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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*

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Posaconazole is a new investigational triazole with broad-spectrum antifungal activity. The in vitro activities of posaconazole were compared with those of itraconazole and fluconazole against 3,685 isolates of *Candida* spp. (3,312 isolates) and *C. neoformans* (373 isolates) obtained from over 70 different medical centers worldwide. The MICs of the antifungal drugs were determined by broth microdilution tests performed according to the National Committee for Clinical Laboratory Standards method using RPMI 1640 as the test medium. Posaconazole was very active against all *Candida* spp. (MIC at which 90% of the isolates were inhibited [MIC_{90}], 0.5 μg/ml; 97% of MICs were ≤1 μg/ml) and *C. neoformans* (MIC_{90}, 0.5 μg/ml; 100% of MICs were ≤1 μg/ml). *Candida albicans* was the most susceptible species of *Candida* (MIC_{90}, 0.06 μg/ml), and *Candida glabrata* was the least susceptible (MIC_{90}, 4 μg/ml). Posaconazole was more active than itraconazole and fluconazole against all *Candida* spp. and *C. neoformans*. These results provide further evidence for the spectrum and potency of posaconazole against a large and geographically diverse collection of clinically important fungal pathogens.

The triazole antifungal agents fluconazole and itraconazole offer several advantages over amphotericin B, including decreased toxicity and the versatility of oral and intravenous administration, and they are frequently used in the treatment of fungal infections due to *Candida* spp., *Cryptococcus neoformans*, and miscellaneous yeast species (14). However, failure of fluconazole and itraconazole therapy has been reported, and acquired or intrinsic resistance to these agents is well known (12, 16). There is clearly a need for additional agents with an enhanced potency and spectrum of activity to improve the treatment of fungal infections.

Posaconazole (Sch 56592) is an investigational triazole antifungal agent that is presently in Phase III clinical trials. Posaconazole exhibits linear pharmacokinetics and has demonstrated comparable safety and efficacy to those of fluconazole in both human and animal studies (3, 7, 8, 10; A. Catanzaro, G. Cloud, D. Stevens, B. Levine, P. Williams, et al., 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1417, 2000; R. Y. Hachem, I. I. Raad, C. M. Afif, R. Negroni, J. Graybill, et al., 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1109, 2000; L. Nieto, R. Northland, P. Pittisuttithum, C. Firnhaber, S. Jacobson, et al., 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1108, 2000; J. A. Vazquez, R. Northland, S. Miller, G. Dickinson, and G. Wright, 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1107, 2000). In vitro studies have documented a potency and spectrum of activity similar to those of itraconazole and superior to those of fluconazole against clinically important isolates of *Candida* spp., *C. neoformans*, and *Aspergillus* spp. (1, 3–5, 8–11).

Because previous in vitro studies have included a limited number of isolates of various yeast species and have generally been restricted in terms of the geographic distribution of the tested strains, we determined the in vitro activities of posaconazole against an international collection (from more than 70 institutions in 22 nations) of 3,685 clinical isolates of *Candida* spp. (14 species, 3,312 isolates) and *C. neoformans* (373 isolates). The comparison agents tested were the licensed triazole antifungal agents fluconazole and itraconazole. The in vitro susceptibility testing method employed was the microdilution broth method described in the National Committee for Clinical Laboratory Standards (NCCLS) document M27-A (6).

MATERIALS AND METHODS

Organisms. A total of 3,685 clinical isolates of *Candida* spp. (3,312 isolates) and *C. neoformans* (373 isolates) obtained from more than 70 different medical centers in North America, South America, and Europe were tested. The collection of isolates included 1,992 *C. albicans*, 421 *C. glabrata*, 468 *C. parapsilosis*, 243 *C. tropicalis*, 70 *C. dubliniensis*, 20 *C. guilliermondii*, 12 *C. famata*, 47 *C. krusei*, 24 *C. lusitaniae*, 6 *C. kefyr*, 4 *C. rugosa*, 2 *C. lambica*, and 2 *C. inconspicua* isolates and 1 *C. lipolytica* isolate. The isolates were all recent clinical isolates and the majority were from blood or normally sterile body fluids. Only the *C. dubliniensis* isolates were from mucosal sites. The isolates were identified by standard methods (15) and were stored as water suspensions until they were used. Prior to testing, each isolate was passaged at least twice on potato dextrose agar (Remel, Lenexa, Kans.) to ensure optimal growth characteristics.

