ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 2006, p. 917–921 Vol. 50, No. 3
0066-4804/06/$08.00+0 doi:10.1128/AAC.50.3.917–921.2006

Head-to-Head Comparison of the Activities of Currently Available Antifungal Agents against 3,378 Spanish Clinical Isolates of Yeasts and Filamentous Fungi

Manuel Cuenca-Estrella,* Alicia Gomez-Lopez, Emilia Mellado, Maria J. Buitrago, Araceli Monzon, and Juan Luis Rodriguez-Tudela

Servicio de Micología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Madrid, Spain

Received 16 November 2005/Returned for modification 10 December 2005/Accepted 3 January 2006

We have compared the activities of posaconazole and other currently available antifungal agents against a collection of 3,378 clinical isolates of yeasts and filamentous fungi. A total of 1,997 clinical isolates of *Candida* spp., 359 of other yeast species, 697 strains of *Aspergillus* spp., and 325 nondermatophyte non-*Aspergillus* spp. were included. The average geometric means of the MICs of agents that were tested against *Candida* spp. were 0.23 μg/ml for amphotericin B, 0.29 μg/ml for flucytosine, 0.97 μg/ml for fluconazole, 0.07 μg/ml for itraconazole, 0.04 μg/ml for voriconazole, 0.15 μg/ml for caspofungin, and 0.03 μg/ml for posaconazole. Voriconazole and posaconazole were active in vitro against the majority of isolates, with resistance to fluconazole and itraconazole, and against *Cryptococcus neoformans* and other *Basidiomycota* yeasts. Posaconazole was the most active of antifungal agents tested against *Aspergillus* spp., with an average geometric mean of 0.10 μg/ml. It was active against *Paecilomyces* spp., *Penicillium* spp., *Scedosporium apiospermum*, and some black fungi, such as *Alternaria* spp. Multiresistant filamentous fungi, such as *Scedosporium prolificans*, *Scopulariopsis brevicaulis*, and *Fusarium solani*, were also resistant to voriconazole, caspofungin, and posaconazole. Amphotericin B and posaconazole were found to be active against most of the *Mucorales* strains tested. Posaconazole and currently available antifungal agents exhibit a potent activity in vitro against the majority of pathogenic fungal species.

Posaconazole is a new triazole agent with an extended spectrum of in vitro activity. It is active against opportunistic, endemic, and dermatophytic fungi (10, 13, 14, 15) as well as *Candida*, *Cryptococcus*, and other yeast species (1, 3, 17, 22, 23), including isolates that exhibit resistance to fluconazole and itraconazole (3, 10, 23). It also has a potent activity in vitro against *Aspergillus* species; posaconazole appears to be more active in vitro against *Aspergillus* than are amphotericin B, itraconazole, voriconazole, and ravuconazole, inhibiting 95% of isolates at a MIC of ≤1 μg/ml (2, 9, 24). In addition, the new triazoles have been shown to have antifungal activity against other species of filamentous fungi, such as *Penicillium* spp., *Paecilomyces* spp., and *Acremonium* spp., and *Sporothrix schenckii* as well as some isolates of *Fusarium* spp., *Scedosporium apiospermum*, and black fungi (9, 10, 13, 18, 24, 30). Most notably, posaconazole is more active than the other triazole compounds against zygomycetes, with MICs around or below 1 μg/ml. Within the class of *Zygomycetes*, posaconazole appears to be more active against *Rhizopus* spp. and *Absidia corymbifera* than against *Mucor* spp., which is consistent with the prior observation that the zygomycetes appear to be a heterogeneous group with regard to their susceptibilities to antifungal agents (7, 9, 16, 24, 29).

We have analyzed the in vitro activity of posaconazole and other currently available antifungal agents against a collection of 3,378 clinical isolates of yeasts and filamentous fungi. A total of 1,997 clinical isolates of *Candida* spp., 359 of other yeast species, 697 strains of *Aspergillus* spp., and 325 nondermatophyte non-*Aspergillus* spp. were included, representing one of the largest and most diverse panels of fungal species that have been tested to date against posaconazole. Comparisons between susceptibility results per species were done in order to better understand the activity profile of this novel antifungal agent.

* Corresponding author. Mailing address: Servicio de Micología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Ctra Majadahonda-Pozuelo Km 2, 28220 Majadahonda (Madrid), Spain. Phone: 34-91-5097961. Fax: 34-91-5097966. E-mail: mcuenca-estrella@isciii.es.

MATERIALS AND METHODS

The strains were recovered from 102 Spanish hospitals through a period of five years, from 2001 to 2006. The isolates were obtained from blood (1,327/3,378, 39.3%), respiratory tract specimens (804/3,378, 23.8%), biopsies and other deep sites, (365/3,378, 10.8%), skin samples (247/3,378, 7.3%), and other locations (635/3,378, 18.8%). *Candida parapsilosis* ATCC 22019, *Candida krusei* ATCC 6258, *Cryptococcus neoformans* ATCC 90112, *Aspergillus fumigatus* ATCC 204306, and *Aspergillus flavus* ATCC 204304 were included as control isolates.

