, AMB, CAS, or VOR, with an MIC range, MIC<sub>50</sub>, and MIC<sub>90</sub> of 0.001-64, 0.5, and 8.0 µg/ml, respectively. The MIC ranges of AMN against filamentous fungi were species specific (Table 2). The range, MIC<sub>50</sub>, and MIC<sub>90</sub> of AMN against *A. fumigatus* were 0.12-0.5, 0.25, and 0.5 µg/ml, respectively. The MIC range of AMN against *Scedosporium* was 4.0-8.0, while the MIC<sub>50</sub> and MIC<sub>90</sub> were both equal to 8.0 µg/ml. The range, MIC<sub>50</sub>, and MIC<sub>90</sub> of AMN against the zygomycetes were 4.0- >16, 16 and >16 µg/ml, respectively. AMN showed no activity against the *Fusarium* isolates tested (MIC range 128->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 0.016-0.06, 0.03, and 0.06 µg/ml, respectively. VOR had an MIC range, MIC<sub>50</sub>, and MIC<sub>90</sub> of 0.06-0.5, 0.12, and 0.25 µg/ml, respectively, against *A. fumigatus*, while the MIC<sub>90</sub> of AMB and CAS was 1.0 and 0.5 µg/ml, respectively, against this organism. AMB and VOR demonstrated similar activity (MIC<sub>90</sub> of 4.0 µg/ml) against the *Fusarium* and *Scedosporium* isolates tested, while neither CAS nor MFN showed activity against either of these species. Finally, AMB demonstrated the most potent activity against the zygomycetes, with an MIC range, MIC<sub>50</sub>, and MIC<sub>90</sub> of 0.06-1.0, 0.25, and 0.5 µg/ml, respectively. VOR had an MIC<sub>50</sub> value of 4.0 µg/ml, while neither CAS nor MFN showed activity against the zygomycetes tested.

**DISCUSSION**

Our data showed that AMN demonstrated potent antifungal activity against all of the yeast isolates tested, with overall MIC<sub>50</sub> and MIC<sub>90</sub> values identical to those of AMB.
and CAS. This data is in agreement with published data describing the antifungal activity of other echinocandins, including CAS, MFN, and anidulafungin. (5,8,14) Further, AMN was active against the C. krusei and C. guilliermondii isolates, which are generally known to have lowered susceptibility to fluconazole. Interestingly, the MIC\textsubscript{90} of AMN against all yeasts was three-fold lower than that of MFN, demonstrating that differences in activity against non-\textit{albicans} strains exist among members of this drug class.

Further, AMN demonstrated potent activity against the \textit{A. fumigatus} isolates tested, with MIC\textsubscript{90} values similar to those of AMB, VOR, and CAS. Again, the activity of AMN against \textit{A. fumigatus} is similar to that of the other member of the echinocandin class. (5,6,8)

As with the other two echinocandins tested, AMN demonstrated no activity against the zygomycete and \textit{Fusarium} strains, which is similar to echinocandin MIC data from earlier studies. (6,12,16,17) The mechanisms underlying the lack of activity of echinocandins against zygomycetes and \textit{Fusarium} spp. are believed to be attributable to differences in their cell wall composition, as these organisms contain largely 1,3-alpha-glucan and glycuronomannoproteins instead of 1,3-beta-D-glucan. (8) AMN did show limited activity against the \textit{Scedosporium} isolates (MIC\textsubscript{90} equal to 8 µg/ml); this agrees with the limited \textit{in vitro} activity of MFN against dematiaceous fungi, including \textit{Scedosporium, Cladosporium, Exophiala}, and \textit{Fonsecaea} spp. reported by Nakai et al. (12)

This data suggests that AMN possesses potent activity against non-\textit{albicans} \textit{Candida} spp. as well as \textit{Aspergillus} and could be an important addition to our arsenal of antifungals for the treatment of invasive fungal disease. Further \textit{in vivo} and clinical testing is warranted.
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    antifungal activity of a novel lipopeptide antifungal agent, FK463, against

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Table 1. MIC range, MIC$_{50}^a$, and MIC$_{90}^b$ (in µg/ml) of AMN and comparators against *Candida* spp.

