

In Vitro Susceptibility of Clinical Isolates of *Aspergillus* spp. to Anidulafungin, Caspofungin, and Micafungin: a Head-to-Head Comparison Using CLSI Broth Microdilution Methods

M.A. Pfaller, L. Boyken, R.J. Hollis, J. Kroeger, S.A. Messer, S. Tendolkar, and D.J. Diekema

Departments of Pathology, Epidemiology, and Internal Medicine

Roy J. and Lucille A. Carver College of Medicine and College of Public Health, University of Iowa, Iowa City, IA 52242

ABSTRACT (revised)

Background: The echinocandins are recently introduced antifungal agents that inhibit the (1,3)- β -D-glucan synthase activity of *Candida* and *Aspergillus* spp. Although each of the 3 available agents has been studied for the treatment of invasive aspergillosis (IA), only caspofungin has been approved for treatment of IA in patients refractory to or intolerant of other agents. Whereas the relative activity of these agents against *Candida* is well studied, there is a lack of head-to-head comparisons of the in vitro activity of all three agents against *Aspergillus* spp.

Methods: We determined the in vitro activity of anidulafungin, caspofungin and micafungin against 526 isolates of *Aspergillus* spp. (64 *A. flavus*, 391 *A. fumigatus*, 46 *A. niger*, and 25 *A. terreus*) collected from over 60 centers worldwide from 2001 through 2007. Susceptibility testing was performed according to CLSI M38-A2 broth microdilution (BMD) methods using RPMI 1640 broth, 48-h incubation and the minimum effective concentration (MEC) endpoint criterion.

Results: All three echinocandins were very active against *Aspergillus*: anidulafungin (MEC₅₀ 0.007 μ g/ml; MEC₉₀ 0.015), caspofungin (MEC₅₀ 0.015 μ g/ml; MEC₉₀ 0.03 μ g/ml), micafungin (MEC₅₀ 0.007 μ g/ml; MEC₉₀ 0.015 μ g/ml). More than 99% of all isolates were inhibited by 0.06 μ g/ml of all three agents. Results by species (expressed as the percentages of isolates inhibited by \leq 0.06 μ g/ml of anidulafungin, caspofungin, and micafungin, respectively) were: for *A. flavus*, 100%, 100%, and 100%; for *A. fumigatus*, 100%, 99%, and 100%; for *A. niger*, 100%, 100%, and 100%; and for *A. terreus*, 100%, 100%, and 100%.

Conclusions: All 3 echinocandins have excellent in vitro activities against clinical isolates of *Aspergillus* spp. from centers worldwide. Our prospective sentinel surveillance reveals no evidence of emerging echinocandin resistance among the *Aspergillus* spp.

MATERIALS and METHODS

Organisms. Between January 2001 and December 2007, 526 unique patient isolates of *Aspergillus* spp. (64 *A. flavus*, 391 *A. fumigatus*, 46 *A. niger*, and 25 *A. terreus*) were obtained from more than 60 different medical centers worldwide for testing against the three echinocandins. The isolates were obtained from a variety of sources, including sputum, bronchoscopy, and tissue biopsy specimens. All isolates were identified using standard microscopic morphology and were stored as spore suspensions in sterile distilled water at room temperature until they were used in the study. Before testing, each isolate was subcultured at least twice on potato dextrose agar (Remel, Lenexa, KS) to ensure viability and purity.

Antifungal susceptibility testing. Reference powders of anidulafungin (Pfizer), caspofungin (Merck), and micafungin (Astellas) were obtained from their respective manufacturers. Stock solutions were prepared in water (caspofungin and micafungin) or dimethyl sulfoxide (anidulafungin) and serial twofold dilutions in RPMI 1640 medium (Sigma, St. Louis) buffered to pH 7.0 with 0.165M MOPS (morpholinepropanesulfonic acid) buffer (Sigma) were made.

BMD testing was performed in accordance with the guidelines in CLSI document M38-A2 (4) by using RPMI 1640 medium, an inoculum of 0.4×10^4 to 5×10^4 CFU/ml, and incubation at 35°C. MECs were determined, after 48-h of incubation, as the lowest concentration of drug at which short, stubby, and highly branched hyphae were observed (4,11).

Quality control (QC). QC was ensured by testing the following strains recommended in M38-A2 (4): *C. parapsilosis* ATCC 22019, *C. krusei* ATCC 6258, and *A. flavus* ATCC 204304.