The antifungal agents used in the study were posaconazole (Schering-Plough, Kenilworth, NJ), amphotericin B (Sigma-Aldrich Química, SA, Madrid, Spain), flucytosine (Sigma-Aldrich), fluconazole (Pfizer, SA, Madrid, Spain), itraconazole (Janssen Pharmaceutica, Madrid, Spain), voriconazole (Pfizer, Ltd., Sandwich, United Kingdom), and caspofungin (Merck & Co., Inc., Rahway, NJ).

Susceptibility testing was performed by broth microdilution. For *Candida* species, MICs were determined by using the reference procedure of the Antifungal Susceptibility Testing Subcommittee of the European Committee on Antibiotic Susceptibility Testing for testing fermentative yeasts (AFST-EUCAST, document 7.1) (25). Briefly, testing was performed in flat-bottomed microdilution plates by using RPMI 1640 medium supplemented with 2% glucose and an inoculum size of 10^6 CFU/ml. MIC end points were determined spectrophotometrically at 24 and 48 h. For amphotericin B, the MIC end points were defined as the lowest drug concentration that resulted in a reduction in growth of 90% or more compared with that of a drug-free-growth control well. For flucytosine and azoles, the MIC end point was defined as a 50% reduction in optical density. Caspofungin susceptibility testing was done following the recommendations of Odds et al. (21).

For *C. neoformans* and other species of nonfermentative yeasts, susceptibility testing strictly followed the recommendations proposed by the EUCAST, with

TABLE 1. Susceptibility in vitro to posaconazole and other antifungal agents of yeast species

Species	No. of isolates	Susceptibility (MIC,[a] μg/ml) of indicated agent to yeast													
		Amphotericin B		Flucytosine		Fluconazole		Itraconazole		Voriconazole		Posaconazole		Caspofungin	
		MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
Candida species															
Candida albicans	940	0.06	0.12	0.12	0.50	0.12	0.25	0.02	0.03	0.02	0.02	0.02	0.02	0.12	0.25
Candida parapsilosis	387	0.12	0.25	0.12	0.25	0.50	0.50	0.03	0.06	0.02	0.03	0.02	0.03	0.50	1.0
Candida tropicalis	202	0.12	0.25	0.12	0.25	0.25	0.50	0.02	0.06	0.02	0.06	0.02	0.06	0.12	1.0
Candida glabrata	244	0.12	0.25	0.12	0.25	4.0	16.0	0.25	1.0	0.25	1.0	0.25	1.0	0.12	0.25
Candida krusei	94	0.25	0.50	4.0	4.0	32.0	64.0	0.25	0.25	0.25	0.50	0.12	0.25	0.25	0.50
Candida guilliermondii	52	0.12	0.25	0.12	0.25	4.0	8.0	0.25	0.50	0.06	0.12	0.06	0.12	1.0	16.0
Candida lusitaniae	21	0.06	0.12	0.12	0.25	0.12	0.50	0.02	0.06	0.02	0.02	0.02	0.02	0.50	1.0
Candida kefyr	13	0.12	0.25	1.0	>64.0	0.25	1.0	0.03	0.03	0.02	0.02	0.02	0.03	0.06	0.12
Candida famata	10	0.50	1.0	0.25	64.0	16.0	>64.0	0.50	>8.0	0.12	>8.0	0.12	>8.0	8.0	>16.0
Candida pelliculosa	10	0.06	0.50	0.12	16.0	8.0	>64.0	0.50	>8.0	0.25	>8.0	1.0	>8.0	0.25	0.50
Other *Candida* spp.[b]	24	0.12	2.0	0.25	16.0	8.0	>64.0	0.06	4.0	0.06	8.0	0.03	8.0	0.50	16.0
Other *Ascomycota* yeasts															
Dipodascus capitatus	25	0.12	8.0	0.12	16.0	8.0	16.0	0.12	0.25	0.12	0.50	0.12	0.25	16.0	>16.0
Saccharomyces cerevisiae	15	0.12	0.25	0.12	8.0	2.0	4.0	0.25	0.50	0.06	0.12	0.12	0.50	0.50	1.0
Yarrowia lipolytica	13	0.50	0.50	16.0	>64.0	8.0	16.0	0.25	2.0	0.12	2.0	0.12	0.50	>16.0	>16.0
Galactomyces geotrichum	12	0.25	0.25	0.12	1.0	16.0	16.0	1.0	8.0	1.0	4.0	0.50	1.0	>16.0	>16.0
Basidiomycota yeasts															
C. neoformans var. *neoformans*	183	0.06	0.25	8.0	16.0	16.0	16.0	0.25	0.50	0.25	0.50	0.25	0.50	>16.0	>16.0
C. neoformans var. *gattii*	28	0.12	0.12	1.0	2.0	16.0	16.0	0.50	1.0	0.50	1.0	0.25	0.50	>16.0	>16.0
Trichosporon asahii	17	2.0	>8.0	16.0	>64.0	8.0	32.0	1.0	>8.0	0.50	>8.0	0.25	>8.0	>16.0	>16.0
Rhodotorula mucilaginosa	24	0.12	0.25	0.25	0.25	16.0	>64.0	4.0	>8.0	4.0	>8.0	2.0	>8.0	>16.0	>16.0
Trichosporon inkin	10	0.25	0.50	>64.0	>64.0	2.0	4.0	0.12	0.50	0.25	2.0	0.12	0.25	>16.0	>16.0
Other *Basidiomycota* yeasts[c]	32	0.25	16.0	16.0	>64.0	16.0	>64.0	0.25	>8.0	0.25	>8.0	0.12	>8.0	>16.0	>16.0

