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Activities of Caspofungin, Itraconazole, Posaconazole, Ravuconazole, Voriconazole, and Amphotericin B against 448 Recent Clinical Isolates of Filamentous Fungi

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We examined the in vitro activity of caspofungin, posaconazole, voriconazole, ravuconazole, itraconazole, and amphotericin B against 448 recent clinical mold isolates. The endpoint for reading caspofungin was the minimum effective concentration (MEC). Among the triazoles, posaconazole was most active, inhibiting 95% of isolates at ≤1 μg/ml, followed by ravuconazole (91%), voriconazole (90%), and itraconazole (79%). Caspofungin and amphotericin B inhibited 93% and 89% of isolates at ≤1 μg/ml, respectively, with caspofungin demonstrating an MEC 90 of 0.12 μg/ml. All three new triazoles and caspofungin inhibited >95% of *Aspergillus* spp. at ≤1 μg/ml compared to 83% for itraconazole and 91% for amphotericin B. Amphotericin B inhibited only 38% of *Aspergillus terreus* isolates at ≤1 μg/ml, whereas the three new triazoles and caspofungin inhibited all *A. terreus* at ≤0.5 μg/ml. The new triazoles and caspofungin have excellent in vitro activity against a very large collection of recent clinical isolates of *Aspergillus* spp., and some in vitro activity against selected other filamentous fungi.

Invasive infections due to *Aspergillus* spp. and other filamentous fungi have emerged as prominent causes of infectious morbidity and mortality in the United States and worldwide (6, 7, 20). Treatment of these infections with available antifungal agents still results in an unacceptably high associated mortality (18).

Two new antifungal agents recently have been introduced for treatment of invasive aspergillosis or other invasive mold infections. Voriconazole and caspofungin offer new alternatives for therapy of these difficult infections (10, 11). In addition, the investigational triazoles ravuconazole and posaconazole have been demonstrated to have in vitro potency against *Aspergillus* spp. and selected other filamentous fungi (8, 9, 24).

Since the availability of these agents represents an exciting opportunity for improving the outcome of invasive infections due to filamentous fungi, their in vitro activity against contemporary clinical isolates is of great interest. We performed a 2-year, 20-center survey of filamentous fungal infections from January 2000 through December 2001. We previously reported preliminary results of the activity of new triazoles against molds collected during the first year of this survey (24). We now report final 2-year results, including the in vitro activity of caspofungin, the new triazoles, amphotericin B, and itraconazole against over 400 recent clinical isolates of filamentous fungi collected from January 2000 through December of 2001. In the process, we present the largest collection of clinical mold isolates tested against caspofungin yet reported.

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MATERIALS AND METHODS

Organisms. A total of 448 unique clinical isolates (one per patient, duplicate patient isolates excluded) of filamentous fungi were obtained from 20 different medical centers in the United States and Canada between January 2000 and December 2001. These centers were participants in the SENTRY Antimicrobial Surveillance Program. The isolates were obtained from sputum, bronchoscopy, and tissue biopsy specimens. The collection of isolates included 372 isolates of *Aspergillus* spp., including 256 *Aspergillus fumigatus*, 30 *A. flavus*, 29 *A. niger*, 20 *A. versicolor*, 16 *A. terreus*, 4 *A. nidulans*, and 3 *A. ustus* isolates, 1 *A. oryzae* isolate, 1 *A. glaucus* isolate, and 12 isolates of unspecified *Aspergillus* spp. The collection included 76 other filamentous fungi, including 35 *Penicillium* spp., 11 *Fusarium* spp., 6 *Paecilomyces* spp., 5 *Rhizopus* spp., 3 *Mucor* spp., and 2 *Scedosporium apiospermum* isolates and one isolate each of *Absidia* sp., *Acremonium* sp., *Bipolaris* sp., *Chrysosporium* sp., *Cladophialophora bantiana*, *Curvularia* sp., *Exophiala dermatitidis*, *Fonsecaeae pedrosoi*, *Geotrichum* sp., *Pithomyces* sp., *Trichophyton mentagrophytes*, *Trichophyton* sp., *Ulocladium* sp., and *Wangiella* sp. All isolates were sent on Amies charcoal transport media to the central laboratory at the University of Iowa for reference identification and susceptibility testing. They were stored as spore suspensions in sterile distilled water at room temperature. Before being tested, each isolate was subcultured at least twice on potato dextrose agar (Remel, Lenexa, Kans.) to ensure viability and purity.

