The Effect of Ketoconazole and Itraconazole on the Filamentous Form of *Pityrosporum ovale*

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The effect of ketoconazole and itraconazole on the filamentous form of *Pityrosporum ovale in vitro* was studied. In a recently developed model, using human stratum corneum *in vitro*, *P. ovale* transformed into the filamentous form in 25–30 % of the cells. Ketoconazole and itraconazole in concentrations of 0.01, 0.1 and 1 µg/ml were incubated together with *P. ovale* cells on human stratum corneum pieces placed on a lipid-enriched culture medium. Both agents effectively blocked the production of hyphae. From the low concentration onwards, the changes consisted of a diminishing transformation into hyphae. With transmission electron mi-

croscopy, the interior of many cells was often in an advanced stage of necrosis. Exposure to 1 μg/ml itraconazole causes a disorganization of the internal organelles in 83 % of the cells. This model for the production of hyphae of *P. ovale* in vitro proved very valuable in screening the activity of antimycotic agents against the filamentous form of this yeast. *Key words: Light microscopy; SEM; TEM.*

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Pityrosporum ovale is a member of the normal human cutaneous flora (1). It is also the aetiological agent in pityriasis versicolor (2), Pityrosporum folliculitis (3) and seborrhoeic dermatitis (4). The aetiological role in atopic dermatitis of the head and neck (5) and sebopsoriasis (6) has also been discussed.

In an earlier study we have been able to produce hyphae in *P. ovale* on human stratum corneum *in vitro* (7). Recently we have improved this model by inoculation on stratum corneum pieces placed on a lipid-enriched selective culture medium (8).

Ketoconazole is a broad spectrum, orally active imidazole derivative (9). It has a very high activity against *P. ovale in vitro* and is also very effective in various *Pityrosporum*-associated diseases (9). Itraconazole is a new, orally active broad spectrum triazole derivative active against *P. ovale* both *in vitro* and *in vivo* (10). In the present investigation we have studied the activity of both ketoconazole and itraconazole against the filamentous form of *P. ovale* with light microscopy, scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

MATERIALS AND METHODS

Light microscopy

The model for filamentous growth of *P. ovale* has been described in detail earlier (8). The strain used in this study was *P. ovale* ATCC 44341. Ketoconazole and itraconazole were dissolved in dimethyl formamide to give a stock solution containing 1 000 μg/ml of the two drugs. Further dilutions were made in phosphate-buffered saline (pH 7.2). Immediately after inoculation on the stratum corneum, the samples were exposed to 0.01, 0.1 and 1.0 μg/ml doses of ketoconazole and itraconazole and then incubated in a microaerophilic environment at 37°C for 6 days. Control cultures were incubated at the same time. Specimens were then stained by the periodic acid Schiff reaction (PAS) for light microscopy examinations.

Scanning (SEM) and transmission electron microscopy (TEM)

At the end of treatment, samples for electron microscopy were immediately fixed at room temperature in 3 % gluataraldehyde buffered to pH 7.5 with 0.1 M sodium cacodylate for several days and rinsed in the same buffer supplemented with 0.22 M sucrose. Samples were postfixed in 2 % OsO_4 buffered to pH 7.4 with 0.05 M veronal acetate at 4°C for 1 hour. After a brief rinse in buffer, the samples were impregnated for 40 min with 0.5 % uranyl acetate buffered to pH 5.2 with veronal acetate. Thereafter, the samples were rinsed in the same buffer and dehydrated in graded series of ethanol. At this point, the samples were divided into two parts. One part was routinely embedded in Epon. Ultrathin sections of the cells were stained with uranyl acetate and lead citrate prior to examination in a Philips EM 300 transmission electron microscope (11). The other part for SEM was passed through a series of graded ethanol–acetone mixtures until 100 % acetone was reached, then critical point dried and covered with a thin layer of gold before examination in a Philips SEM 500 microscope (11). For ultrastructural quantification, a total of 100 cells from each sample were evaluated.

