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THE ACTIVITY *IN VITRO* OF FIVE DIFFERENT ANTIMYCOTICS AGAINST *PITYROSPORUM ORBICULARE*

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Abstract. The activity *in vitro* of miconazole, clotrimazole, econazole, sodium omadine, and sodium thiosulphate against *Pityrosporum orbiculare* was found to correlate with the good clinical results these drugs produce in tinea versicolor. In addition many substances used as solvents or in vehicles had an inhibitory effect *in vitro* against *P. orbiculare*. The influence of the culture medium, especially lipids, on the action of imidazole derivatives is discussed.

Key words: Pityrosporum orbiculare; Antimycotics; MIC

It is now generally accepted that *Pityrosporum orbiculare* and the dimorphic fungus seen in tinea versicolor are one and the same organism (3, 10, 18).

The clinical effect of miconazole, clotrimazole, econazole, zinc omadine (pyrithione), and sodium thiosulphate in tinea versicolor has long been established (5, 6, 14, 4, 13). Only a few reports have appeared on the activity of antifungal agents against *P. orbiculare in vitro*. Van Cutzem & Thienpont studied the antimycotic activity of miconazole against *P. orbiculare* and found a MIC of less than 1 μg/ml (19). The lipophilic yeast *P. orbiculare* does not thrive on lipid-free media, and the medium used by Van Cutzem & Thienpont was a Sabouraud's liquid medium. In studies with other yeasts, Sabouraud's medium has an inhibitory effect on the antimycotic activity of miconazole and clotrimazole (7, 8).

Propylene glycol is used as a vehicle in many dermatological formulations. It has an antimicrobial activity against many bacteria and fungi (14). Seidel et al. found no activity of ethanol, propyl alcohol, isopropyl alcohol, dimethylformamide, and acetone against *P. orbiculare in vitro* (16).

In this study, the *in vitro* activity of miconazole, clotrimazole, econazole, sodium omadine, and sodium thiosulphate against *P. orbiculare* was investigated. The activity of some substances used as solvents or in vehicles, namely ethanol, methanol, isopropyl alcohol, propylene glycol, acetone, dimethylformamide, and dimethyl sulphoxide was also investigated *in vitro*.

MATERIALS AND METHODS

Microorganisms

P. orbiculare was cultured on a medium containing neopeptone (Difco) 10 g/l, Bacto agar (Difco) 18 g/l, glucose 40 g/l, yeast extract (Difco) 0.1 g/l, Tween 80 2 ml/l, and glycerol monostearate 2.5 g/l; pH adjusted to 5.6. After autoclave sterilization, chloramphenicol (50 mg/l) and gentamycin (100 mg/l) were added. As a control, one strain of *Candida albicans*, obtained from the National Bacteriological Laboratory, Stockholm, Sweden, was used. *C. albicans* was grown on Sabouraud's solid medium. The *P. orbiculare* strains were obtained from patients with tenea versicolor. A fungal isolate was considered to be *P. orbiculare* if it was lipophilic and if the microscopical morphology conformed with descriptions of Lodder (13).

Antifungal agents

Miconazole base, clotrimazole base, econazole base, sodium omadine, and sodium thiosulphate were used for MIC investigations. These drugs were obtained from regular commercial sources. A screening test for the activity of ethanol, methanol, isopropyl alcohol, propylene glycol, acetone, dimethylformamide, and dimethyl sulphoxide against *P. orbiculare in vitro* was also perfomed.

Test medium

The medium (DST, Oxoid) used was compounded as follows: proteose peptone 10 g/l, veal infusion solids 10 g/l, glucose 2 g/l, sodium chloride 3 g/l, disodium phosphate 2 g/l, sodium acetate 1 g/l, adenine sulphate 0.01 g/l, guanine hydrochloride 0.01 g/l, uracil 0.01 g/l, xanthine 0.01 g/l, aneurine 0.00002 g/l, agar no. 1 (Difco) 12 g/l; pH adjusted to 5.6. In order to enable growth of *P. orbiculare*, glycerol monostearate 2.5 g/l and Tween 80 2 ml/l were added to the medium. *C. albicans* was tested on the same medium with and without these additives.

Procedures

P. orbiculare was harvested after 4 days of growth at 37°C. *C. albicans* was harvested after 2 days' growth at 37°C.

1. For MIC investigations of miconazole, clotrimazole, econazole, and sodium omadine, DST medium containing

Table I. *The* in vitro *activity of miconazole, clotrimazole, econazole, and sodium omadine against* Pityrosporum orbiculare

P. orbiculare Strain no.	MIC, μg/ml			
	Miconazole	Econazole	Clotrimazole	Sodium omadine
1	0.4	0.2	0.1	0.75
2	0.75	0.4	0.4	0.75
3	0.2	0.2	0.1	0.75
4	0.75	0.4	0.4	0.75
5	0.2	0.2	0.1	0.75
6	0.75	0.4	0.4	1.5
7	0.75	0.4	0.4	1.5
8	1.5	0.4	0.4	1.5
9[a]	50	12.5	12.5	3
10[a]	50	12.5	12.5	3
11	0.4	0.2	0.1	0.75
12	0.4	0.2	0.1	0.75
13[a]	0.05	0.05	0.0125	0.2
14[a]	0.4	0.1	0.0125	0.2
15[a]	50	12.5	25	6
16	0.4	0.4	0.2	1.5
17	0.75	0.75	0.2	1.5
18	0.75	0.75	0.4	1.5
19	0.75	0.4	0.4	1.5
Mean value	0.63	0.38	0.26	1.13

