M-1796

Antifungal Susceptibility Results from a Worldwide Anidulafungin Surveillance Program (2002-2003)

P.R. Rhomberg, R.N. Jones, S.A. Messer, J.T. Kirby
The JONES Group/JMI Laboratories, North Liberty, IA

Phone: 319-665-3370
Fax: 319-665-3371
Email: Ronald-Jones@JMIlabs.com

ABSTRACT

Background: In the past two decades there have been significant increases in serious fungal infections, and *Candida* is now the 4th leading cause of positive blood cultures (USA). This rise stimulated the development of newer antifungal agents with unique modes of action and the standardization of antifungal susceptibility testing. Anidulafungin (ANID) is a novel derivative in the echinocandin class that inhibits cell wall synthesis by blocking the formation of 1,3-β-D glucan. **Materials and Methods:** Broth microdilution susceptibility testing was performed following NCCLS methods (M27-A2, M38-A) against 882 yeast and 68 mould isolates collected in 2002-2003 from 64 medical centers in 20 countries. Antifungal agents included ANID, amphotericin B (AmB), 5-fluorocytosine, fluconazole, itraconazole (ITRA), ketoconazole (KETO), and voriconazole (VORI). **Results:** Against *Aspergillus* spp. (30), ANID was the most potent agent (MEC_{90}, 0.03), 16-fold more active than VORI and 64-fold greater than AmB or ITRA. When testing *Candida*, there was a 2-fold shift for 50% inhibition (lower ANID MIC) endpoints compared to complete inhibition results. VORI and KETO were the most potent agents against 500 *C. albicans* (CA; MIC_{90}, ≤0.008 and 0.016, respectively) followed by ANID (MIC_{90}, 0.06). ANID MIC_{90} results by species were: *C. glabrata* (CG, 105; 0.12), *C. krusei* (CK, 23; 0.06) and *C. tropicalis* (CT, 106; 0.06). All CA, CG, CK and CT had ANID MIC results at ≤2 μg/ml. *C. parapsilosis* (CPAR; 106) showed the highest ANID MICs with $MIC_{50/90}$ values at 2/4 μg/ml. *C. guilliermondii* and *C. lusitaniae* ANID MICs (MIC_{90}, 1) were more similar to those of CPAR than CA. **Conclusions:** ANID shows potent activity against a wide variety of yeast and mould isolates recovered worldwide. MICs were higher for CPAR isolates, as previously described for the echinocandin class, but MIC_{90} for the remaining common *Candida* spp. isolates was very low (0.06 μg/ml), without potency variations across three continents.

INTRODUCTION

Opportunistic fungal infections represent a significant risk to immunocompromised individuals and are associated with high rates of morbidity and mortality. Currently, only a limited number of antifungal agents are available for therapeutic use in these infections. New and alternative antifungal agents have been successfully used in the treatment of these problematic infections. In addition, due to the new realities in healthcare, the general trend is to admit only the most seriously ill patients, who tend to have a longer duration of hospitalization, and among whom nosocomial infections have become a greater health concern. *Candida* species currently rank as the fourth most frequent source of nosocomial bloodstream infections in the United States and elsewhere. The emergence of mould pathogens and yeast species with decreased susceptibility to current antifungal regimens demonstrates the need for new agents to manage these infections.

The echinocandins offer an alternative treatment and have the additional advantage of low toxicity. Anidulafungin (ANID) is a novel semisynthetic echinocandin antifungal agent that compromises cell wall structural integrity through non-competitive inhibition of β-1,3-D-glucan biosynthesis, causing cell death. It has demonstrated excellent broad spectrum *in vitro* and *in vivo* activity against a wide variety of fungal pathogens. We present initial data from a longitudinal study comparing the antifungal activity of ANID, AmB, 5-fluorocytosine (FC) and four azole agents.

