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In vitro activities of isavuconazole against opportunistic filamentous and dimorphic fungi

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The *in vitro* activity of isavuconazole was compared to those of amphotericin B, fluconazole, itraconazole, voriconazole, posaconazole, and ravuconazole against 300 clinical isolates of *Pseudallescheria boydii*, *Paecilomyces lilacinus*, *Fusarium* spp., *Bipolaris spicifera*, *Curvularia lunata*, *Alternaria alternata*, *Exophiala* spp., *Rhizopus arrhizus*, *Mucor circillenoides*, *Absidia corymbifera*, *Blastomyces dermatitidis*, *Histoplasma capsulatum* and *Coccidioides posadasii*. MICs were determined by a broth macrodilution method based on the CLSI M38-A procedure. The triazoles were relatively uniform in that they showed strong *in vitro* inhibitory activity against most of the tested fungi. *In vitro* activity was variable with strains of *P. lilacinus* while with *Fusarium* spp., the triazoles were found to have limited *in vitro* activity and amphotericin B was moderately active. The results suggest that isavuconazole is a broad-spectrum antifungal agent, effective against a wide range of moulds *in vitro*.

Keywords Isavuconazole, triazoles, filamentous fungi, *in vitro* activity

Introduction

Fungal infections are found with relatively high frequency in several patient groups, especially those with cancer having chemotherapy-induced neutropenia, HIV-infected patients, transplant recipients and other individuals receiving immunosuppressive treatments. While opportunistic infections with organisms such as *Candida* spp. and *Aspergillus* spp. are more commonly seen in neutropenic patients, hyalohyphomycetes, phaeohyphomycetes and zygomycetes are emerging as the causes of a variety of infections in humans [1,2]. Dimorphic fungi such as *Histoplasma capsulatum*, *Coccidioides* spp. and *Blastomyces dermatitidis* may also be the agents of severe disease [3,4].

Amphotericin B, fluconazole, and itraconazole have been the most important drugs used for the treatment of serious fungal infections. The limitations of amphotericin B, fluconazole, and itraconazole involving their efficacy and tolerability are well known. Therefore, systemic antifungal triazoles, i.e., voriconazole, posaconazole, and ravuconazole were introduced. The newer triazoles have similar enhanced potency and broad-spectrum antifungal activity against *Candida* spp., *Trichosporon* spp., *Cryptococcus neoformans*, *Aspergillus* spp., *Fusarium* spp., dematiaceous as well as dimorphic moulds and some zygomycetes [5–8].

Isavuconazole (BAL4815) is a novel triazole agent that acts by inhibiting fungal cytochrome P-450, 14-alpha –sterol demethylase-mediated synthesis of ergosterol [9]. It appears to have a broad spectrum of activity against opportunistic, as well as dimorphic fungi, although isolates tested to date are limited in number and diversity. Warn *et al*. evaluated the *in vitro* activity of isavuconazole against 118 isolates of *Aspergillus* spp. and demonstrated that isavuconazole had potent antifungal action against four different *Aspergillus* species (*A. fumigatus*, *A. terreus*, *A. flavus* and A. *niger*) including strains resistant to itraconazole, amphotericin B, and caspofungin [10].

In this study the activity of isavuconazole was compared with amphotericin B, fluconazole, itraconazole, voriconazole, posaconazole and ravuconazole

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against 300 opportunistic filamentous and dimorphic fungi.

Materials and methods

Isolates

A total of 300 clinical isolates of opportunistic filamentous and dimorphic fungi were selected for this study, including *Pseudallescheria boydii* (28), *Paecilomyces lilacinus* (22), *Fusarium* spp. (30), *Bipolaris spicifera* (30), *Curvularia lunata* (24), *Alternaria alternata* (30), *Exophiala* spp. (12), *Rhizopus arrhizus* (27), *Mucor circillenoides* (16), *Absidia corymbifera* (17), *Blastomyces dermatitidis* (6), *Histoplasma capsulatum* (28) and *Coccidioides posadasii* (30). The isolates were identified at the Departamento de Microbiología, Facultad de Medicina, Universidad Autónoma de Nuevo León, México. *C. posadasii* isolates were identified by molecular methods [11], while standard morphologic methods were used in the identification of all other isolates. The isolates were stored as a water suspension at ambient temperature until used for this study.

