

In Vitro and In Vivo Activities of Ketoconazole and Itraconazole Against *Pityrosporum orbiculare*

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The MICs of ketoconazole and itraconazole against *Pityrosporum orbiculare* were 0.02 to 0.05 and 0.1 to 0.2 $\mu\text{g ml}^{-1}$, respectively. In a rabbit model, orally administered ketoconazole (1 mg kg^{-1}) afforded protection against experimental pityriasis (tinea) versicolor in all animals. Itraconazole (5 mg kg^{-1}) was effective in four of five rabbits.

Experimental pityriasis (tinea) versicolor-like lesions have been produced with *Pityrosporum orbiculare* (*P. ovale*) under plastic occlusion in both rabbits and humans (1, 5). A method for the in vitro estimation of the MICs of potential therapeutic agents against *P. orbiculare* (*P. ovale*) has also been described (2, 4).

Ketoconazole, an imidazole derivative, is effective against many fungi both in vivo and in vitro (6). It is effective in many dermatomycoses, including pityriasis versicolor (3).

Itraconazole is an orally active triazole derivative recently developed by Janssen Pharmaceutica, Beerse, Belgium (7) (Fig. 1). It is active in vitro and, in various animal models, it is active against dermatophytes, yeasts, *Aspergillus* spp., dimorphic fungi, and various other fungi (J. Van Cutzem, F. Van Gerven, R. Zaman, J. Heeres, and P. A. J. Janssen, Abstr. Int. Congr. Chemother. 13th, Vienna, Austria, 1983). In pilot studies it was found to be effective against pityriasis versicolor in daily oral doses of 50 mg (G. Cauwenbergh, Abstr. Int. Congr. Chemother. 13th, Vienna, Austria, 1983).

To investigate the antimycotic activities of ketoconazole and itraconazole, I determined the MICs of these compounds against *P. orbiculare* and studied their prophylactic activities against experimental *P. orbiculare* infections in rabbits.

In vitro activity of ketoconazole and itraconazole. *P. orbiculare* ATCC 42132, 44337, 44338, 44339, 44340, and 44031 were used. They were grown on glucose-neopeptone-yeast extract medium with glycerol monostearate (2.5 g liter⁻¹), olive oil (20 ml liter⁻¹), and Tween 80 (2 ml liter⁻¹) at 37°C for 3 days before use (4).

The test agar for MIC determinations was DST (Oxoid Ltd., Basingstoke, England) with glycerol monostearate (2.5 g liter⁻¹) and Tween 80 (2 ml liter⁻¹). The details of the technique used have been described previously (2, 4). Ketoconazole and itraconazole were dissolved in *N,N*-dimethylformamide and diluted in water to yield stock solutions containing 1,000 μg of ketoconazole and itraconazole ml⁻¹ and 20% *N,N*-dimethylformamide. From the stock solutions dilutions were made with distilled water to yield concentrations of 0.005 to 100 μg of ketoconazole and itraconazole ml⁻¹ in the agar. The plates were inoculated with 10⁸ cells ml⁻¹, incubated at 37°C, and read after 1, 2, 3, and 4 days of growth. The experiment was done in duplicate, and the MIC endpoint was defined as the lowest concentration of drug that inhibited growth.

The MICs of ketoconazole against *P. orbiculare* were 0.02 $\mu\text{g ml}^{-1}$ for one isolate and 0.05 $\mu\text{g ml}^{-1}$ for the other five

isolates. The MICs of itraconazole were 0.1 $\mu\text{g ml}^{-1}$ for five isolates and 0.2 $\mu\text{g ml}^{-1}$ for the other isolate.

In vivo experiments. Two groups each of 18 male New Zealand White rabbits weighing 2.3 to 3.3 kg were used. Ketoconazole and itraconazole were suspended in polyethylene glycol 200 and administered orally by gavage once daily for 7 days. Six rabbits in each group received 1 mg of drug kg^{-1} , and six received 5 mg kg^{-1} . Six rabbits in each group served as controls.

