

## In Vitro Activity of Posaconazole against Clinical Isolates of Dermatophytes

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**A broth macrodilution method following the recommendations established by the National Committee for Clinical Laboratory Standards was used to compare the in vitro activity of posaconazole (PCZ) with that of itraconazole (ITC) against 30 clinical isolates of dermatophytes belonging to six different species. In terms of MICs, PCZ showed an activity equal to that of ITC. MICs of PCZ at which 50% (MIC<sub>50</sub>) and 90% (MIC<sub>90</sub>) of the isolates were inhibited were 0.5 and >4.0 µg/ml, respectively. The MIC<sub>50</sub> and MIC<sub>90</sub> of ITC were 1.0 and >4.0 µg/ml, respectively. However, PCZ showed a more potent fungicidal activity than that of ITC against isolates belonging to the genus *Microsporum* ( $P = 0.03$ ). PCZ merits further investigation as a potentially useful agent for treatment of dermatophytosis.**

Cases of dermatophytosis have increased over the past few decades (11). These infections are often recalcitrant to therapy (4, 11). In the last few years, several antifungal agents, such as itraconazole (ITC), have become available for the treatment of these infections. Posaconazole (PCZ) is a new antifungal triazole with potent activity against yeasts and filamentous fungi that cause systemic infections (1, 7, 10). Whether this new triazole is active against dermatophytes is still unknown. In this study, we compared the in vitro activity of PCZ with that of ITC against clinical isolates of dermatophytes.

Thirty clinical isolates were collected over a 1-year period in the Department of Dermatology of the University of Ancona, Ancona, Italy. They included 16 strains of *Microsporum canis*, 6 strains of *Trichophyton rubrum*, 4 strains of *Trichophyton mentagrophytes*, 2 strains of *Epidermophyton floccosum*, and 1 strain each of *Trichophyton verrucosum* and *Microsporum gypsum*. All isolates were identified by standard methods, which included identification based on the macroscopic and microscopic characteristics of the cultured strains (5). Additional tests included those for the ability to produce a red pigment when the strains were grown on potato dextrose agar and for the ability to produce urease, as well as the hair perforation test. Both ITC (Janssen, Beerse, Belgium) and PCZ (Schering-Plough Research Institute, Kenilworth, N.J.) were provided as pure powders by their respective manufacturers. Stock solutions of both drugs were prepared in polyethylene glycol 400 (Janssen Chimica, Geel, Belgium). Further dilutions were prepared in the test medium. Both triazoles were tested at concentrations ranging from 0.0078 to 4.0 µg/ml. Testing was performed by a broth macrodilution method following the recommendations of the NCCLS (2, 6). In brief, stock inocula of the molds were prepared from 7- to 14-day cultures grown on potato dextrose agar at 30 to 35°C. Mature colonies were covered with approximately 2 to 3 ml of sterile water, and

suspensions were made by gently scraping the colony with the tip of a sterile pipette. The resulting suspended mixture was withdrawn and transferred to a sterile tube. Heavy particles of the suspension were allowed to settle for 5 min, and the upper homogeneous suspension was used for further testing. After the suspensions were mixed with a vortex mixer, their densities were read at a wavelength of 530 nm and adjusted to 95% transmittance. The suspensions containing conidia and hyphal fragments were diluted 1:10 with RPMI 1640 medium buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS) to obtain an inoculum size of approximately 10<sup>4</sup> CFU/ml. Tubes were incubated at 30°C. Readings were performed every day until the control tube showed visible growth. Incubation ranged from 6 to 10 days for isolates belonging to the genus *Microsporum*, *E. floccosum*, and *T. mentagrophytes*. Incubation ranged from 15 to 20 days for isolates of *T. rubrum* and *T. verrucosum*. The MIC of both triazoles was defined as the lowest drug concentration which resulted in an 80% reduction in turbidity compared to that of a drug-free control tube (2, 3, 8, 9). For determining the minimal fungicidal concentration (MFC), 100 µl of suspension was taken from those tubes exhibiting no growth and subcultured onto Sabouraud dextrose agar plates. The plates were incubated at 30°C until the growth of subcultures was visible. The MFC was defined as the lowest drug concentration at which no fungal growth was visible. A clinical isolate of *T. rubrum* was included in each experiment. All isolates were tested twice against both drugs.