[a] MIC_{50} (MIC causing inhibition of 50% of isolates) and MIC_{90} (MIC causing inhibition of 90% of isolates) values were calculated for those species with 10 or more isolates.

[b] Other *Candida* species include species with less than 10 isolates: *Candida pintolopesii* (4 isolates), *Candida haemulonii* (4), *Candida dubliniensis* (4), *Candida colliculosa* (3), *Candida rugosa* (3), *Candida sake* (2), and *Candida norvegensis* (2).

[c] Other *Basidiomycota* yeasts include species with less than 10 isolates: *Trichosporon ovoides* (7 isolates), *Trichosporon dermatis* (7), *Cryptococcus albidus* (6), *Rhodotorula glutinis* (5), *Cryptococcus laurentii* (3), other *Trichosporon* spp. (4).

the following minor modification: microdilution plates were sealed to limit evaporation, attached to an electrically driven wheel inside the incubator, and agitated at 350 rpm at 30°C for 48 h (26).

For filamentous fungi broth, microdilution testing was performed following the Clinical Laboratory Standards Institute (CLSI) reference method (19), with the following minor modifications: RPMI 1640 with L-glutamine (buffered to pH 7 with 0.165 M morpholinepropanesulfonic acid [MOPS] and 10 M NaOH) supplemented with 2% glucose (RPMI-2% glucose; OXOID, Madrid, Spain) and an inoculum size of 1×10^6 to 5×10^6 CFU/ml (8, 12, 27). Denning et al. (8) demonstrated that inoculum sizes higher than those proposed by CLSI document M38-A (1×10^4 to 5×10^4 CFU/ml) also generate reproducible in vitro susceptibility data for *Aspergillus* spp. that can predict clinical outcome. Gomez-Lopez et al. (12) did not find significant differences in MICs using RPMI 1640 or RPMI 1640 supplemented with glucose, and MICs were not falsely elevated even if an inoculum size of 10^6 CFU/ml was used. Inoculum suspensions were prepared from plates that were 3 to 5 days old. The plates were incubated at 35°C for 48 h in a humid atmosphere, and visual readings were performed with the help of a mirror. The MIC was defined as the lowest concentration of drug that completely inhibited growth. Caspofungin was not tested since a reference method for filamentous fungi has not been defined.

Statistical analysis was done with the Statistical Package for the Social Sciences (SPSS, version 13.0.) (SPSS, SL, Madrid, Spain).

RESULTS

The MICs for all antifungal agents are summarized in Tables 1 and 2. The average geometric mean (GM) for posaconazole against *Candida* spp. was 0.03 μg/ml, and the GMs for the other drugs were as follows: amphotericin B, 0.23 μg/ml; flucytosine, 0.29 μg/ml; fluconazole, 0.97 μg/ml; itraconazole, 0.07 μg/ml; voriconazole, 0.04 μg/ml; and caspofungin, 0.15 μg/ml. Posaconazole and voriconazole were active against the majority of isolates belonging to species which can frequently exhibit decreased susceptibility or resistance to fluconazole, such as *Candida glabrata*, *Candida krusei*, *Candida guilliermondii*, *Candida famata*, and *Candida pelliculosa*. In addition, the panel that was tested included a number of fluconazole-resistant strains of species that are normally susceptible to fluconazole. For this subset of isolates, posaconazole and voriconazole were active in vitro against 12 out of 15 (80%) strains of *Candida albicans*, 2 out of 5 (40%) strains of *Candida tropicalis*, and all 3 (100%) of the *C. parapsilosis* isolates.

In reference to other species of yeasts, posaconazole was highly active against *Dipodascus capitatus*, *Saccharomyces cerevisiae*, *Yarrowia lipolytica*, and *Galactomyces geotrichum*. Posaconazole was also active against species of *Basidiomycota*. The GM of posaconazole for *C. neoformans* var. *neoformans* was 0.16 μg/ml, and only one strain had a MIC of >1 μg/ml. The GM for tested isolates of *C. neoformans* var. *gattii* was 0.25, and all strains were inhibited at a MIC of ≤0.5 μg/ml. Posaconazole was less active against *Trichosporon* spp., with a GM that was significantly higher than those of other species. A total of 11 out of 45 (24.4%) *Trichosporon* strains tested had posaconazole MICs of ≥2 μg/ml; 6 of these strains were *Trichosporon asahii*. Notably, 12 of 24 *Rhodotorula mucilaginosa* organisms had posaconazole MICs of ≤1 μg/ml; the GM for all 24 strains was 1.68 μg/ml. The majority of *R. mucilaginosa* isolates were resistant to fluconazole, itraconazole, and voriconazole.