Inoculum preparation

Prior to testing, each isolate was subcultured at least twice on in-house prepared potato dextrose agar slants [12] at 35°C for 7–15 days to ensure purity and optimal growth. Cultures for *Fusarium* spp. were grown at 35°C for 48–72 h and then at 25–28°C for the remainder of a 7-day period.

Antifungal agents

Isavuconazole (Basilea Pharmaceutica, Basel, Switzerland), fluconazole and voriconazole (Pfizer, New York, NY), itraconazole (Janssen Pharmaceutica, Beerse, Belgium), ravuconazole (Eisai Co. Ltd, Tokyo, Japan) and posaconazole (Schering-Plough Research Institute, Kenilworth, NJ) were obtained as reagent-grade powders from their respective manufacturers. Stock solutions were prepared in polyethylene glycol (itraconazole, voriconazole, and posaconazole), water (fluconazole), and dimethylsulfoxide (isavuconazole and ravuconazole). Amphothericin B was purchased as the sodium deoxycholate formulation (Bristol-Myers Squibb, Princeton, NJ) and dissolved in 5% dextrose to produce a stock solution of 5 mg/ml. The drug stock was diluted in sterile water to produce the required concentrations.

Serial twofold dilutions of each antifungal agent were prepared to obtain final drug concentrations that ranged from 0.125–64 μg/ml for fluconazole and voriconazole and from 0.015–8 μg/ml for itraconazole, isavuconazole, posaconazole, ravuconazole, and amphotericin B. The drug solutions were dispensed as 0.1ml volumes into sterile polystyrene 12×75 mm tubes and stored at -20°C until used.

Media

Triazoles were tested in RPMI-1640 with L-glutamine and morpholinepropanesulfonic acid buffer at a concentration of 165 mmol/l (Angus, Niagara Falls, NY). Amphotericin B studies were conducted in Antibiotic Medium 3 (Difco, Detroit, Mich.) buffered with 10 mM phosphate.

Broth macrodilution method

Isolates were evaluated using the Clinical and Laboratory Standards Institute broth macrodilution approved standard reference method M38-A for broth dilution antifungal susceptibility testing of conidium-forming filamentous fungi [13]. The results of the first collaborative evaluation of testing parameters for *in vitro* susceptibility studies of nine species of uncommon dematiaceous and moniliaceous moulds were incorporated into the present testing [14]. For the rest of the isolates, inoculum suspensions of 10^6 CFU/ml were prepared through the use hemocytometer counting and then the conidial suspensions were diluted to obtain a final organism concentration of $1–5 \times 10^4$ CFU/ml. The inoculum size for all tests was verified by inoculating 10 μl of each inoculum suspension onto Sabouraud dextrose agar plates, incubating the plates at 35°C, and counting the number of colonies.

Previously prepared frozen drug samples containing 0.1 ml of each drug were allowed to thaw and inoculated with 0.9-ml volumes of the inoculum suspensions. A drug-free growth control tube was included for each isolate. Tubes were incubated at 35°C and examined at 24 h (*R. arrhizus*, *M. circinelloides* and *A. corymbifera*), 48 h (*P. lilacinus*, *Fusarium* spp., *B. spicifera*, *C. lunata*, *A. alternata*, *C. posadasii* and *Exophiala* spp.), 72 h (*P. boydii*), and up to 120 h (*H. capsulatum* and *B. dermatitidis*). The MIC endpoint for fluconazole was defined as the lowest drug concentration that had turbidity corresponding to 50% inhibition of that of the growth control tube. The MIC endpoint for amphotericin B and the rest of

Table 1 *In vitro* susceptibilities of 300 isolates to isavuconazole, amphotericin B, itraconazole, fluconazole, voriconazole, posaconazole and ravuconazole