MATERIALS AND METHODS

Clinical isolates: A total of 882 yeast and 68 mould clinical isolates from 64 medical centers in North America, Latin America and Europe were tested. The isolates were collected in 2002 and 2003, and were sent to a central reference laboratory for testing. The collection of yeasts included *Candida albicans* (n=500), *C. parapsilosis* (n=106), *C. tropicalis* (n=106), *C. glabrata* (n=105), *C. krusei* (n=23), *C. lusitaniae* (n=13), *C. guilliermondii* (n=8), *C. kefyr* (n=5), *C. lipolytica* (n=5), *C. pulcherrima* (n=3), *C. dubliniensis* (n=2), *C. famata* (n=2), and one strain each of *C. humicola*, *C. rugosa*, *C. pulcherrima*, and *C. sake*. The collection of moulds included *Aspergillus fumigatus* (n=30), *A. flavus* (n=7), *A. niger* (n=6), *Penicillium* spp. (n=6), *A. terreus* (n=4), *Trichosporon* spp. (n=3), *A. nidulans* (n=2), *A. versicolor* (n=2), *Curvularia* spp. (n= 2), *Paecilomyces* spp. (n=2), *Fusarium* spp. (n= 2), and *Rhizopus* spp. (n=2).

Antifungal agents: ANID (Vicuron, Inc., King of Prussia, PA, USA) was obtained as a standard powder and comparator stocks were prepared according to NCCLS guidelines at TREK Diagnostics (Westlake, OH). Final concentration ranges were as follows: ANID (0.008-16 μg/ml), AmB (0.016-16 μg/ml), 5-fluorocytosine (FC) (0.03-64 μg/ml), fluconazole (FLUCON) (0.12-256 μg/ml), ITRA (0.008-16 μg/ml), KETO (0.008-16 μg/ml), and VORI (0.008-16 μg/ml). Sterile 96-well round-bottomed panels (Corning, New York, NY, USA) containing 100 μl of two-fold antifungal dilutions in RPMI 1640 growth medium buffered to a pH of 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS) were used for testing.

Broth microdilution tests: Testing of yeasts followed standard conditions described in the NCCLS M27-A2 approved standard. Filamentous fungi were tested using conditions described in the NCCLS M38-A approved standard. Quality control (QC) isolates *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 from the American Type Culture Collection were used as recommended (NCCLS) and all results were within published ranges. All micro-dilution panels were incubated in enclosed, humid containers at 35°C and visualized using a reading mirror at both 24h and 48h.

Yeasts: Following incubation of yeasts, the MIC values for ANID, FC, FLUCON, ITRA, KETO, and VORI were read at the lowest concentration at which a significant decrease in turbidity (≥50%) was detected compared to the growth control. AmB MICs were determined as the lowest concentration at which no visible growth was discerned. In addition, a second MIC value for ANID was determined as the lowest concentration at which no visible growth was detected.

Interpretive breakpoints for susceptibility (S) to FLUCON (S at ≤8 μg/ml; S dose dependent [SDD] at 16-32 μg/ml; and resistant [R] at ≥64 μg/ml), FC (S at ≤4 μg/ml; intermediate [I] at 8-16 μg/ml; R at ≥32 μg/ml) and ITRA (S at ≤0.12 μg/ml; SDD at 0.25-0.5 μg/ml; R: ≥1 μg/ml) were those published by Rex et al. and the NCCLS. Interpretive criteria for ANID, AmB, KETO and VORI have not been established.

Determination of MIC and MEC values for moulds: MIC values for FC, FLUCON and KETO were defined as the lowest concentration at which no visible growth was detected. MIC values for FC, fluconazole and KETO were detected as the lowest concentration at which a prominent decrease in growth (≥50%) was visualized as compared to the growth control. As described for the echinocandins, the minimum effective concentration (MEC) for ANID was determined as the lowest concentration at which a pronounced morphological change from filamentous growth to non-filamentous growth was observed.

RESULTS

- As shown in **Table 1**, the *in vitro* antifungal activity of ANID was superior or equivalent to AmB, FLUCON, ITRA and FC for *C. albicans*, *C. tropicalis*, *C. glabrata*, and *C. krusei* and also superior to KETO and VORI, except against *C. tropicalis*. ANID also had potent activity against less frequently encountered yeast species (**Table 3**; data for comparators not shown).