For the filamentous fungi, posaconazole was the most active

TABLE 2. Susceptibility in vitro to posaconazole and other antifungal agents of filamentous fungi included in the study

Species	No. of isolates	Susceptibility (MIC,[a] μg/ml) of indicated agent to fungi							
		Amphotericin B		Itraconazole		Voriconazole		Posaconazole	
		MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
Aspergillus spp.									
Aspergillus fumigatus	375	0.25	0.50	0.25	0.50	0.25	1.0	0.06	0.25
Aspergillus flavus	110	1.0	2.0	0.25	1.0	0.50	2.0	0.12	0.50
Aspergillus terreus	74	1.0	8.0	0.25	0.50	0.50	1.0	0.06	0.25
Aspergillus niger	55	0.25	0.25	0.50	4.0	1.0	1.0	0.25	0.50
Aspergillus nidulans	29	0.50	2.0	0.25	>8.0	0.25	>8.0	0.12	>8.0
Aspergillus sydowii	21	0.50	1.0	0.50	1.0	0.50	2.0	0.06	0.50
Aspergillus versicolor	13	0.50	2.0	0.50	1.0	1.0	2.0	0.25	0.50
Other *Aspergillus* spp.	20	0.50	>16.0	0.50	>8.0	0.50	>8.0	0.12	>8.0
Other hyaline fungi									
Scedosporium apiospermum	65	4.0	>16.0	8.0	>8.0	1.0	4.0	1.0	8.0
Penicillium spp.	45	0.50	4.0	0.50	8.0	2.0	8.0	0.25	2.0
Scopulariopsis brevicaulis	19	4.0	16.0	>8.0	>8.0	>8.0	>8.0	>8.0	>8.0
Fusarium solani	18	1.0	4.0	>8.0	>8.0	>8.0	>8.0	>8.0	>8.0
Fusarium oxysporum	15	0.50	1.0	>8.0	>8.0	4.0	>8.0	4.0	>8.0
Fusarium verticillioides	11	2.0	>16.0	>8.0	>8.0	>8.0	>8.0	>8.0	>8.0
Paecilomyces lilacinus	11	>16.0	>16.0	8.0	>8.0	0.25	8.0	0.25	0.50
Paecilomyces variotii	10	0.12	1.0	0.06	0.25	1.0	4.0	0.03	0.50
Acremonium spp.	10	2.0	16.0	8.0	>8.0	2.0	4.0	1.0	4.0
Other hyaline fungi[b]	27	0.25	4.0	0.50	>8.0	1.0	>8.0	0.25	>8.0
Black fungi									
Scedosporium prolificans	37	16.0	>32.0	>8.0	>8.0	>8.0	>8.0	>8.0	>8.0
Alternaria spp.	11	0.25	0.50	0.25	>8.0	1.0	>8.0	0.12	>8.0
Other black fungi[c]	15	0.12	0.50	0.25	>8.0	0.12	>8.0	0.06	>8.0
Mucorales									
Rhizopus oryzae	15	0.50	2.0	>8.0	>8.0	>8.0	>8.0	0.50	8.0
Other *Mucorales* species[d]	16	0.12	2.0	1.0	>8.0	8.0	>8.0	1.0	>8.0

[a] MIC_{50} (MIC causing inhibition of 50% of isolates) and MIC_{90} (MIC causing inhibition of 90% of isolates) values were calculated for those species with 10 or more isolates.

[b] Other hyaline fungi include species with less than 10 isolates: *Arthrographis kalrae* (5 isolates), *Trichoderma* spp. (4), *Scopulariopsis* spp. (2), *Arthrographis cuboidea* (2), *Cylindrocarpon* spp. (2), *Hormographiella verticillata* (2), *Chrysosporium* spp. (2), *Arthrinium phaeospermum* (2), *Phialemonium curvatum* (2), *Paecilomyces fumosoroseus* (1), *Chrysonilia sitophila* (1), *Beauveria bassiana* (1), and *Myceliophthora thermophila* (1).

[c] Other black fungi include species with less than 10 isolates: *Rhinocladiella aquaspersa* (3 isolates), *Aureobasidium pullulans* (2), *Exophiala jeanselmei* (1), *Exophiala dermatitidis* (1), *Cladosporium* spp. (1), *Cladophialophora bantiana* (1), *Curvularia lunata* (1), *Nattrassia mangiferae* (1), *Ochroconis gallopava* (1), *Scytalidium hyalinum* (1), *Scytalidium infestans* (1), and *Lecythophora* spp. (1).