The details of the experimental infections have been described previously (1, 5). *P. orbiculare* ATCC 44031 was used. The cells were taken with an ordinary loop directly from the culture plate (mean, 1.7×10^7 cells, as determined in a counting chamber). The rabbits were inoculated on the insides of both ears in an area measuring 1 by 1.5 cm. The areas were occluded for 7 days with Scotch 3M plastic wrap (1 by 1.5 cm) held in place by Scanpor tape (Norgesplaster A/S, Norway) covered with Leukoplast tape (Beiersdorf AG, Hamburg, West Germany). After 7 days, the inoculated areas were investigated clinically, under Wood's light, and microscopically, and skin scrapings were taken with a curette for culturing. The criteria for positive microscopy included round and budding cells and the presence of hyphae (1, 5). The rabbits were examined at weekly intervals for 3 weeks.

The results after 1 week are as follows. Three rabbits, two receiving 1 mg of ketoconazole kg^{-1} and one receiving 5 mg of itraconazole kg^{-1} , died because of technical problems with the gavage. In the ketoconazole group, all animals were without signs of infection, but cultures were positive for all receiving 1 mg kg^{-1} and for four of six receiving 5 mg kg^{-1} . In the itraconazole group, infections developed in four of six rabbits receiving 1 mg kg^{-1} and in one of five rabbits receiving 5 mg kg^{-1} . The infections were less pronounced than in the control group (12 of 12 rabbits infected).

Ketoconazole was highly effective against *P. orbiculare*, both in vitro and in vivo. The in vitro activity of ketoconazole was comparable to that found in an earlier study (3). In vivo ketoconazole at doses as low as 1 mg kg^{-1} afforded complete protection against experimental *P. orbiculare* infections. This is comparable to its good clinical effect in the treatment of pityriasis versicolor (3).

Although the activity of itraconazole was lower than that of ketoconazole, itraconazole was still very potent in vitro with MICs against *P. orbiculare* between 0.1 and 0.2 $\mu\text{g ml}^{-1}$. The in vivo activity was also lower than that of ketoconazole, but in the group receiving 5 mg kg^{-1} only one

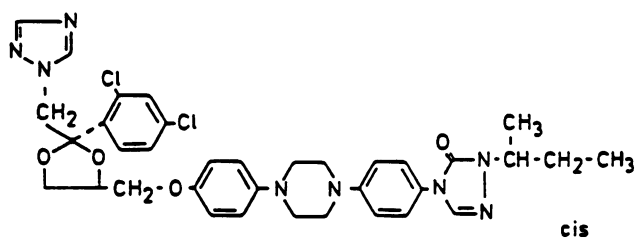


FIG. 1. Chemical structure of itraconazole.

of five animals developed an infection. This infection was less pronounced than that in the control animals and healed within 1 week, as compared with 2 to 3 weeks for control animals. These results are in contrast to preclinical data indicating that the effect of itraconazole at a daily dose of 50 mg for 2 to 3 weeks is comparable to that of ketoconazole against pityriasis versicolor (G. Cauwenbergh 13th ICC).

For ketoconazole, the peak concentration in plasma after a single oral dose of 10 mg kg⁻¹ reached 1.0 µg ml⁻¹ after 2 h (6). For itraconazole, the peak concentration in plasma after a single oral dose of 10 mg kg⁻¹ reached 0.89 µg ml⁻¹ after 6 h (data on file at Janssen Pharmaceutica), and after a single oral dose of 5 mg kg⁻¹ it reached 0.21 µg ml⁻¹ after 4 h (unpublished data). All of these data are for rabbits.

As with other experimental infections, the problem is that the lesions heal spontaneously. Therefore, a prophylactic

infection model was thought to be most relevant. This model for pityriasis versicolor, described in detail previously (1, 5), opens possibilities for screening new antimycotics for their activity against *P. orbiculare* in vivo.

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