Fungus (no. tested) and drug	MIC[a] range	Geometric Mean MIC	MIC_{50}	MIC_{90}
P. boydii (28)				
Isavuconazole	0.5–4	1.561	2	4
Amphotericin B	1–8	2.561	2	4
Itraconazole	0.5–8	1.640	2	4
Fluconazole	4–64	13.791	16	32
Voriconazole	0.125–2	1.189	2	2
Posaconazole	0.125–2	1.104	1	2
Ravuconazole	0.5–8	2.263	2	4
P. lilacinus (22)				
Isavuconazole	0.2–2	1.327	1	2
Amphotericin B	4–>16		4	>16
Itraconazole	1–>16		2	>16
Fluconazole	16–>64		32	>64
Voriconazole	0.5–4	1.327	1	4
Posaconazole	0.5–2	1	1	2
Ravuconazole	0.25–2	1	1	2
Fusarium spp (30)				
Isavuconazole	4–>8		8	>8
Amphotericin B	1–4	1.624	2	2
Itraconazole	4–>16		8	>16
Fluconazole	>64		>64	>64
Voriconazole	2–>16		8	8
Posaconazole	2–>8		8	>8
Ravuconazole	2–>8		8	>8
B. spicifera (30)				
Isavuconazole	0.5–4	1.741	2	4
Amphotericin B	0.25–4	0.675	0.5	4
Itraconazole	0.5–8	2.297	2	4
Fluconazole	8–64	20.158	16	64
Voriconazole	0.5–4	1.148	1	2
Posaconazole	0.5–2	1.122	1	2
Ravuconazole	0.5–4	1.587	2	4
C. lunata (24)				
Isavuconazole	1–4	1.943	2	4
Amphotericin B	0.5–2	0.793	1	2
Itraconazole	0.5–4	1.681	2	4
Fluconazole	8–64	26.908	32	64
Voriconazole	0.5–2	1.090	1	2
Posaconazole	0.5–2	1.029	1	2
Ravuconazole	0.5–4	1.834	2	4
A. alternata (30)				
Isavuconazole	0.5–2	0.911	1	1
Amphotericin B	0.5–4	1.096	1	2
Itraconazole	0.5–2	0.615	0.5	1
Fluconazole	8–64	23.156	16	64
Voriconazole	0.125–1	0.353	0.5	0.5
Posaconazole	0.125–1	0.425	0.5	0.5
Ravuconazole	0.5–2	0.850	1	1
B. dermatitidis (6)				
Isavuconazole	0.5–4	1.259	1	
Amphotericin B	0.06–0.5	0.197	0.25	
Itraconazole	0.25–4	0.890	0.5	
Fluconazole	4–32	10.079	8	
Voriconazole	0.125–2	0.707	1	
Posaconazole	0.25–1	0.707	0.5	
Ravuconazole	0.125–4	1.000	1	

Table 1 (*Continued*)

Fungus (no. tested) and drug	MIC[a] range	Geometric Mean MIC	MIC_{50}	MIC_{90}
H. capsulatum (28)				
Isavuconazole	0.125–2	0.609	0.5	2
Amphotericin B	0.06–0.25	0.163	0.125	0.25
Itraconazole	0.25–2	0.464	0.5	1
Fluconazole	4–32	6.092	4	16
Voriconazole	0.06–2	0.430	0.25	1
Posaconazole	0.03–2	0.380	0.25	2
Ravuconazole	0.125–2	0.452	0.5	1
C. posadasii (30)				
Isavuconazole	0.125–1	0.280	0.25	0.5
Amphotericin B	0.03–0.125	0.056	0.06	0.125
Itraconazole	0.03–0.5	0.149	0.125	0.5
Fluconazole	2–64	8.774	8	32
Voriconazole	0.06–1	0.193	0.125	0.5
Posaconazole	0.06–1	0.183	0.125	0.5
Ravuconazole	0.125–1	0.261	0.25	0.5
Exophiala spp. (12)				
Isavuconazole	0.125–0.5	0.374	0.5	0.5
Amphotericin B	0.06–0.25	0.124	0.125	0.25
Itraconazole	0.125–0.5	0.264	0.25	0.5
Fluconazole	2–16	4.237	4	8
Voriconazole	0.06–0.25	0.109	0.125	0.25
Posaconazole	0.06–0.25	0.103	0.125	0.25
Ravuconazole	0.125–0.5	0.353	0.5	0.5
R. arrhizus (27)				
Isavuconazole	1–8	2.393	2	4
Amphotericin B	0.25–1	0.554	0.5	1
Itraconazole	0.25–4	0.835	0.5	2
Fluconazole	4–32	9.332	8	16
Voriconazole	1–8	2.585	2	8
Posaconazole	0.5–2	1.000	1	2
Ravuconazole	1–8	2.585	2	4
M. circinelloides (16)				
Isavuconazole	2–8	3.668	4	8
Amphotericin B	0.25–1	0.569	0.5	1
Itraconazole	0.5–2	0.878	1	1
Fluconazole	8–64	19.869	16	32
Voriconazole	4–8	7.336	8	8
Posaconazole	0.5–2	1.138	1	2
Ravuconazole	2–8	4.362	4	8
A. corymbifera (17)				
Isavuconazole	2–8	4.520	4	8
Amphotericin B	0.25–1	0.588	0.5	1
Itraconazole	0.5–2	1.000	1	2
Fluconazole	8–32	17.359	16	32
Voriconazole	4–16	9.809	8	16
Posaconazole	1–2	1.130	1	2
Ravuconazole	2–8	5.321	4	8