Table. In vitro susceptibilities of 526 clinical isolates of *Aspergillus* to anidulafungin, caspofungin, and micafungin

Species	No. tested	Antifungal agent	No. of isolates for which the MEC (μ g/ml) was:										
			0.007	0.015	0.03	0.06	0.12	0.25	0.5	1	2	>=4	
<i>A. flavus</i>	64	Anidulafungin	52	10	2								
		Caspofungin	15	36	10	3							
		Micafungin	50	12	2								
<i>A. fumigatus</i>	391	Anidulafungin	264	84	40	3							
		Caspofungin	24	191	138	34	4						
		Micafungin	339	47	5								
<i>A. niger</i>	46	Anidulafungin	42	4									
		Caspofungin	32	11	3								
		Micafungin	40	6									
<i>A. terreus</i>	25	Anidulafungin	22	3									
		Caspofungin	9	11	5								
		Micafungin	24	1									

RESULTS and DISCUSSION

The MEC distributions for each of the three echinocandins and the four species of *Aspergillus* are shown in the Table. First of all, it should be noted that all three echinocandins demonstrate excellent potency and spectrum with more than 99% of all isolates inhibited by \leq 0.06 μ g/ml of all three agents. The respective MEC₅₀ and MEC₉₀ values for all isolates combined were 0.007 μ g/ml and 0.015 μ g/ml for anidulafungin and micafungin and 0.015 μ g/ml and 0.03 μ g/ml for caspofungin. Results by species (expressed as the percentages of isolates inhibited by \leq 0.06 μ g/ml of anidulafungin, caspofungin, and micafungin, respectively) were as follows: for *A. flavus*, 100%, 100% and 100%; for *A. fumigatus* 100%, 99%, and 100%; for *A. niger*, 100%, 100%, and 100%; and for *A. terreus*, 100%, 100%, and 100%.

The results of this study constitute the largest head-to-head comparison of the in vitro activities of anidulafungin, caspofungin and micafungin against *Aspergillus* spp. that has been reported to date. Antachopoulos et al (1) have compared the MECs and inhibition of metabolic activity for the three echinocandins against a much smaller collection (27 isolates) of *Aspergillus* spp. using both germinated and ungerminated conidia. They found that anidulafungin exhibited the lowest MEC values and caspofungin exhibited the highest MEC values against nongerminated conidia. This difference was minimized when germinated conidia were tested. There was a significant correlation between the degrees of maximal metabolic inhibition caused by the different echinocandins at both the species level (greater inhibition for *A. flavus*) and the strain level. Furthermore, for each drug and species, the maximal metabolic inhibition values obtained for germinated and nongerminated conidia did not differ significantly, suggesting that the degree of metabolic inhibition induced by the echinocandins was not significantly altered in the presence of germinated conidia in comparison to that in the presence of nongerminated conidia (1).

The CLSI M38-A2 BMD uses an ungerminated conidial inoculum and as such supports the findings of Antachopoulos et al (2008) showing excellent and broad-spectrum activity of all three echinocandins against a large collection of *Aspergillus* isolates. Both anidulafungin and micafungin were slightly more active than caspofungin; however, 99% to 100% of all isolates were inhibited at the low MEC of \leq 0.06 μ g/ml for all three agents. MECs of all agents tended to be slightly higher (1 log, dilution) for *A. fumigatus* versus the other three species.

In summary we have performed a head-to-head challenge of anidulafungin, caspofungin, and micafungin against a large globally diverse collection of *Aspergillus* species using the CLSI M38-A2 BMD method. The results of the study demonstrate the comparable and excellent level of inhibitory activity of each agent and the distinct lack of isolates with significantly decreased susceptibility to one or more of the echinocandins. These data provide a baseline level of in vitro activity of these agents against *Aspergillus* spp. that may be used to add perspective to other studies of clinical and in vitro echinocandin activity. For example, Madureira et al (9) reported four cases of breakthrough IA in patients undergoing empirical or prophylactic therapy with caspofungin for which MECs to all three echinocandins were obtained. The MECs for caspofungin ranged from 0.25 μ g/ml to 8 μ g/ml whereas those for anidulafungin were 0.0125 μ g/ml and those for micafungin ranged from 0.25 μ g/ml to 4 μ g/ml. In each case the MECs for each of the echinocandins were outside of the MEC distributions shown in the Table with the greatest deviations seen with caspofungin. Continued surveillance using the CLSI BMD method is warranted to monitor the activities of these agents against *Aspergillus* spp. and to detect those unusual isolates with reduced susceptibility for further study.

