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Determination of MICs of Aminocandin for *Candida* spp. and Filamentous Fungi[▽]

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***Candida* and A*spergillus* 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 of azole 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. (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 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 0.5×10^3 to 2.5×10^3 CFU/ml and 0.4×10^4 to 5×10^4 CFU/ml for the *Candida* spp. and the filamentous fungi, respectively. Microdilution plates

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TABLE 1. MIC ranges, MIC_{50}s, and MIC_{90}s of AMN and comparator agents for *Candida* spp.

Candida sp.[a]	AMN		AMB		VOR		CAS		MFN	
	MIC range (μg/ml)	MIC_{50}/MIC_{90} (μg/ml)	MIC range (μg/ml)	MIC_{50}/MIC_{90} (μg/ml)	MIC range (μg/ml)	MIC_{50}/MIC_{90} (μg/ml)	MIC range (μg/ml)	MIC_{50}/MIC_{90} (μg/ml)	MIC range (μg/ml)	MIC_{50}/MIC_{90} (μg/ml)
C. parapsilosis	0.03–2.0	1.0/2.0	0.25–0.5	0.25/0.5	<0.016–0.12	<0.016/0.03	0.12–1.0	0.5/1.0	0.12–8.0	4.0/8.0
C. krusei	0.03–4.0	0.12/0.5	0.25–2.0	0.5/1.0	<0.016–0.25	0.12/0.25	0.25–16	0.5/1.0	0.016–64	0.25/2.0
C. guilliermondii	0.12–1.0	0.5/1.0	0.12–1.0	0.25/0.5	<0.016–0.25	0.03/0.06	0.25–2.0	0.5/1.0	0.12–4.0	2.0/2.0
C. tropicalis	0.06–2.0	0.25/1.0	0.25–4.0	1.0/1.0	<0.016–32	<0.016/0.06	0.06–8.0	0.25/1.0	0.001–8.0	0.06.0.5
All yeasts	0.03–4.0	0.5/1.0	0.12–4.0	0.5/1.0	<0.016–32	0.03/0.12	0.06–16	0.5/1.0	0.001–64	0.5/8.0

[a] Twenty-five isolates of each species were tested.

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 *Candida* spp. and 100% for the filamentous fungi, while the AMB endpoint was 100% inhibition for all strains.

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_{50}s 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_{90}s 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_{50}, and MIC_{90} 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_{50}s and MIC_{90}s 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_{50}, and MIC_{90} 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_{50}, and MIC_{90} 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_{50} and the MIC_{90} were both equal to 8.0 μg/ml. The MIC range, MIC_{50}, and MIC_{90} 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_{50}, and MIC_{90} of 0.016 to 0.06, 0.03, and 0.06 μg/ml, respectively. VOR had an MIC range, MIC_{50}, and MIC_{90} of 0.06 to 0.5, 0.12, and 0.25 μg/ml, respectively, for *A. fumigatus*, while the MIC_{90}s of AMB and CAS were 1.0 and 0.5 μg/ml, respectively, for this organism. AMB and VOR demonstrated similar activities (MIC_{90}s = 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_{50}, and MIC_{90} of 0.06 to 1.0, 0.25, and 0.5 μg/ml, respectively. VOR had an MIC_{50} 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_{50}s and MIC_{90}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_{90} 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_{90}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 two echinocandins tested, AMN demonstrated no activity against the zygomycete or *Fusarium* strains, which is similar to echinocandin MIC data from earlier studies

TABLE 2. MIC ranges, MIC_{50}s, and MIC_{90}s of AMN and comparator agents against filamentous fungi

Isolate[a]	AMN		AMB		VOR		CAS		MFN	
	MIC range (μg/ml)	MIC_{50}/MIC_{90} (μg/ml)	MIC range (μg/ml)	MIC_{50}/MIC_{90} (μg/ml)	MIC range (μg/ml)	MIC_{50}/MIC_{90} (μg/ml)	MIC range (μg/ml)	MIC_{50}/MIC_{90} (μg/ml)	MIC range (μg/ml)	MIC_{50}/MIC_{90} (μg/ml)
A. fumigatus	0.12–0.5	0.25/0.5	0.5–1.0	0.5/1.0	0.06–0.5	0.12/0.25	0.5	0.5/0.5	0.016–0.06	0.03/0.06
Fusarium	128–>256	>256/>256	1.0–4.0	1.0/4.0	1.0–4.0	2.0/4.0	64–256	128/256	>256	>256/>256
Scedosporium	4.0–8.0	8.0/8.0	1.0–8.0	4.0/4.0	0.25–8.0	4.0/4.0	1.0–16	16/16	2.0–>256	64/>256
Zygomycetes	4.0–>16	16/>16	0.06–1.0	0.25/0.5	2.0–>8.0	4.0/>8.0	4.0–>16	>16/>16	1.0–>256	>256/>256

[a] Twenty-five isolates of each species were tested.

(6, 12, 16, 17). 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_{90} = 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.

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