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In Vitro Activities of Posaconazole, Itraconazole, Voriconazole, Amphotericin B, and Fluconazole against 37 Clinical Isolates of Zygomycetes

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In vitro antifungal susceptibility testing results of a new antifungal triazole, posaconazole (POS), were compared to results with amphotericin B (AMB), itraconazole (ITC), voriconazole (VRC), and fluconazole (FLC) against clinical agents of zygomycosis. The MICs of POS at which 50% and 90% of the isolates were inhibited were 0.25 and 4 μg/ml, respectively. POS was significantly more active than VRC and FLC and slightly more active than ITC. The results suggest that POS has significant potential for clinical development against the zygomycetes.

Invasive mycoses have increased dramatically over the past several years, largely as a result of increasing numbers of immunosuppressed patients. The zygomycetes are less common causes of invasive human infection compared to *Aspergillus* spp. and *Candida* spp. In several large studies of the occurrence of fungal infection in high-risk populations, infections with zygomycetes represented 5 to 12% of all fungal infections (1, 14), while in another study, zygomycosis represented up to 25 to 44% of all invasive fungal diseases (6, 9). The manifestations of zygomycosis include primary rhinocerebral, pulmonary, gastrointestinal, cutaneous or subcutaneous, or allergic disease and disseminated disease (11). Amphotericin B (AMB) is the first-line therapy of choice for most cases of zygomycosis, but its use is limited by its potentially severe side effects.

In the study described here, we compared the in vitro activities of four triazoles and AMB against 37 clinical isolates of zygomycetes. The isolates included 7 isolates of *Mucor* spp., 10 isolates of *Rhizopus* spp., 5 isolates of *Absidia coryambifera*, 5 isolates of *Cunninghamella* spp., 4 isolates of *Apophysomyces elegans*, 2 isolates of *Cokeromyces recurvatus*, and 4 isolates of *Saksenaea vasiformis*. All isolates came from the Fungus Testing Laboratory at The University of Texas Health Science Center (UTHSC) at San Antonio. Isolates were retrieved from storage at −70°C or from water stocks and were subcultured onto slants of potato flake agar with incubation at room temperature until adequate growth was obtained. *Paecilomyces variotii* strain UTHSC 90-459 was used as a control. MIC endpoints were read visually after 24 and 48 h of incubation. Standard antifungal powders of posaconazole (POS; Schering-Plough), voriconazole (VRC; Pfizer), fluconazole (FLC; Pfizer), itraconazole (ITC; Janssen), and AMB (Bristol-Myers Squibb) were obtained from their respective manufacturers. Stock solutions of POS, VRC, and ITC were prepared in polyethylene glycol 400, while AMB and FLC were dissolved in water. Final dilutions of POS, VRC, FLC, and ITC were made in RPMI 1640 medium with L-glutamine and morpholinepropanesulfonic acid buffer (Angus Chemical Co., Niagara Falls, N.Y.). AMB was made with Antibiotic Medium 3 (Difco). Serial twofold dilutions of each antifungal agent were prepared to the following final drug concentrations: AMB, 0.03 to 16 μg/ml; POS and ITC, 0.015 to 8 μg/ml; FLC and VRC, 0.125 to 64 μg/ml. Stock solutions of these drugs were stored at −70°C until use. Fungal strains were cultured on potato flake agar slants at room temperature for 1 week. Tested strains were overlaid with sterile distilled water and suspensions were obtained by gently scraping the surface of colonies with the tip of a sterile wooden applicator stick. Large fragments were allowed to settle, and homogeneous suspensions of conidia were removed and adjusted to a concentration of 10^5 conidia/ml by adding sterile distilled water and counting with a hemacytometer. Fungal suspensions were diluted 1:10 with RPMI medium or Antibiotic Medium 3 to obtain final suspensions. A drug-free growth control was included for each isolate.

MICs were determined by a broth microdilution method by following NCCLS recommendations for filamentous fungi (standard M38-P [7]). Previously prepared aliquots containing 0.1 ml of the various drug concentrations were allowed to thaw at room temperature and inoculated with 0.9 ml of each suspension. Tubes were incubated at 35°C for 24 and 48 h. MICs were read visually and were defined at 48 h of incubation as the lowest concentration resulting in 80% inhibition of growth compared with that of the growth control for POS, ITC, VRC, and FLC and as the first tube that was optically clear for AMB. The control strain *P. variotii* UTHSC 90-459 was used in all tests. Testing of these isolates was performed in duplicate.