Table 1 summarizes the in vitro susceptibilities of 30 clinical isolates of dermatophytes to ITC and PCZ. The MICs of ITC and PCZ ranged from 0.06 to >4.0 µg/ml and from 0.015 to >4.0 µg/ml, respectively. ITC MICs at which 50% and 90% of the isolates were inhibited were 1.0 and >4.0 µg/ml, respectively. For PCZ, these concentrations were 0.5 and >4.0 µg/ml, respectively. When the Mann-Whitney U test was used to determine the distribution of triazole MICs, no significant difference was found ( $P = 0.136$ ). In general, isolates belonging to the genus *Microsporum* proved to be significantly more susceptible than those belonging to the genus *Trichophyton* to

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TABLE 1. In vitro activities of ITC and PCZ against dermatophytes

Isolate(s) (no.)	MIC ( $\mu\text{g/ml}$ ) <sup>a</sup>						MFC ( $\mu\text{g/ml}$ ) <sup>a</sup>					
	ITC			PCZ			ITC			PCZ		
	Range	50%	90%	Range	50%	90%	Range	50%	90%	Range	50%	90%
<i>Microsporium</i> spp. <sup>b</sup> (17)	0.06–2.0	0.25	1.0	0.015–1.0	0.5	0.5	1.0–>4.0	2.0	2.0	0.5–4.0	0.5	1.0
<i>Trichophyton</i> spp. <sup>c</sup> (11)	0.125–>4.0	1.0	>4.0	0.5–>4.0	1.0	>4.0	0.25–>4.0	>4.0	>4.0	0.5–4.0	2.0	>4.0
<i>Epidermophyton floccosum</i> (2)	2.0–>4.0			2.0			ND <sup>d</sup>			ND		
Total	0.06–>4.0	1.0	>4.0	0.015–>4.0	0.5	>4.0	0.25–>4.0	2.0	>4.0	0.5–>4.0	1.0	>4.0

<sup>a</sup> 50% and 90%, MICs and MFCs at which 50 and 90% of isolates are inhibited, respectively.

<sup>b</sup> Includes *M. canis* (16 isolates) and *M. gypseum* (1 isolate).

<sup>c</sup> Includes *T. rubrum* (six isolates), *T. mentagrophytes* (four isolates), and *T. verrucosum* (one isolate).

<sup>d</sup> ND, not done.

both ITC ( $P = 0.001$ ) and PCZ ( $P = 0.0001$ ). The MFCs of ITC and PCZ ranged from 2 to >4.0  $\mu\text{g/ml}$  and from 0.5 to >4.0  $\mu\text{g/ml}$ , respectively. Although both drugs were equally effective against isolates belonging to the genus *Trichophyton* in terms of fungicidal activity, PCZ exerted a more potent activity than ITC against isolates belonging to the genus *Microsporium* ( $P = 0.03$ ).

To our knowledge, this is the first study in which the in vitro activity of the new antifungal agent PCZ against clinical isolates of dermatophytes has been investigated. Its activity was compared with that of ITC, a triazole whose activity against this group of fungi is well recognized. Our data showed that this new molecule appears to be as active in vitro as ITC against dermatophytes commonly encountered in clinical practice. In addition, PCZ showed a higher fungicidal activity than that of ITC against isolates belonging to the genus *Microsporium*. It must be noted that the correlation between in vitro results and clinical outcomes of cases of dermatophytosis is still to be established, and we therefore caution against extrapolating these results to clinical situations without additional testing of a larger sample of dermatophytes. Nevertheless, based on our study, PCZ merits further investigation as a potentially useful agent for the treatment of dermatophytosis.

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