Antifungal agents. Standard antifungal powders of posaconazole (Sch 56592; Schering-Plough), fluconazole (Pfizer), and itraconazole (Janssen) were obtained from their respective manufacturers. Stock solutions were prepared in polyethylene glycol (posaconazole and itraconazole) or water (fluconazole). Serial twofold dilutions were prepared exactly as outlined in NCCLS document M27-A (6). Final dilutions were made in RPMI 1640 medium (Sigma, St. Louis, Mo.) buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS) buffer (Sigma). The final concentration of solvent did not exceed 1% in any well.

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TABLE 1. In vitro susceptibilities of 3,312 clinical isolates of *Candida* spp. to posaconazole, itraconazole, and fluconazole

Organism (no. tested) and antifungal agent	MIC (μg/ml)			% Susceptible at MIC (μg/ml) of:			
	Range	50%	90%	0.12	0.25	0.5	1
C. albicans (1,992)							
Posaconazole	0.007–>8	0.03	0.06	98	98	99	99
Itraconazole	0.007–>8	0.03	0.12	94[a]	97	98	99
Fluconazole	0.12–>128	0.25	0.5	99 (≤8)[b]			
C. glabrata (421)							
Posaconazole	0.015–>8	1	4	5	15	46	80
Itraconazole	0.03–>8	1	8	6[a]	20	43	70
Fluconazole	0.25–>128	16	>128	42 (≤8)[b]			
C. parapsilosis (468)							
Posaconazole	0.015–2	0.06	0.12	96	99	99	99
Itraconazole	0.015–2	0.12	0.25	61[a]	91	99	99
Fluconazole	0.12–>128	0.5	2	98 (≤8)[b]			
C. tropicalis (243)							
Posaconazole	0.015–8	0.06	0.25	85	94	96	99
Itraconazole	0.015–8	0.12	0.5	58[a]	82	94	98
Fluconazole	0.12–>128	0.5	2	96 (≤8)[b]			
C. dubliniensis (70)							
Posaconazole	0.015–0.25	0.03	0.06	99	100	100	100
Itraconazole	0.015–0.5	0.06	0.25	89[a]	99	100	100
Fluconazole	0.12–>128	0.12	0.5	94 (≤8)[b]			
C. guilliermondii (20)							
Posaconazole	0.015–1	0.25	0.5	35	75	95	100
Itraconazole	0.03–1	0.25	1	20[a]	55	70	100
Fluconazole	0.25–8	2	8	100 (≤8)[b]			
C. famata (12)							
Posaconazole	0.015–1	0.25	1	42	58	83	100
Itraconazole	0.06–2	0.5	1	17[a]	42	58	92
Fluconazole	0.25–16	4	16	75 (≤8)[b]			
C. krusei (47)							
Posaconazole	0.12–0.5	0.5	0.5	2	36	100	100
Itraconazole	0.12–2	0.5	1	2[a]	30	72	96
Fluconazole	8–>128	32	>128	6 (≤8)[b]			
C. lusitaniae (24)							
Posaconazole	0.015–0.5	0.03	0.12	96	96	100	100
Itraconazole	0.03–0.5	0.12	0.5	67[a]	88	100	100
Fluconazole	0.12–8	1	2	100 (≤8)[b]			
Candida spp. (15)[c]							
Posaconazole	0.015–8	0.06	0.5	67	75	93	93
Itraconazole	0.015–8	0.06	1	73[a]	80	87	93
Fluconazole	0.25–>128	1	>128	80 (≤8)[b]			
Total (3,312)							
Posaconazole	0.007–>8	0.03	0.5	83	86	92	97
Itraconazole	0.007–>8	0.06	0.5	73[a]	84	90	95
Fluconazole	0.12–>128	0.25	16	90 (≤8)[b]			

[a] Percent susceptible at NCCLS breakpoint for itraconazole (≤0.12 μg/ml).
[b] Percent susceptible at NCCLS breakpoint for fluconazole (≤8 μg/ml).
[c] Includes six *Candida kefyr*, four *Candida rugosa*, two *Candida lambica*, and two *Candida inconspicua* isolates and one *Candida lipolytica* isolate.