<table>
<thead>
<tr>
<th><em>Candida</em> spp.</th>
<th>AMN</th>
<th>AMB</th>
<th>VOR</th>
<th>CAS</th>
<th>MFN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC Range</td>
<td>MIC$<em>{50}^a$/MIC$</em>{90}^b$</td>
<td>MIC Range</td>
<td>MIC$<em>{50}^a$/MIC$</em>{90}^b$</td>
<td>MIC Range</td>
</tr>
<tr>
<td><em>parapsilosis</em></td>
<td>0.03-1.0/0.25-0.25</td>
<td>0.12-0.16/0.03</td>
<td>0.5-1.0/0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>krusei</em></td>
<td>0.03-0.12/0.25-0.25</td>
<td>0.12-0.25/0.5</td>
<td>0.5-1.0/0.5</td>
<td></td>
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<tr>
<td></td>
<td>0.12-0.5/0.25-0.25</td>
<td>0.25-0.5</td>
<td>1.0-4.0/0.25</td>
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<td></td>
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<tr>
<td><em>guilliermondii</em></td>
<td>0.12-1.0/0.5-0.5</td>
<td>0.25-0.25</td>
<td>0.5-1.0</td>
<td>0.12-2.0</td>
<td></td>
</tr>
<tr>
<td><em>tropicalis</em></td>
<td>0.06-0.25/0.25-1.0</td>
<td>0.06-0.16/0.06</td>
<td>0.5-1.0</td>
<td>0.12-0.06</td>
<td></td>
</tr>
<tr>
<td><em>All yeasts</em></td>
<td>0.03-1.0/0.5-0.5</td>
<td>0.12-1.0/0.5</td>
<td>0.03-0.03</td>
<td>0.06-0.06</td>
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<td><em>n=100</em></td>
<td>4.0-1.0/4.0</td>
<td>1.0-4.0</td>
<td>4.0-16</td>
<td>4.0-64</td>
<td></td>
</tr>
</tbody>
</table>

$^a$Defined as the lowest concentration to inhibit 50% of the isolates tested

$^b$Defined as the lowest concentration to inhibit 90% of the isolates tested
Table 2. MIC range, MIC$_{50}$\textsuperscript{a}, and MIC$_{90}$\textsuperscript{b} (in µg/ml) of AMN and comparators against filamentous fungi.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>AMN</th>
<th>AMB</th>
<th>VOR</th>
<th>CAS</th>
<th>MFN</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>MIC Range</td>
<td>MIC$<em>{50}$/MIC$</em>{90}$</td>
<td>MIC Range</td>
<td>MIC$<em>{50}$/MIC$</em>{90}$</td>
<td>MIC Range</td>
</tr>
<tr>
<td><em>A. fumigatus</em></td>
<td>0.12-0.5</td>
<td>0.5-0.5</td>
<td>0.5-0.5</td>
<td>0.06-0.12</td>
<td>0.5-0.5</td>
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<tr>
<td><em>n=25</em></td>
<td>0.5</td>
<td>0.5</td>
<td>1.0</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td><em>Fusarium</em></td>
<td>128- &gt;256</td>
<td>&gt;256-4.0</td>
<td>1.0-4.0</td>
<td>1.0-4.0</td>
<td>2.0-64</td>
</tr>
<tr>
<td><em>n=25</em></td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td><em>Scedosporium</em></td>
<td>4.0-8.0</td>
<td>8.0-8.0</td>
<td>1.0-8.0</td>
<td>0.25-0.25</td>
<td>4.0-4.0</td>
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<tr>
<td><em>n=25</em></td>
<td>8.0</td>
<td>8.0</td>
<td>8.0</td>
<td>8.0</td>
<td>4.0</td>
</tr>
<tr>
<td><em>Zygomycetes</em></td>
<td>4.0-16</td>
<td>16-16</td>
<td>0.06-0.06</td>
<td>0.25-0.25</td>
<td>2.0-4.0</td>
</tr>
<tr>
<td><em>n=25</em></td>
<td>&gt;16</td>
<td>&gt;16</td>
<td>1.0</td>
<td>0.5</td>
<td>&gt;8.0</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Defined as the lowest concentration to inhibit 50\% of the isolates tested

\textsuperscript{b} Defined as the lowest concentration to inhibit 90\% of the isolates tested