[d] Other *Mucorales* include species with less than 10 isolates: *Rhizopus microsporus* (5 isolates), *Absidia corymbifera* (4), *Mucor circinelloides* (2), *Rhizomucor pusillus* (2), *Rhizomucor miehei* (1), *Rhizomucor variabilis* (1), and *Cunninghamella bertholletiae* (1).

of the antifungal agents tested against *Aspergillus* spp. Its average GM was 0.10 μg/ml, and the GMs of amphotericin B, itraconazole, and voriconazole were 0.41, 0.33, and 0.48, respectively. Posaconazole was active in vitro particularly against *A. fumigatus* and *Aspergillus terreus*. The collection that was tested included nine *Aspergillus* isolates with itraconazole resistance in vitro (MIC > 8 μg/ml). Posaconazole exhibited activity against five of these nine isolates (55.5%). More precisely, posaconazole was active against one of the three *A. fumigatus* strains, the *A. terreus* isolate, and all three *Aspergillus niger* isolates; however, it lacked activity against the itraconazole-resistant *Aspergillus nidulans* strains.

Other species of hyaline filamentous fungi were also susceptible in vitro to posaconazole, and almost all of the isolates from the following genera were inhibited by the triazole: *Penicillium* spp., *Paecilomyces* spp., *Acremonium* spp., and *Arthrographis* spp. The GM of posaconazole against *S. apiospermum* was 1.3 μg/ml, and 40 out of 65 (61.5%) isolates analyzed were inhibited by the agent. However, the most active compound against *S. apiospermum* was voriconazole, with a GM of 0.89 μg/ml and a MIC of ≤1 μg/ml for 58 of 65 (89.2%) organisms tested. Posaconazole was inactive in vitro against most of *Scopulariopsis* spp., *Fusarium* spp., and *Trichoderma* spp., with an average GM of >4 μg/ml. In the case of *Fusarium* spp., posaconazole exhibited activity in vitro for only 1 of 15 *Fusarium oxysporum* isolates and for 3 of 11 *Fusarium verticilloides* isolates studied.

None of the antifungal agents that were tested appeared to be active against the black fungus *Scedosporium prolificans*. With regard to other black fungi, posaconazole was active against the majority of *Alternaria* isolates (9/11, 82%) and the three *Rhinocladiella* strains. The limited number of isolates analyzed for the other species prevents significant conclusions from being drawn.

Finally, posaconazole was shown to have an inhibitory effect for some *Mucorales* isolates. The majority of these isolates were susceptible in vitro to amphotericin B and resistant to itraconazole and voriconazole. The average GM of posaconazole for 31 isolates tested was 1.1 μg/ml. Nine of 31 (29%) strains were resistant in vitro to posaconazole, with MICs as

TABLE 3. MIC ranges (μg/ml) for control strains by reference procedures and by the modified methods[a]

Antifungal agent	MIC obtained by indicated method for:									
	Candida parapsilosis ATCC 22019		*Candida krusei* ATCC 6258		*Cryptococcus neoformans* ATCC 90112		*Aspergillus flavus* ATCC 204304		*Aspergillus fumigatus* ATCC 204305	
	EUCAST[b] method	CLSI procedure	EUCAST[b] method	CLSI procedure	Modified[c] method	CLSI procedure	Modified[d] method	CLSI procedure	Modified[d] method	CLSI procedure
Amphotericin B	0.12 – 0.50	0.25 – 1.0	0.12 – 0.50	0.25 – 1.0	0.12 – 0.50	0.12 – 0.50	0.25 – 1.0	0.50 – 2.0	0.25 – 1.0	0.50 – 2.0
Flucytosine	0.12 – 0.50	0.12 – 0.50	1.0 – 4.0	4.0 – 16.0	8.0 – 32.0	4.0 – 16.0	ND	ND	ND	ND
Fluconazole	1.0 – 4.0	2.0 – 8.0	8.0 – 32.0	16.0 – 64.0	8.0 – 32.0	8.0 – 32.0	ND	ND	ND	ND
Itraconazole	0.03 – 0.12	0.06 – 0.25	0.06 – 0.25	0.12 – 0.50	0.25 – 1.0	0.25 – 1.0	0.12 – 0.50	0.12 – 0.50	0.12 – 1.0	0.25 – 1.0
Voriconazole	0.03 – 0.12	0.015 – 0.12	0.06 – 0.25	0.06 – 0.50	0.25 – 1.0	0.25 – 1.0	0.50 – 2.0	0.50 – 1.0	0.25 – 1.0	0.25 – 1.0
Caspofungin	0.50 – 2.0	0.50 – 4.0	0.12 – 0.50	0.25 – 1.0	>16.0	>16.0	4.0 – 16.0	4.0 – 16.0	0.12 – 0.50	0.12 – 0.50
Posaconazole	0.015 – 0.06	0.03 – 0.12	0.03 – 0.12	0.06 – 0.50	0.12 – 0.50	0.25 – 1.0	0.12 – 0.25	0.12 – 0.25	0.06 – 0.25	0.12 – 0.50

[a] Results show the MICs after 30 repetitions on different days. ND, not determined.
[b] See reference 21.
[c] See reference 22.
[d] See references 5, 8, and 23.

high as >8 μg/ml. The compound was more active against *Rhizopus oryzae*, *Absidia corymbifera*, and *Rhizomucor pusillus*, with MICs of ≤1 μg/ml for 12 of 15 (80%) *R. oryzae* isolates and for all *A. corymbifera* organisms tested. However, the majority of isolates of *Mucor* spp. and of *Rhizopus microsporus* were resistant in vitro to the triazole.