Aliquots (0.1 ml) of each antifungal agent at a 2× final concentration were dispensed into the wells of plastic microdilution trays by using a Quick Spense II system (Dynatech Laboratories, Chantilly, Va.). The trays were sealed and frozen at −70°C until they were used.

Antifungal susceptibility studies. Broth microdilution testing was performed in accordance with the guidelines in NCCLS document M27-A (6) by using the spectrophotometric method of inoculum preparation, an inoculum concentration of $(1.5 \pm 1.0) \times 10^3$ cells/ml, and RPMI 1640 medium buffered to pH 7.0 with MOPS. A 100-μl yeast inoculum was added to each well of the microdilution trays. The final concentrations of the antifungal agents were 0.007 to 8.0 μg/ml for posaconazole and itraconazole and 0.12 to 128 μg/ml for fluconazole. The trays were incubated at 35°C, and MIC endpoints were read after 48 h of incubation (for both *Candida* spp. and *C. neoformans)*. Drug-free and yeast-free controls were included.

Following incubation, the broth microdilution wells were examined with the aid of a reading mirror; the growth in each well was compared with that of the growth control (drug-free) well. The MIC of each triazole was defined as the lowest concentration that produced a prominent decrease in turbidity (approximately 50% reduction in growth) compared with that of the drug-free control (6). The interpretive criteria for fluconazole and itraconazole were those published by Rex et al. (13) and the NCCLS (6).

Quality control. Quality was controlled by testing the following strains recommended by NCCLS standard M27-A (6): *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 (2).

RESULTS AND DISCUSSION

Table 1 summarizes the in vitro susceptibilities of 3,312 isolates of *Candida* spp. to posaconazole, fluconazole, and itraconazole. Overall, posaconazole was quite active (MIC at which 90% of the isolates were inhibited [MIC_{90}], 0.5 μg/ml; 97% of isolates were inhibited by ≤1 μg/ml). *C. albicans* and *C. dubliniensis* were the most susceptible (MIC_{90}, 0.06 μg/ml; 99 to 100% of MICs were ≤1 μg/ml) and *C. glabrata* was the least susceptible (MIC_{90}, 4 μg/ml; 80% of MICs were ≤1 μg/ml). Posaconazole was more active than both fluconazole and itraconazole against all species of *Candida*. Notably, 100% of *C. krusei* isolates were inhibited by ≤0.5 μg of posaconazole/ml, compared with 72% of isolates being inhibited by itraconazole.

Among the 3,312 *Candida* spp. isolates studied, a total of 76 were resistant to both fluconazole and itraconazole. The MICs of posaconazole (0.03 to ≥8 μg/ml; mode, ≥8 μg/ml) were also elevated with these isolates (82% of MICs were ≥4 μg/ml) (data not shown). In contrast, among 32 isolates resistant to fluconazole but not itraconazole, 31 (97%) were inhibited by ≤1 μg/ml of posaconazole. Isolates inhibited by 16 to 32 μg of fluconazole/ml (dose-dependent susceptibility) were all susceptible to ≤1 μg of posaconazole/ml.

The in vitro susceptibilities of 373 isolates of *C. neoformans* to posaconazole, fluconazole, and itraconazole are shown in Table 2. Fluconazole had MICs of ≤8 μg/ml for 78% of the *C. neoformans* isolates tested, 16 to 32 μg/ml for 21% of the isolates, and ≥64 μg/ml for 1% of the isolates. Overall, posaconazole and itraconazole demonstrated comparable activities (MIC_{90}, 0.5 μg/ml; 100% of isolates were inhibited by ≤1 μg/ml); however, 78% of isolates were inhibited by ≤0.12 μg of posaconazole/ml versus only 11% with itraconazole.