The MIC results are summarized in Table 1. The mean of the MIC of POS was 1.22 μg/ml, which was slightly more active

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TABLE 1. In vitro activities of five antifungal agents against 37 strains of zygomycetes

Organism (no. of isolates) and agent	MIC (μg/ml)			
	Mean	Range	50%[a]	90%[a]
Mucor spp. (7)				
POS	1.54	0.125–8	0.5	1
ITC	2.18	0.25–8	1	2
VRC	59	32–>64	>64	>64
FLC	>64	>64	>64	>64
AMB	0.24	0.06–0.5	0.25	0.25
Rhizopus spp. (10)				
POS	2.73	0.25–8	1	8
ITC	3.93	0.25–8	1	8
VRC	51.2	32–>64	>64	>64
FLC	>64	>64	>64	>64
AMB	0.33	0.06–2	0.125	0.5
Absidia coryambifera (5)				
POS	0.13	0.03–0.25	0.03	0.25
ITC	0.14	0.03–0.25	0.06	0.25
VRC	48	16–>64	>64	>64
FLC	>64	>64	>64	>64
AMB	0.30	0.25–0.5	0.25	0.25
Cunninghamella spp. (5)				
POS	0.36	0.03–1	0.25	1
ITC	0.60	0.125–2	0.25	0.5
VRC	43.2	8–>64	16	>64
FLC	>64	>64	>64	>64
AMB	0.55	0.125–2	0.25	0.25
Apophysomyces elegans (4)				
POS	1.57	0.03–4	0.25	2
ITC	2.63	0.03–8	0.5	2
VRC	44	16–>64	32	>64
FLC	>64	>64	>64	>64
AMB	0.33	0.03–1	0.03	0.25
Cokeromyces recurvatus (2)				
POS	2.13	0.25–4	0.25	4
ITC	4.13	0.25–8	0.25	8
VRC	40	16–>64	16	>64
FLC	>64	>64	>64	>64
AMB	0.31	0.125–2	0.125	2
Saksenaea vasiformis (4)				
POS	0.11	0.015–0.25	0.06	0.125
ITC	0.05	0.015–0.03	0.015	0.03
VRC	2.62	0.5–4	2	4
FLC	20.74	1–64	2	16
AMB	0.23	0.125–2	0.125	0.25
All isolates (37)				
POS	1.22	0.015–8	0.25	4
ITC	1.95	0.015–8	0.5	8
VRC	41.14	0.5–>64	>64	>64
FLC	57.82	1–>64	>64	>64
AMB	0.33	0.03–2	0.25	0.5

[a] MIC at which 50% or 90% of the isolates were inhibited.

than ITC (MIC mean, 1.95 μg/ml) and considerably more active than VRC (MIC mean, 41.14 μg/ml) and FLC (MIC mean, 57.82 μg/ml). POS had a MIC of <8 μg/ml for 91.9% of isolates tested, and ITC had a MIC of <8 μg/ml for 81.1% of the isolates. Overall, the isolates were generally susceptible to POS, ITC, and AMB. Most isolates were resistant to FLC, while only one isolate was susceptible to VCR. Both POS and ITC were more active than VRC and FLC against this group of fungi, and POS was slightly more active than ITC.

Organisms within the class *Zygomycetes* are found in two orders: *Mucorales* and *Entomophthorales*. For the most part, the *Mucorales* organisms are considered to be opportunistic pathogens, with members of the genera *Rhizopus*, *Mucor*, *Rhizomucor*, *Abisdea*, *Apophysomyces*, *Saksenaea*, *Cunninghamella*, and *Cokeromyces* all having been implicated as causing human disease (4, 13). Because zygomycosis is less common than aspergillosis and the course is progressively rapid, the effectiveness of antifungal chemotherapy in small case studies is difficult to evaluate. AMB is the first-line drug of choice for most cases of zygomycosis caused by the *Mucorales*, but it is not an effective treatment in many cases, particularly if the patient presents late in the disease course or has disseminated disease (11). Also, there is no clinical support available for the use of FLC or VRC in zygomycosis, and experience with ITC is limited to scattered case reports (2, 10). POS has been shown to have potent in vitro and in vivo activities against *Candida* spp., *Cryptococcus neoformans*, *Aspergillus* spp., *Blastomyces dermatitidis*, and *Coccidioides immitis* (3, 5, 8, 12). In our study, POS also had good activity against zygomycetes. In that the MICs (means) of POS were about 1.6-fold lower than those of ITC, 33-fold lower than those of VRC, and 47-fold lower than those of FLC, this suggests that POS may be useful in the treatment of zygomycosis. However, further in vivo studies with experimental animal models are needed to confirm this activity.

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