Table 3 displays the susceptibility results for reference strains per antifungal agent tested. Table 3 includes MIC data after 30 repetitions on different days. In addition, Table 3 shows MICs that were obtained for reference strains by the reference procedures of CLSI documents M27-A2 and M38-A (19, 20).

DISCUSSION

We present the results of one of the largest and the most diversified surveys on the profile of activity in vitro of posaconazole and other currently available antifungal agents. The triazole showed a good activity against the majority of species studied. The GM of MICs of posaconazole for the 3,378 isolates was 0.11 μg/ml. Breakpoints for posaconazole have not been established, but pharmacokinetic studies have shown that after the administration of posaconazole oral suspension (800 mg), the resulting maximum serum concentrations ranged between 1 and 2 μg/ml (4, 11, 16).

Posaconazole exhibited potent activity against *Candida* spp., extending to species and isolates with decreased susceptibility or resistance to fluconazole. Of the 186 isolates with a fluconazole MIC of ≥16 μg/ml, only 27 had a posaconazole MIC of >1 μg/ml; put another way, 85.5% of strains that exhibited decreased susceptibility in vitro to fluconazole were susceptible to posaconazole. In addition, 32 of the 59 (54.3%) itraconazole-resistant isolates (MIC ≥ 1 μg/ml), and 10 of the 37 (27%) strains with decreased susceptibility to voriconazole (MIC ≥ 2 μg/ml), exhibited posaconazole MICs of ≤1 μg/ml.

Posaconazole was particularly active against *Cryptococcus neoformans* isolates; its activity was similar to that of amphotericin B and superior to that of either itraconazole or voriconazole. In addition, posaconazole was active against many of the strains belonging to species which cause infections that are difficult to treat, such as *Dipodascus*, *Saccharomyces*, *Trichosporon*, and *Rhodotorula* spp. These results indicate that the new triazole exhibits a more extended spectrum than currently licensed antifungal agents and that it could be a useful alternative for the treatment of these mycoses.

Consistent with previous data, we found that posaconazole was the most active agent against *Aspergillus* spp. (9, 24). The compound was also active against these species in animal models of infection (31) and demonstrated clinically relevant activity in patients with invasive aspergillosis who were refractory to or intolerant of other antifungal therapy in an open-label, multicenter, phase III study (16). These data suggest that this new azole could play a significant role in the treatment of aspergillosis.

Regarding other hyphomycetes species, posaconazole and voriconazole appeared to be equally active. Posaconazole was active in vitro against the majority of strains of some species, such as *Paecilomyces* spp., *Penicillium* spp., and *S. apiospermum*. Notably, it exhibited a potent effect (GM, 0.16 μg/ml) against *Paecilomyces lilacinus*, a rare emerging hyphomycete that can cause disseminated and invasive infections in immunocompromised patients (28). However, multidrug-resistant filamentous fungi, such as *S. prolificans*, *Scopulariopsis brevicaulis*, and *Fusarium solani*, exhibited reduced susceptibility (MICs > 8 μg/ml) to posaconazole (5, 6).

Many of the *Mucorales* isolates studied were susceptible to posaconazole and resistant to other azole agents. Posaconazole was active against the 71% of the strains tested. *R. oryzae* and *A. corymbifera* were the most susceptible organisms, a result that is consistent with the results published by Dannaoui et al. (7). These data suggest that posaconazole may be a useful alternative to amphotericin B for treating these mycoses. In this regard, it should be noted that posaconazole had an overall success rate of 54% in preliminary studies on the treatment of mucormycosis (16).

In conclusion, this study demonstrated that posaconazole is an extended-spectrum triazole. This triazole and other currently available antifungal agents, such as voriconazole and caspofungin, exhibit potent in vitro activity against the majority of the isolates that were tested. Posaconazole has the potential to be a useful therapeutic option for treating infections due to rare emerging species or for those mycoses caused by resistant isolates.

ACKNOWLEDGMENTS

A. Gomez-Lopez holds a contract from the RESITRA (Spanish Research Network of Infection in Transplantation) fund of the Fondo de Investigaciones Sanitarias, G03/75. E. Mellado holds the contract Ramón y Cajal from the Ministry of Science and Technology.

We thank Pfizer, Janssen Pharmaceutical, Schering-Plough, and Merck & Co., Inc. for supplying antifungal powders.