[a]All MICs are in micrograms per milliliter.

triazoles was defined as the lowest drug concentration that prevents any discernible growth. All testing were performed in triplicate. The isolates *Candida parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were included in each set of susceptibility tests to ensure quality control [14,15].

Analysis of the results

The MIC ranges, geometric mean MIC and MIC of isavuconazole, amphotericin B, itraconazole, fluconazole, voriconazole, posaconazole, and ravuconazole necessary to inhibit 50% and 90% of the isolates were calculated.

Results and discussion

Table 1 summarizes the *in vitro* susceptibilities of 300 clinical isolates to antifungal compounds as determined by the M38-A method. The data are presented as MIC ranges, geometric mean and the drug concentration necessary to inhibit 50% and 90% of the isolates of each species (MIC_{50} and MIC_{90}). The MIC ranges for the two quality control strains were within the recommended CLSI limits [15,16].

Overall, the triazoles itraconazole, voriconazole, posaconazole, ravuconazole and isavuconazole had uniform *in vitro* activity against this group of filamentous fungi and the fluconazole MIC was generally greater than the others triazoles.

In comparison with those of the other antifungal agents, the MICs of voriconazole and posaconazole aginst *P. boydii* were low, followed by isavuconazole. Our data seem to confirm the general trend that has been reported in the literature about the *in vitro* susceptibilities of *P. boydii* [5,6,17–19].

Earlier studies by Aguilar *et al*. showed that amphotericin B, miconazole, itraconazole, fluconazole and flucytosine had poor activity against *Paecilomyces* spp. [20]. One of the noteworthy aspects of this study is the activity displayed by voriconazole, posaconazole, ravuconazole, and isavuconazole against this fungus.

The results also showed that itraconazole, voriconazole, posaconazole, ravuconazole, and isavuconazole, when tested by the broth macrodilution method, had potent activity against the dimorphic fungi *B. dermatitidis*, *H. capsulatum* and *C. posadasii*. The MICs of isavuconazole were very similar for all the dimorphic fungi and were lower than those for fluconazole. Specifically, for *C. posadasii*, the MICs of isavuconazole are hopeful and indicate that this compound is a potent antifungal agent and that further clinical evaluation is recommended.

With regard to *Fusarium* spp., the triazoles had poor *in vitro* activity and amphotericin B was moderately active. On the other hand, the triazoles tested in this study were active against dematiaceous fungi.

Isavuconazole, voriconazole and ravuconazole exhibited similar *in vitro* activity against zygomycetes. The greater activity of posaconazole compared to ravuconazole, voriconazole, and isavuconazole observed in other studies was confirmed [21]. Posaconazole, amphotericin B, and itraconazole were most active against this group of fungi.

The results suggest that isavuconazole is a broad-spectrum antifungal agent, effective against a wide range of moulds *in vitro*. Further *in vivo* studies are required in order to confirm the efficacy for treatment of severe infections caused by these fungi in immunocompromised patients.

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