Table 1: Comparative activity of anidulafungin and six other antifungal agents tested against 883 strains of yeast and mould species with at least 10 isolates

Organism (no. tested)	Antifungal agent	MIC or MEC (μg/ml) Range	50%	90%	% susceptible[a]
C. albicans (500)	Anidulafungin	≤0.008-0.12	0.03	0.06	–[c]
	Amphotericin B	0.12-2	1	1	99.4
	5-fluorocytosine	≤0.03->64	0.25	2	98.0
	Fluconazole	≤0.12-128	0.25	0.5	99.4
	Itraconazole	0.016-2	0.06	0.12	92.6
	Ketoconazole	≤0.008-2	≤0.008	0.016	–[c]
	Voriconazole	≤0.008-4	≤0.008	≤0.008	99.8
C. parapsilosis (106)	Anidulafungin	0.12-8	2	4	–[c]
	Amphotericin B	0.12-1	1	1	100.0
	5-fluorocytosine	≤0.03-2	0.12	0.25	100.0
	Fluconazole	0.25-64	1	2	97.2
	Itraconazole	0.03-1	0.25	0.5	39.6
	Ketoconazole	0.016-1	0.06	0.25	–[c]
	Voriconazole	≤0.008-1	0.03	0.12	100.0
C. tropicalis (106)	Anidulafungin	≤0.008-2	0.03	0.06	–[c]
	Amphotericin B	0.25-2	1	1	96.2
	5-fluorocytosine	≤0.03->64	0.25	>64	89.6
	Fluconazole	0.25-64	1	2	98.1
	Itraconazole	0.06-1	0.25	0.5	30.2
	Ketoconazole	≤0.008-0.5	0.03	0.12	–[c]
	Voriconazole	≤0.008-0.12	0.06	0.12	100.0
C. glabrata (105)	Anidulafungin	0.016-0.25	0.06	0.12	–[c]
	Amphotericin B	0.25-2	1	2	89.5
	5-fluorocytosine	≤0.03-2	0.06	0.12	100.0
	Fluconazole	1-128	8	64	52.4
	Itraconazole	0.25->16	1	2	0.0
	Ketoconazole	0.12-4	0.5	2	–[c]
	Voriconazole	0.03-4	0.25	2	89.5
C. krusei (23)	Anidulafungin	0.03-0.12	0.06	0.06	–[c]
	Amphotericin B	0.5-2	1	2	87.0
	5-fluorocytosine	8->64	32	64	0.0
	Fluconazole	16-128	64	128	0.0
	Itraconazole	0.25-2	1	1	0.0
	Ketoconazole	0.5-2	1	2	–[c]
	Voriconazole	0.12-1	0.5	0.5	100.0
C. lusitaniae (13)	Anidulafungin	0.016-4	0.5	1	–[c]
	Amphotericin B	0.12-1	1	1	100.0
	5-fluorocytosine	≤0.03->64	0.06	16	84.6
	Fluconazole	≤0.12-2	1	2	100.0
	Itraconazole	0.06-1	0.25	0.5	38.5
	Ketoconazole	≤0.008-0.25	0.03	0.12	–[c]
	Voriconazole	≤0.008-0.06	0.016	0.03	100.0
A. fumigatus (30)	Anidulafungin[b]	≤0.008-8	0.016	0.03	–[c]
	Amphotericin B	0.5-2	1	2	60.0
	5-fluorocytosine	4->64	>64	>64	3.3
	Fluconazole	128->256	256	>256	0.0
	Itraconazole	0.25-2	0.5	2	0.0
	Ketoconazole	1-8	4	8	–[c]
	Voriconazole	0.12-1	0.5	0.5	100.0

a. NCCLS criteria for susceptibility were used where available; ≤1 μg/ml for amphotericin B and voriconazole was used for comparisons only. (Diag. Microbiol. Infect. Dis. 2004; 48:101; Antimicrob. Agents Chemother. 2002; 46:1032)

b. Endpoint was a prominent decrease in growth at 48 hours, MEC.

c. Susceptibility breakpoints not established

Table 2. Variations in anidulafungin MIC results at two incubation intervals (24 or 48 hours [h]) and two endpoint interpretation criteria (complete and 50% inhibition) tested against the four most frequently isolated Candida spp. (817 strains)