REFERENCES

1. **Barchiesi, F., D. Arzeni, A. W. Fothergill, L. F. Di Francesco, F. Caselli, M. G. Rinaldi, and G. Scalise.** 2000. In vitro activities of the new antifungal triazole SCH 56592 against common and emerging yeast pathogens. Antimicrob. Agents Chemother. **44:**226–229.
2. **Cacciapuoti, A., D. Loebenberg, E. Corcoran, F. Menzel, Jr., E. L. Moss, Jr., C. Norris, M. Michalski, K. Raynor, J. Halpern, C. Mendrick, B. Arnold, B. Antonacci, R. Parmegiani, T. Yarosh-Tomaine, G. H. Miller, and R. S. Hare.** 2000. In vitro and in vivo activities of SCH 56592 (posaconazole), a new triazole antifungal agent, against *Aspergillus* and *Candida*. Antimicrob. Agents Chemother. **44:**2017–2022.
3. **Carrillo-Munoz, A. J., G. Quindos, M. Ruesga, R. Alonso, O. del Valle, J. M. Hernandez-Molina, P. McNicholas, D. Loebenberg, and P. Santos.** 2006. Antifungal activity of posaconazole compared with fluconazole and amphotericin B against yeasts from oropharyngeal candidiasis and other infections. J. Antimicrob. Chemother. **55:**317–319.
4. **Courtney, R., D. Wexler, E. Radwanski, J. Lim, and M. Laughlin.** 2004. Effect of food on the relative bioavailability of two oral formulations of posaconazole in healthy adults. Br. J. Clin. Pharmacol. **57:**218–222.
5. **Cuenca-Estrella, M., A. Gomez-Lopez, E. Mellado, M. J. Buitrago, A. Monzon, and J. L. Rodriguez-Tudela.** 2003. *Scopulariopsis brevicaulis*, a fungal pathogen resistant to broad-spectrum antifungal agents. Antimicrob. Agents Chemother. **47:**2339–2341.
6. **Cuenca-Estrella, M., B. Ruiz-Diez, J. V. Martinez-Suarez, A. Monzon, and J. L. Rodriguez-Tudela.** 1999. Comparative in-vitro activity of voriconazole (UK-109,496) and six other antifungal agents against clinical isolates of *Scedosporium prolificans* and *Scedosporium apiospermum*. J. Antimicrob. Chemother. **43:**149–151.
7. **Dannaoui, E., J. Meletiadis, J. W. Mouton, J. F. Meis, and P. E. Verweij.** 2003. In vitro susceptibilities of zygomycetes to conventional and new antifungals. J. Antimicrob. Chemother. **51:**45–52.
8. **Denning, D. W., S. A. Radford, K. L. Oakley, L. Hall, E. M. Johnson, and D. W. Warnock.** 1997. Correlation between in-vitro susceptibility testing to itraconazole and in-vivo outcome of *Aspergillus fumigatus* infection. J. Antimicrob. Chemother. **40:**401–414.
9. **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.
10. **Espinel-Ingroff, A.** 2003. In vitro antifungal activities of anidulafungin and micafungin, licensed agents and the investigational triazole posaconazole as determined by NCCLS methods for 12,062 fungal isolates: review of the literature. Rev. Iberoam. Micol. **20:**121–136.
11. **Ezzet, F., D. Wexler, R. Courtney, G. Krishna, J. Lim, and M. Laughlin.** 2006. Oral bioavailability of posaconazole in fasted healthy subjects: comparison between three regimens and basis for clinical dosage recommendations. Clin. Pharmacokinet. **44:**211–220.
12. **Gomez-Lopez, A., A. Aberkane, E. Petrikkou, E. Mellado, J. L. Rodriguez-Tudela, and M. Cuenca-Estrella.** 2006. Analysis of the influence of Tween concentration, inoculum size, assay medium, and reading time on susceptibility testing of *Aspergillus* spp. J. Clin. Microbiol. **43:**1251–1255.
13. **Gonzalez, G. M., A. W. Fothergill, D. A. Sutton, M. G. Rinaldi, and D. Loebenberg.** 2006. In vitro activities of new and established triazoles against opportunistic filamentous and dimorphic fungi. Med. Mycol. **43:**281–284.
14. **Groll, A. H., and T. J. Walsh.** 2006. Posaconazole: clinical pharmacology and potential for management of fungal infections. Expert Rev. Anti. Infect. Ther. **3:**467–487.
15. **Gupta, A. K., Y. Kohli, and R. Batra.** 2006. In vitro activities of posaconazole, ravuconazole, terbinafine, itraconazole and fluconazole against dermatophyte, yeast and non-dermatophyte species. Med. Mycol. **43:**179–185.
16. **Keating, G. M.** 2006. Posaconazole. Drugs **65:**1553–1567.
17. **Laverdiere, M., D. Hoban, C. Restieri, and F. Habel.** 2002. In vitro activity of three new triazoles and one echinocandin against *Candida* bloodstream isolates from cancer patients. J. Antimicrob. Chemother. **50:**119–123.