Susceptibility testing. Posaconazole (Schering-Plough Research Institute, Kenilworth, N.J.), ravuconazole (Bristol-Myers Squibb, Wallingford, Conn.), voriconazole (Pfizer Pharmaceutical Group, New York, N.Y.), caspofungin (Merck Research Laboratories, Rahway, N.J.), and amphotericin B (Sigma Chemical Co., St. Louis, Mo.) were all obtained as reagent-grade powders from their respective manufacturers. The broth microdilution method was performed according to NCCLS guidelines (21). Stock solutions of voriconazole, ravuconazole, and amphotericin B were prepared in dimethyl sulfoxide (Sigma), itraconazole and posaconazole were prepared in polyethylene glycol, and caspofungin was prepared in water; all were diluted to 100 times their final concentrations, further diluted in RPMI 1640 medium buffered to pH 7.0 with morpholinepropanesulfonic acid buffer, and dispensed into 96-well microdilution trays. Trays containing a 0.1-ml aliquot in each well of the appropriate drug solution (2× final concentration) were subjected to quality control and then sealed and stored at −70°C until used. The final concentrations of the drugs in the wells ranged from 0.008 to 8.0 μg/ml. The stock conidial suspension (10^6 spores/ml) was diluted to a final inoculum concentration of 0.4×10^4 to 5×10^4 CFU/ml and dispensed

TABLE 1. In vitro susceptibilities of filamentous fungi isolated during 2000 and 2001