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A copy of this poster can be found at the following website:

<http://www.healthcare.uiowa.edu/pathology/site/research/ICAAC.html>

REFERENCES

- Antachopoulos, C., J. Meletiadi, T. Sein, E. Roilides, and T.J. Walsh. 2008. Comparative in vitro pharmacodynamics of caspofungin, micafungin, and anidulafungin against germinated and nongerminated *Aspergillus* conidia. *Antimicrob. Agents Chemother.* 52:321-328.
- Arendrup, M.C., S. Perkhof, S.J. Howard, G. Garcia-Effron, A. Vishukumar, D. Perlin, and C. Lass-Flörl. 2008. Establishing in vitro – in vivo correlations for *Aspergillus fumigatus*: the challenge of azoles versus echinocandins. *Antimicrob. Agents Chemother.* 52:3504-3511.
- Cappalerty, D., and K. Eiselstein-McKittrick. 2007. The echinocandins. *Pharmacotherapy* 27:369-388.
- Clinical and Laboratory Standards Institute. 2008. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; Approved standard, 2nd Ed. M38-A2. Clinical and Laboratory Standards Institute, Wayne, PA.
- Clinical Trials.gov. 2008. Anidulafungin plus voriconazole versus voriconazole for the treatment of invasive aspergillosis. N.I.O. Health, ed.
- Denning, D.W., K.A. Marr, W.M. Lau, et al. 2008. Micafungin (FK463), alone or in combination with other systemic antifungal agents, for the treatment of acute invasive aspergillosis. *J. Infect.* 53:337-349.
- Diekema, D.J., S.A. Messer, R.J. Hollis, R.N. Jones, and M.A. Pfaller. 2003. Activities of caspofungin, itraconazole, posaconazole, ravuconazole, voriconazole, and amphotericin B against 448 recent clinical isolates of filamentous fungi. *J. Clin. Microbiol.* 41:3623-3626.
- Kahn, J.N., M.J. Hsu, F. Racine, R. Giacobbe, and M. Motyl. 2006. Caspofungin susceptibility in *Aspergillus* and non-*Aspergillus* molds: inhibition of glucan synthase and reduction of β -D-1, 3 glucan levels in culture. *Antimicrob. Agents Chemother.* 50:2214-2216.
- Madureira, A., A. Bergeron, C. Lacroix, M. Robin, V. Rocha, R. Peffault de Latour, C. Ferry, A. Devergie, J. Lapalu, E. Gluckman, G. Socie, M. Ghannoum, and P. Ribaud. 2007. Breakthrough invasive aspergillosis in allogeneic hematopoietic stem cell transplant recipients treated with caspofungin. *Int. J. Antimicrob. Agents* 30:551-554.
- Maertens, J., I. Raad, G. Petrikos, M. Boogaerts, D. Sleselag, F.B. Petersen, C.A. Sable, N.A. Kartsonis, A. Ngai, A. Taylor, T.F. Patterson, D.W. Denning, and T.J. Walsh. 2003. Efficacy and safety of caspofungin for treatment of invasive aspergillosis in patients refractory to or intolerant of conventional antifungal therapy. *Clin. Infect. Dis.* 39:1563-1571.
- Odds, F.C., M. Motyl, R. Andrade, et al. 2004. Interlaboratory comparison of results of susceptibility testing with caspofungin against *Candida* and *Aspergillus* species. *J. Clin. Microbiol.* 42:3475-3482.
- Patterson, T.F. 2007. The role of echinocandins, extended-spectrum triazoles, and polyenes to treat opportunistic moulds and *Candida*. *Curr. Fungal Infect. Reports* 1:5-11.
- Perlin, D.S. 2007. Resistance to echinocandins-class antifungal drugs. *Drug Resist. Updat.* 10:121-130.
- Pfaller, M.A., L. Boyken, R.J. Hollis, J. Kroeger, S.A. Messer, S. Tendolkar, and D.J. Diekema. 2008. In vitro susceptibility of invasive isolates of *Candida* spp. to anidulafungin, caspofungin, and micafungin: six years of global surveillance. *J. Clin. Microbiol.* 46:150-156.
- Rocha, E.M.F., G. Garcia-Effron, S. Park, and D.S. Perlin. 2007. A Ser678Pro substitution in Fks1p confers resistance to echinocandin drugs in *Aspergillus fumigatus*. *Antimicrob. Agents Chemother.* 51:4174-4176.
- Serrano Mdel, C., A. Valverde-Conde, M.M. Chavez, S. Bernal, R.M. Claro, J. Peman, M. Ramirez, and E. Martin-Mazuelos. 2003. In vitro activity of voriconazole, itraconazole, caspofungin, anidulafungin (VER002, LY303366) and amphotericin B against *Aspergillus* spp. *Diagn. Microbiol. Infect. Dis.* 45:131-135.
- Walsh, T.J., E.J. Anaissie, D.W. Denning, R. Herbrecht, D.P. Kontoyiannis, K.A. Marr, V.A. Morrison, B.H. Segal, W.J. Steinbach, D.A. Stevens, J.A. van Burik, J.R. Wingard, and T.F. Patterson. 2008. Treatment of aspergillosis: clinical practice guidelines of the Infectious Diseases Society of America. *Clin. Infect. Dis.* 46:327-360.