18. **Meletiadis, J., J. F. Meis, J. W. Mouton, J. L. Rodriquez-Tudela, J. P. Donnelly, and P. E. Verweij.** 2002. In vitro activities of new and conventional antifungal agents against clinical *Scedosporium* isolates. Antimicrob. Agents Chemother. **46:**62–68.
19. **National Committee for Clinical Laboratory Standards.** 2002. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi. Approved standard. NCCLS document M38-A. National Committee for Clinical Laboratory Standards, Wayne, Pa.
20. **National Committee for Clinical Laboratory Standards.** 2002. Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved standard. NCCLS document M27-A2. National Committee for Clinical Laboratory Standards, Wayne, Pa.
21. **Odds, F. C., M. Motyl, R. Andrade, J. Bille, E. Canton, M. Cuenca-Estrella, A. Davidson, C. Durussel, D. Ellis, E. Foraker, A. W. Fothergill, M. A. Ghannoum, R. A. Giacobbe, M. Gobernado, R. Handke, M. Laverdiere, W. Lee-Yang, W. G. Merz, L. Ostrosky-Zeichner, J. Peman, S. Perea, J. R. Perfect, M. A. Pfaller, L. Proia, J. H. Rex, M. G. Rinaldi, J. L. Rodriguez-Tudela, W. A. Schell, C. Shields, D. A. Sutton, P. E. Verweij, and D. W. Warnock.** 2004. Interlaboratory comparison of results of susceptibility testing with caspofungin against *Candida* and *Aspergillus* species. J. Clin. Microbiol. **42:**3475–3482.
22. **Paphitou, N. I., L. Ostrosky-Zeichner, V. L. Paetznick, J. R. Rodriguez, E. Chen, and J. H. Rex.** 2002. In vitro antifungal susceptibilities of *Trichosporon* species. Antimicrob. Agents Chemother. **46:**1144–1146.
23. **Pfaller, M. A., S. A. Messer, R. J. Hollis, and R. N. Jones.** 2001. In vitro activities of posaconazole (Sch 56592) compared with those of itraconazole and fluconazole against 3,685 clinical isolates of *Candida* spp. and *Cryptococcus neoformans*. Antimicrob. Agents Chemother. **45:**2862–2864.
24. **Pfaller, M. A., S. A. Messer, R. J. Hollis, and R. N. Jones.** 2002. Antifungal activities of posaconazole, ravuconazole, and voriconazole compared to those of itraconazole and amphotericin B against 239 clinical isolates of *Aspergillus* spp. and other filamentous fungi: report from SENTRY Antimicrobial Surveillance Program, 2000. Antimicrob. Agents Chemother. **46:**1032–1037.
25. **Rodriguez-Tudela, J. L., F. Barchiesi, J. Bille, E. Chryssanthou, M. Cuenca-Estrella, D. Denning, J. P. Donnelly, B. Dupont, W. Fegeler, C. Moore, M. Richardson, P. E. Verweij, and Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST).** 2003. Method for the determination of minimum inhibitory concentration (MIC) by broth dilution of fermentative yeasts. Clin. Microbiol. Infect. **9:**I–VIII.
26. **Rodriguez-Tudela, J. L., M. Cuenca-Estrella, L. Rodero, Y. Carpintero, and B. Gorgojo.** 2000. Influence of shaking on antifungal susceptibility testing of *Cryptococcus neoformans*: a comparison of the NCCLS standard M27A medium, buffered yeast nitrogen base, and RPMI-2% glucose. Antimicrob. Agents Chemother. **44:**400–404.
27. **Rodriguez-Tudela, J. L., E. Chryssanthou, E. Petrikkou, J. Mosquera, D. W. Denning, and M. Cuenca-Estrella.** 2003. Interlaboratory evaluation of hematocytometer method of inoculum preparation for testing antifungal susceptibilities of filamentous fungi. J. Clin. Microbiol. **41:**5236–5237.
28. **Safdar, A.** 2002. Progressive cutaneous hyalohyphomycosis due to *Paecilomyces lilacinus*: rapid response to treatment with caspofungin and itraconazole. Clin. Infect. Dis. **34:**1415–1417.
29. **Sun, Q. N., L. K. Najvar, R. Bocanegra, D. Loebenberg, and J. R. Graybill.** 2002. In vivo activity of posaconazole against *Mucor* spp. in an immunosuppressed-mouse model. Antimicrob. Agents Chemother. **46:**2310–2312.
30. **Uchida, K., N. Yokota, and H. Yamaguchi.** 2001. In vitro antifungal activity of posaconazole against various pathogenic fungi. Int. J. Antimicrob. Agents **18:**167–172.
31. **Walsh, T. J., V. Petraitis, R. Petraitiene, A. Field-Ridley, D. Sutton, M. Ghannoum, T. Sein, R. Schaufele, J. Peter, J. Bacher, H. Casler, D. Armstrong, A. Espinel-Ingroff, M. G. Rinaldi, and C. A. Lyman.** 2003. Experimental pulmonary aspergillosis due to *Aspergillus terreus*: pathogenesis and treatment of an emerging fungal pathogen resistant to amphotericin B. J. Infect. Dis. **188:**306–319.