Species (no. of isolates tested)	Antifungal agent	MIC or MEC[a] (μg/ml)		MIC or MEC[a] range (μg/ml)	% Total at ≤1 μg/ml	Species (no. of isolates tested)	Antifungal agent	MIC or MEC[a] (μg/ml)		MIC or MEC[a] range (μg/ml)	% Total at ≤1 μg/ml
		50%	90%					50%	90%		
A. fumigatus (256)	Amphotericin	1	1	0.5–>8	96.1	*Fusarium* spp. (11)	Amphotericin	1	2	1–2	81.8
	Caspofungin	0.03	0.06	0.015–2	99.2		Caspofungin	>8	>8	>8–>8	0
	Itraconazole	1	2	0.12–2	87.1		Itraconazole	>8	>8	2–>8	0
	Posaconazole	0.25	0.5	0.03–2	99.6		Posaconazole	>8	>8	0.5–>8	18.2
	Ravuconazole	0.25	0.5	0.06–2	98.8		Ravuconazole	8	>8	0.25–>8	18.2
	Voriconazole	0.25	0.5	0.06–4	98.8		Voriconazole	4	>8	0.25–>8	18.2
A. flavus (30)	Amphotericin	1	2	1–2	70.0	*Paecilomyces* spp. (6)	Amphotericin	0.5		0.06–>8	66.7
	Caspofungin	0.03	0.06	0.015–0.12	100		Caspofungin	0.06		0.03–8	83.3
	Itraconazole	0.5	1	0.25–2	96.7		Itraconazole	0.25		0.06–2	83.3
	Posaconazole	0.25	0.5	0.12–1	100		Posaconazole	0.12		0.03–0.5	100
	Ravuconazole	0.5	1	0.06–1	100		Ravuconazole	0.25		0.03–4	83.3
	Voriconazole	0.5	1	0.12–1	100		Voriconazole	0.25		0.03–2	83.3
A. niger (29)	Amphotericin	1	1	0.25–1	100	*Rhizopus* spp. (5)	Amphotericin	1		0.5–1	100
	Caspofungin	0.03	0.06	0.015–0.06	100		Caspofungin	>8		>8–>8	0
	Itraconazole	2	2	0.5–4	44.8		Itraconazole	4		1–>8	20
	Posaconazole	0.5	1	0.25–1	100		Posaconazole	2		1–4	40
	Ravuconazole	0.5	2	0.25–2	86.2		Ravuconazole	>8		0.5–>8	20
	Voriconazole	1	2	0.12–4	65.5		Voriconazole	2		1–>8	40
A. versicolor (20)	Amphotericin	1	2	1–2	80.0	*Mucor* spp. (3)	Amphotericin	0.5		0.5–1	100
	Caspofungin	0.03	0.12	0.015–4	90.5		Caspofungin	>8		>8–>8	0
	Itraconazole	1	2	0.12–2	65.0		Itraconazole	2		2–>8	0
	Posaconazole	0.5	1	0.06–2	90.0		Posaconazole	1		0.5–>8	66.7
	Ravuconazole	0.25	1	0.03–2	95.2		Ravuconazole	>8		1–>8	33.3
	Voriconazole	0.5	1	0.06–2	95.0		Voriconazole	2		1–>8	33.3
A. terreus (16)	Amphotericin	2	2	1–4	37.5	All *Aspergillus* (372)	Amphotericin	1	1	0.12–>8	90.6
	Caspofungin	0.03	0.06	0.015–0.12	100		Caspofungin	0.03	0.06	0.015–4	98.4
	Itraconazole	0.5	0.5	0.25–1	100		Itraconazole	1	2	0.12–>8	83.1
	Posaconazole	0.12	0.25	0.06–0.25	100		Posaconazole	0.25	0.5	0.03–>8	98.1
	Ravuconazole	0.25	0.5	0.03–0.5	100		Ravuconazole	0.25	1	0.03–>8	96.8
	Voriconazole	0.25	1	0.06–0.5	100		Voriconazole	0.5	1	0.03–>8	95.2
Penicillium spp. (35)	Amphotericin	1	2	0.12–2	85.7	All filamentous fungi (448)	Amphotericin	1.0	2.0	0.06–>8	89.3
	Caspofungin	0.03	0.12	0.015–>8	97.4		Caspofungin	0.03	0.12	0.015–>8	92.8
	Itraconazole	1	2	0.25–2	77.1		Itraconazole	1.0	2.0	0.06–>8	79.0
	Posaconazole	0.5	1	0.06–2	97.1		Posaconazole	0.25	1.0	0.03–>8	94.6
	Ravuconazole	0.5	4	0.015–8	80.0		Ravuconazole	0.25	1.0	0.015–>8	91.3
	Voriconazole	0.5	2	0.03–>8	80.0		Voriconazole	0.5	1.0	0.03–>8	90.2

[a] The MIC is given for all agents except caspofungin, for which the MEC is given (9, 10).

into the microdilution wells. The inoculated microdilution trays were incubated at 35°C and read at 48 h. The MIC endpoint for the azoles and amphotericin B was defined as the lowest concentration that produced complete inhibition of growth, whereas the minimum effective concentration (MEC) endpoint for caspofungin was defined according to published methods (1, 15).

Quality control. Quality control was ensured by testing the following strains (4, 21): *A. flavus* ATCC 204304, *Candida parapsilosis* ATCC 22019, and *Candida krusei* ATCC 6258. All results were within the recommended limits of the NCCLS (21) or other published limits if NCCLS recommended limits were not available (e.g., for caspofungin) (4).

Statistical analysis. MIC distributions for different triazole antifungal agents were compared by using the Wilcoxin signed-rank test. The alpha value was set at 0.05, and all *P* values were two tailed.