Organism (no. tested)	Incubation time	Endpoint criteria	Occurrence at MIC (μg/ml): ≤0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8
C. albicans (500)	24 h	50%	56	53	257	128	6	0	0	0	0	0	0
		Complete	2	27	149	230	90	2	0	0	0	0	0
	48 h	50%	44	47	264	135	10	0	0	0	0	0	0
		Complete	1	20	129	240	106	4	0	0	0	0	0
C. glabrata (105)	24 h	50%	1	2	30	52	16	4	0	0	0	0	0
		Complete	0	1	6	37	43	16	2	0	0	0	0
	48 h	50%	0	1	33	50	17	4	0	0	0	0	0
		Complete	0	0	5	37	45	16	2	0	0	0	0
C. parapsilosis (106)	24 h	50%	0	0	0	0	2	2	12	35	42	12	1
		Complete	0	0	0	0	1	0	6	19	59	18	3
	48 h	50%	0	0	0	0	2	2	10	24	38	24	6
		Complete	0	0	0	0	1	0	5	8	48	34	10
C. tropicalis (106)	24	50%	4	14	54	30	1	1	1	0	1	0	0
		Complete	0	6	32	34	22	8	1	0	3	0	0
	48 h	50%	3	9	52	31	6	3	0	0	2	0	0
		Complete	0	3	22	39	26	5	6	2	3	0	0

Table 3. Distribution of anidulafungin MIC (yeasts) and MEC (moulds) values tested against 67 strains of rarely isolated yeast and moulds

Organism (no. tested)	MIC or MEC (μg/ml) occurrence distributions: ≤0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	≥8
A. flavus (7)	2	4	1	-	-	-	-	-	-	-	-
A. nidulans (2)	-	1	1	-	-	-	-	-	-	-	-
A. niger (6)	4	1	1	-	-	-	-	-	-	-	-
A. terreus (4)	2	1	1	-	-	-	-	-	-	-	-
A. versicolor (2)	1	1	-	-	-	-	-	-	-	-	-
C. dubliniensis (2)	-	-	1	1	-	-	-	-	-	-	-
C. humicolus (1)	-	-	1	-	-	-	-	-	-	-	-
C. kefyr (5)	-	-	3	2	-	-	-	-	-	-	-
C. lipolytica (5)	-	-	2	3	-	-	-	-	-	-	-
C. pelliculosa (3)	2	1	-	-	-	-	-	-	-	-	-
C. rugosa (1)	-	-	-	-	1	-	-	-	-	-	-
C. famata (2)	-	-	-	-	-	-	-	1	1	-	-
C. guilliermondii (8)	-	-	-	-	-	1	1	2	3	1	-
C. pulcherrima (1)	-	-	-	-	-	1	-	-	-	-	-
C. sake (1)	-	-	-	-	-	-	-	-	-	1	-
Penicillium spp. (6)	2	-	2	1	1	-	-	-	-	-	-
Curvularia spp. (2)	-	-	-	-	1	-	1	-	-	-	-
Paecilomyces spp. (2)	-	-	1	-	-	-	-	-	-	-	1
Fusarium spp. (2)	-	-	-	-	-	-	-	-	-	-	2
Rhizopus spp. (2)	-	-	-	-	-	-	-	-	-	-	2
Trichosporon spp. (3)	-	-	-	-	-	-	-	-	-	-	3

- Among the 6 yeast species most frequently isolated in this study, all *C. albicans*, *C. glabrata* and *C. krusei* were inhibited by ≤0.25 μg/ml of ANID; 98% of *C. tropicalis* and 92% of *C. lusitaniae* were inhibited by ≤1 μg/ml.
- Notably, ANID demonstrated excellent activity against those species that are intrinsically resistant to FLUCON and ITRA (*C. krusei*) or in which resistance is frequent (*C. glabrata*, 47.6% non-susceptible to FLUCON in this study).
- Anidulafungin was somewhat less active against *C. parapsilosis*, *C. guilliermondii*, and *C. famata*, as has been reported previously for all echinocandins.
- MIC distributions of ANID using different incubation times and endpoints are shown in **Table 2** for the 4 more commonly encountered *Candida* species. MIC_{50} and MIC_{90} values for *C. albicans*, *C. glabrata*, and *C. parapsilosis* remained constant whether read at 24 or 48 h. For *C. tropicalis*, the MIC_{90} increased two-fold for both endpoint criteria when determined at 48 vs 24 h.
- The data support an incubation time of 24 h as appropriate for testing ANID against yeasts. A recent multi-laboratory QC study demonstrated better inter-laboratory reproducibility with the 50% inhibition endpoint as compared with complete inhibition.
- ANID and VORI exhibited greater activity against *A. fumigatus* than FLUCON and ITRA (**Table 1**).
- Excellent ANID activity was observed against other *Aspergillus* spp., *Penicillium* spp., and *Curvularia* spp. (**Table 3**). ANID was not active against *Fusarium*, *Rhizopus*, or *Trichosporon* isolates.