Among the isolates of *C. neoformans* inhibited by ≤8 μg of fluconazole/ml, posaconazole was more potent than itraconazole: 96% of these isolates were inhibited by ≤0.25 μg of

TABLE 2. In vitro susceptibilities of 373 clinical isolates of *C. neoformans* to posaconazole, itraconazole, and fluconazole

Antifungal agent	MIC (μg/ml)			% Susceptible at MIC (μg/ml) of:			
	Range	50%	90%	0.12	0.25	0.5	1
Posaconazole	0.015–1	0.12	0.5	78	86	98	100
Itraconazole	0.03–1	0.25	0.5	11	59	94	100
Fluconazole	0.25–>128	8	16	78 (≤8)[a]			

[a] Percent susceptible to fluconazole at ≤8 μg/ml.

posaconazole/ml and 68% were inhibited by ≤0.25 μg of itraconazole/ml. Posaconazole was also more active than itraconazole against the 73 isolates inhibited by 16 to 32 μg of fluconazole/ml. All of these isolates were inhibited by ≤0.5 μg of posaconazole/ml and 75% were inhibited by ≤0.5 μg of itraconazole/ml. Only three isolates required ≥64 μg of fluconazole/ml to inhibit growth in vitro. For these isolates, the posaconazole MICs were 0.12, 0.5, and 1 μg/ml and the itraconazole MICs were 0.5, 1, and 1 μg/ml.

These findings confirm and extend those reported previously regarding the antifungal activity of posaconazole (1, 3–5, 8–11). Posaconazole was as active or more potent than either fluconazole or itraconazole against virtually all of the *Candida* spp. and *C. neoformans* isolates tested. Posaconazole has been reported to be fungicidal against *C. neoformans*, filamentous fungi, and some *Candida* species (4, 8); however, minimum fungicidal concentration determinations were not performed in the present study. Similar to what occurred with fluconazole and itraconazole, some trailing was observed with posaconazole, but this was minimal and did not interfere with MIC endpoint determinations. Although the MICs of posaconazole for *Candida* spp. isolates that were resistant to fluconazole and itraconazole were also found to be elevated, those isolates resistant to fluconazole alone and those for which fluconazole MICs were 16 to 32 μg/ml were susceptible to ≤1 μg of posaconazole/ml. Likewise, posaconazole was active against all of the *C. krusei* isolates tested.

Posaconazole was also more active than either itraconazole or fluconazole against clinical isolates of *C. neoformans*. Both posaconazole and itraconazole were most active against isolates with the greatest susceptibility to fluconazole (MIC, ≤8 μg/ml). In addition, we evaluated the activity of posaconazole against isolates for which fluconazole MICs were increased. As fluconazole MICs increased, the MICs of both posaconazole and itraconazole did likewise. However, a greater proportion of isolates for which fluconazole MICs were 16 to 32 μg/ml remained susceptible at concentrations of ≤0.5 μg/ml for posaconazole (100%) than for itraconazole (75%). The three isolates for which fluconazole MICs were ≥64 μg/ml were all inhibited by ≤1 μg of posaconazole/ml, and the posaconazole MICs for two of the isolates were ≤0.5 μg/ml.

Consistent with these in vitro results, in vivo studies have demonstrated efficacy in treating infections due to *Candida* spp. and *C. neoformans* with posaconazole (3, 8; Hachem et al., 40th ICAAC; Nieto et al., 40th ICAAC; Vazquez et al., 40th ICAAC). Pharmacokinetic studies have demonstrated excellent oral bioavailability and peak concentrations in plasma of >5 μg/ml, with sustained levels exceeding 1 μg/ml for the entire dosing interval (7, 9). Thus, the oral dosing regimens result in concentrations of posaconazole in plasma that exceeded the MICs for 97 to 100% of *Candida* spp. and *C. neoformans* isolates (Tables 1 and 2).

In summary, we have demonstrated posaconazole to be more potent than fluconazole and itraconazole against significant clinical isolates of *Candida* spp. and *C. neoformans*. The emerging in vivo data from experimental models of fungal infection, as well as from clinical trials, appear to support these in vitro data. Posaconazole is a very promising new triazole antifungal agent that merits further clinical investigation.

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