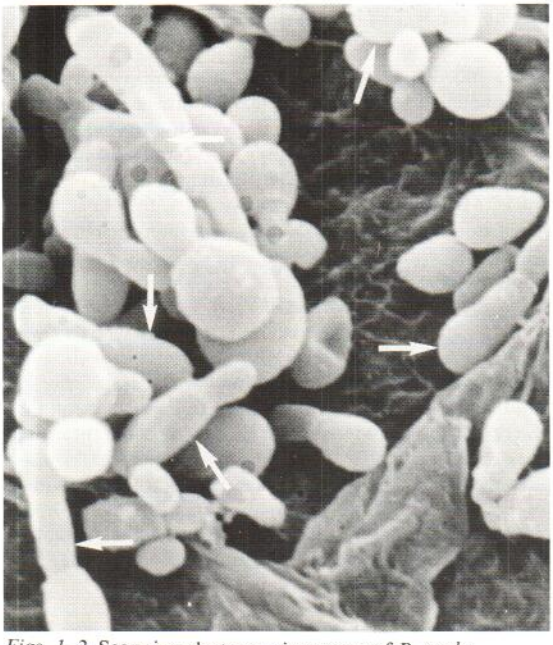
RESULTS

Light microscopy

Table IA shows the light microscopic observations after incubation of *P. ovale* cells with various concen-

Table I. *Effects of ketoconazole and itraconazole on the production of hyphae in Pityrosporum ovale*

	% hypae
A. Light microscopic observations	
Control	26
Ketoconazole	
1 μg/ml	3
0.1 μg/ml	5
0.01 μg/ml	11
Itraconazole	
1 μg/ml	10
0.1 μg/ml	12
0.01 μg/ml	18
B. Scanning-electron microscopic observations	
Control	32
Ketoconazole	
1 μg/ml	2
0.1 μg/ml	2
0.01 μg/ml	7
Itraconazole	
1 μg/ml	5
0.1 μg/ml	13
0.01 μg/ml	14



Figs. 1–2. Scanning electron microscopy of *P. ovale*.
Fig. 1. Control culture. A number of cells adopted the hyphal forms (*arrows*) (×5250).

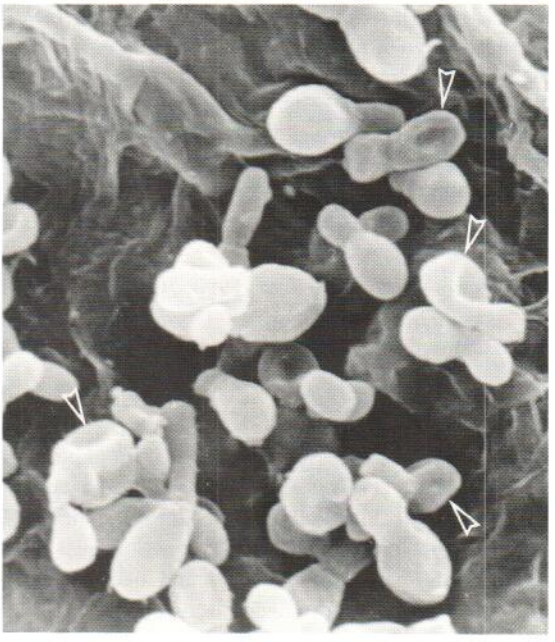
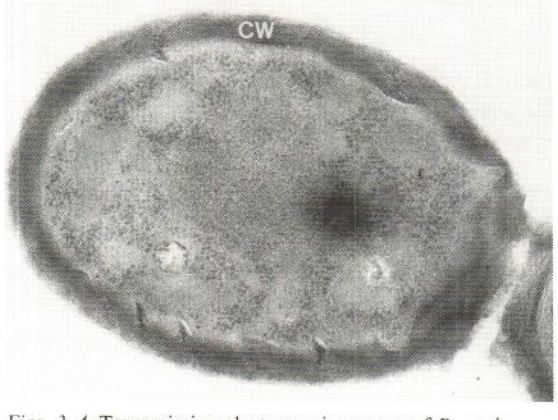


Fig. 2. Culture treated with 1 μg/ml itraconazole. The mycelial outgrowth is strongly suppressed. Note the presence of collapsed cells (arrowheads) (×3900).



Figs. 3–4. Transmission electron microscopy of *P. ovale*.
Fig. 3. Control culture. Section through portions of a developing hypha. Note the uniform density of the cell wall (*cw*) (×41050).