RESULTS AND DISCUSSION

Table 1 presents the in vitro activities of each antifungal agent against the *Aspergillus* spp., including the percentage of isolates of each species that were inhibited at an MIC or MEC of ≤1 μg of the antifungal agents/ml. Each of three new and investigational triazoles (posaconazole, ravuconazole, and voriconazole) had greater in vitro activity than itraconazole against the *Aspergillus* isolates tested ($P < 0.01$ for difference in MIC distribution of new triazoles compared to itraconazole against *A. fumigatus*, *A. niger*, *A. versicolor*, and all *Aspergillus* spp. combined). These data confirm previous reports of the in vitro activity of the new and investigational triazoles against *Aspergillus* spp. (8, 9, 16, 17, 22). The clinical promise of one new triazole, voriconazole, was demonstrated in a recent report of its superiority to amphotericin B as primary therapy of invasive aspergillosis (10).

Caspofungin, using MEC as the in vitro susceptibility testing endpoint (1, 15), also had excellent in vitro activity against all species of *Aspergillus* tested, inhibiting 90% of isolates at an MEC of 0.06 μg/ml and >98% at an MEC of ≤1 μg/ml. Caspofungin has been approved by the U.S. Food and Drug Administration for use in refractory cases of invasive aspergillosis and may hold promise for treatment of amphotericin B-resistant aspergillosis or as part of combination regimens with triazoles or amphotericin (2, 13, 14, 26). Importantly, direct comparisons of MIC and MEC values should not be made, since they represent different inhibition endpoints for different antifungal classes. In particular, the MEC endpoint for echinocandins recognizes that they do not produce complete macroscopic growth inhibition of *Aspergillus* spp. but rather partial inhibition associated with the development of short, stubby, highly branched, and abnormal hyphae (1, 15).

Of particular note, the triazoles and caspofungin all had excellent in vitro activity against *A. terreus* (100% inhibited at an MIC or MEC of $\leq$1 μg/ml), a species against which amphotericin B demonstrated poor in vitro activity (38% inhibited at an MIC of $\leq$1 μg/ml) and against which amphotericin B has poor clinical efficacy (12).

Table 1 also shows the activity of each agent against other species of filamentous fungi that were isolated in sufficient numbers to merit examining individually. All tested agents were less active against these filamentous fungi than against *Aspergillus* spp. Caspofungin, which is not generally considered to be active against molds other than *Aspergillus* spp., demonstrated some in vitro activity against *Penicillium* spp. (MEC90, 0.12 μg/ml; 97% inhibited at an MEC of $\leq$1 μg/ml) and *Paecilomyces* spp. (five of six isolates inhibited at an MEC of $\leq$1 μg/ml) but no activity against *Fusarium* spp. or the zygomycetes. It remains to be seen whether caspofungin or other echinocandins will have any role, either alone or in combination with other agents (2, 3, 27), in the treatment of infections due to molds other than *Aspergillus* spp.

The new and investigational triazoles also demonstrated some activity against miscellaneous molds, particularly *Penicillium* and *Paecilomyces* spp. None of the agents except amphotericin B had good in vitro activity against *Fusarium* spp. (90% MIC [MIC_{90}] or MEC_{90} of >8 μg/ml for all triazoles and caspofungin). These data are consistent with previously published in vitro data (8, 9, 15, 17, 19, 24, 25). Despite poor in vitro activity, the clinical response to voriconazole and posaconazole has been described in some cases of *Fusarium* spp. infection (23; R. Y. Hachem, I. I. Raad, C. M. Afif, et al., 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1009, 2000). We report in vitro results for only eight zygomycetes (five *Rhizopus* and three *Mucor* spp.). The zygomycetes have been demonstrated to be a heterogeneous group with regard to in vitro antifungal susceptibility (5), and more data are needed in order to determine which new or investigational agents are active against individual species.

In summary, caspofungin and the new triazoles posaconazole, ravuconazole, and voriconazole have excellent in vitro activity against *Aspergillus* spp. and variable activity against selected other filamentous fungi. In addition, an in vitro-in vivo correlation is needed for both new and established antifungal agents against the filamentous fungi.

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