CONCLUSIONS

- These results from a longitudinal study expand the information available for ANID activity *in vitro* against a broad range of clinical yeasts and moulds from different geographical areas.
- The study confirms the excellent *in vitro* ANID activity against *Aspergillus* spp., for which there are a limited number of treatment options, as well as against *Candida* spp. with decreased susceptibility to the azoles.
- ANID is in late stage clinical development and recent reports have demonstrated its efficacy in Phase 2 and 3 studies of candidemia and mucosal candidiasis, as well as its tolerability and lack of drug interactions.
- These current results provide additional support for the continuing clinical development of anidulafungin.

SELECTED REFERENCES

Arevalo MP, Carrillo-Munoz AJ, Salgado J. (2003). Antifungal activity of the echinocandin anidulafungin (VER002, LY-303366) against yeast pathogens: A comparative study with M27-A microdilution method. *Journal of Antimicrobial Chemotherapy* 51:163-166.

Marco F, Danes C, Almela M. (2003). Trends in frequency and *in vitro* susceptibilites to antifungal agents, including voriconazole and anidulafungin, of Candida bloodstream isolates. Results from a six year study (1996-2001). *Diagnostic Microbiology and Infectious Disease* 46:259-264.

National Committee for Clinical Laboratory Standards. (2002). *Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved standard, 2nd ed. M27-A2.* Wayne, PA:NCCLS.

National Committee for Clinical Laboratory Standards. (2002). *Reference method for broth dilution antifungal susceptibility testing of filamentous fungi. Approved standard M38-A.* Wayne, PA:NCCLS.

Oakley KL, Moore CB, Denning DW. (1998). *In vitro* activity of the echinocandin antifungal agent LY303,366 in comparison with itraconazole and amphotericin B against *Aspergillus* spp. *Antimicrobial Agents and Chemotherapy* 42:2726-2730.

Odabasi Z, Paetznick VL, Rodriguez JR. (2004). *In vitro* activity of anidulafungin against selected clinically important mold isolates. *Antimicrobial Agents and Chemotherapy* 48:1912-1915.

Pfaller MA, Marco F, Messer SA, Jones RN. (1998). *In vitro* activity of two echinocandin derivatives, LY303366 and MK-0991 (L-743,792), against clinical isolates of *Aspergillus, Fusarium, Rhizopus and other filamentous fungi. Diagnostic Microbiology and Infectious Disease* 30:251-255.

Roberts J, Schock K, Marino S, Andriole VT. (2000). Efficacies of two new antifungal agents, the triazole ravuconazole and the echinocandin LY 303366, in an experimental model of invasive aspergillosis. *Antimicrobial Agents and Chemotherapy* 44:3381-3388.

Krisher K, Brown SD, Traczewski MM. (2004). Quality control parameters for broth microdilution tests of anidulafungin. *Journal of Clinical Microbiology* 42:490.

Klepser ME, Ernst EJ, Ernst ME, Pfaller MA. (1997). Growth medium effect on the antifungal activity of LY 303366. *Diagnostic Microbiology and Infectious Disease* 29:227-231.

Krause DS, Reinhardt J, Vazquez JA. (2004). Phase 2, randomized, dose-ranging study evaluating the safety and efficacy of anidulafungin in invasive candidiasis and candidemia. *Antimicrobial Agents and Chemotherapy* 48:2021-2024.

Krause, D.S., Simjee, A.E., van Rensburg, C., et al. (2004). A randomized double-blind trial of anidulafungin versus fluconazole for the treatment of esophageal candidiasis. In press in Clinical Infectious Disease.

Dowell JA, Knebel W, Ludden T. (2004). Population pharmacokinetic analyses of anidulafungin, an echinocandin antifungal. *Journal of Clinical Pharmacology* 44:590-598.