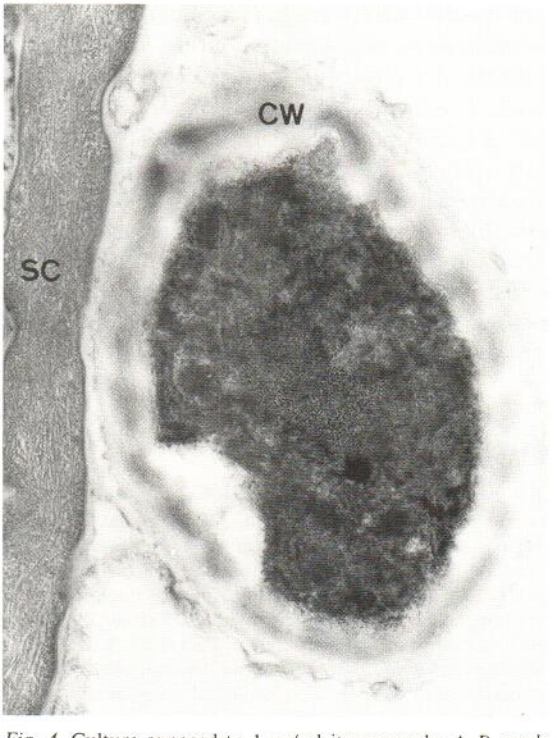


Fig. 4. Culture exposed to 1 μg/ml itraconazole. A *P. ovale* lying close to a stratum corneum cell (*sc*) shows a necrotic cytoplasm and is surrounded by an ill-defined plasma membrane and a partly dissolved cell wall (*cw*) (×79,150).

trations of ketoconazole and itraconazole. Incubation with ketoconazole and itraconazole reduced the number of filaments produced. With a concentration of ketoconazole of 0.1 and 1 μg/ml, only 5% and 3%, respectively, of the cells were transformed into the hyphal form. Besides, many fungi were swollen and stained poorly.

Table II. *Necrosis of Pityrosporum ovale induced by ketoconazole and itraconazole at different doses*

	% necrotic cells
Control	15
Ketoconazole	
1 μg/ml	61
0.1 μg/ml	27
0.01 μg/ml	18
Itraconazole	
1 μg/ml	83
0.1 μg/ml	26
0.01 μg/ml	24

Electron microscopy

Control cultures showed *P. ovale* cells which were partly transformed into the hyphal form (Fig. 1). With TEM, the majority of cells looked healthy with a compact cytosol and a uniformly thick electron-dense cell wall (Fig. 3). A small proportion of the cells were necrotic, possible due to the non-replenishment of the medium. Penetration of *P. ovale* either as yeast or mycelial elements, into keratinocytes, as seen under *in vivo* conditions, was not observed under the current culture conditions.

Observations made with TEM and SEM on *P. ovale* cultured for 6 days on stratum corneum in the presence of 0.01 μg/ml to 1 μg/ml ketoconazole and itraconazole revealed the following changes. From the low concentration (0.01 μg/ml) of ketoconazole onwards, the changes consisted of a diminishing transformation into hyphae (Table IB) (Fig. 2). The data observed with SEM fully corroborate those obtained with the light microscope (Table IA). Many of the treated cells had a wrinkled surface and appeared collapsed (Fig. 2). With TEM the walls of most of the cells were thickened and were loosely organized. The interior of such altered cells was very often in an advanced stage of necrosis so that subcellular organelles were barely identifiable (Fig. 4). Exposure to 1 μg/ml itraconazole resulted in a disorganization of the internal organelles in 83% of the cells (Table II). The degree of necrosis was slightly higher after exposure to itraconazole in the 1 μg/ml concentration, whereas in the lower concentration range there was no difference between ketoconazole and itraconazole. Changes in the cell wall in the form of vesicle deposition, an alteration usually seen in fungi after azole exposure, were not seen in these cultures.

DISCUSSION

In pityriasis versicolor, *P. ovale* changes from the saprophytic round or oval blastospore form to the mycelial form (1). In Pityrosporum folliculitis and seborrhoeic dermatitis the organism usually remains in the blastospore form, though production of hyphae has been reported (3). An in vitro model for filamentous production in *P. ovale* has been described earlier (7), but with difficulties in reproducibility. A new model that consistently enables the production of hyphae in *P. ovale* has been developed recently (8).

Using these culture conditions we found that both ketoconazole and itraconazole were very effective in blocking the production of hyphae in *P. ovale*. This was noticed with the light microscope where we found a significant reduction in the number of cells producing hyphae (from 26% to 3–10%). The efficacy of these azole antifungals in inhibiting the growth of the *P. ovale* yeast form (12, 13) and deteriorating the substructure of the *P. ovale* mycelium form in patients with pityriasis versicolor (11) has been reported.

The present data on the electron microscopic appearance of *P. ovale's* different morphologic forms substantiate and extend these results. There appears to be only a marginal difference in potency between the two antifungals—both inhibit the morphogenic yeast to mycelium transition and induce structural injury to *P. ovale*.

In conclusion, this model for *in vitro* production of hyphae in *P. ovale* is very valuable for screening the activity of antimycotic agents the filamentous form of this yeast.

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