[a] The MIC of these strains are not included in the mean value.

various concentrations of antimycotics was inoculated with 10^8 viable units/ml of *P. orbiculare* (as determined in a counting chamber). As a control, plates were inoculated with 10^6 and 10^8 viable units/ml of *C. albicans*. In addition, DST medium, without additives, containing miconazole was inoculated with 10^6 viable units/ml of *C. albicans*. Nineteen different strains of *P. orbiculare* were tested. The antimycotics miconazole, clotrimazole, and econazole were dissolved in dimethylformamide (DMF) and thereafter diluted with distilled water. Three different stock solutions were made containing 1000, 60, and 4 μg/l of these 3 antimycotics. The highest concentrations of DMF were in the solutions containing 1 000 μg/l, 10% for econazole, 20% for miconazole, and 50% for clotrimazole. From these nine stock solutions dilutions were made, using substrate still in liquid form, giving concentrations of 100, 50, 25, 12.5, 6, 3, 1.5, 0.75, 0.4, 0.2, 0.1, 0.05, 0.025, and 0.012 μg/ml of miconazole, clotrimazole, and econazole. The control plates contained the test medium with and without the various concentrations of DMF used. The same procedure was carried out using sodium omadine, but this substance, being water soluble, was dissolved directly in distilled water.

2. To study the activity of sodium thiosulphate *in vitro*, sodium thiosulphate was diluted in distilled water and added to DST medium to give concentrations of 10000, 1000, 100, 10, and 1 μg/ml. These plates were inoculated with 10^7 and 10^8 viable units/ml of *P. orbiculare*. Five different strains were tested.

3. The activity of ethanol, methanol, isopropyl alcohol, propylene glycol, acetone, dimethylformamide, and dimethyl sulphoxide against *P. orbiculare* was studied in a screening test. Ten, one and 0.1% solutions of each of these substances were made in DST medium. The plates were inoculated with 10^7 and 10^8 viable units/ml of *P. orbiculare*. Five different strains were tested.

In all experiments the plates were incubated at 37°C and read after 1, 2, and 3 days of growth.

RESULTS

1. MIC of miconazole, clotrimazole, econazole, and sodium omadine

Table I shows the results after 3 days. Only minor differences were seen in the MIC of miconazole, clotrimazole, econazole, and sodium omadine against *P. orbiculare*. Strains 9, 10, and 15 were markedly more resistant to miconazole, clotrimazole, and econazole. These strains also had a higher resistance to sodium omadine, though far less pronounced. Strains 13 and 14 were very susceptible to all four antimycotics. DMF showed an inhibitory activity in the concentrations 2.5% and 5%. These concentrations were only present in plates containing 50 and 100 μg/ml of clotrimazole.

The inhibitory activity of miconazole, clotrimazole, and econazole against *C. albicans* was not in evidence in the DST medium supplemented with glycerol monostearate and Tween 80. When the same strain was tested with miconazole, without the additives, it had a MIC of 0.4 μg/ml. The MIC

of sodium omadine on *C. albicans* was 6 μg/ml, comparable to the least sensitive strain of *P. orbiculare*.

2. MIC of sodium thiosulphate

The five strains tested were inhibited by sodium thiosulphate 100 μg/ml, using 10^7 viable units/ml, and by 1 000 μg/ml using 10^8 viable units/ml of *P. orbiculare*.

3. The antifungal activity of ethanol, methanol, isopropyl alcohol, propylene glycol, dimethylformamide, acetone, and dimethyl sulphoxide against P. orbiculare

All of these substances had an inhibitory activity against *P. orbiculare* in a 10% concentration. Isopropyl alcohol, ethanol, and acetone also had an inhibitory effect in a 1% concentration. No inhibitory activity was noted in the 0.1% concentration. The results were not affected by the size of the inoculum.

DISCUSSION

The antifungal effect of the imidazole derivatives is attributable to damage to the cell wall (9, 17) and may be dependent on an inhibition of ergosterol biosynthesis (20). The antimycotic activity of imidazole derivatives may be inhibited by egg lecithin and other phospholipids (11, 21). The difficulty with *P. orbiculare*, in the investigation of MIC, is that it is lipophilic and the addition of a lipid substance to the test medium is necessary to obtain optimal growth. Earlier reports have indicated the influence of the culture medium on the *in vitro* activity of miconazole and clotrimazole (7, 8). These reports show that Sabouraud's medium and brain–heart infusion medium have an inhibitory effects on the action of clotrimazole and miconazole. Another problem when the activity of imidazole derivatives is tested *in vitro*, is the low water solubility of these substances. As shown in this investigation, many of the substances used as solvents have an antifungal activity. Even at low concentrations of the solvent, an additional inhibitory effect may be suspected. In order to obtain low concentrations of the initial solvent, dilutions must be made with water. This procedure may give rise to precipitation, thus producing a false MIC.

The *in vitro* activity of miconazole, clotrimazole, and econazole against *P. orbiculare* corresponds to the good clinical effect of these drugs. The activity of miconazole against *C. albicans* is apparently hampered by the presence of glycerol monostearate and Tween 80. This may reflect differences in the mechanism of action of imidazole derivatives on *C. albicans* and *P. orbiculare*. Zinc omadine is an antifungal and antibacterial substance which has long been used in the treatment of dandruff (1, 2). In addition, it has a good clinical effect in tinea versicolor (4). Zinc omadine has a very low water solubility and also a low solubility in many other solvents. We therefore investigated the *in vitro* activity of a related compound, sodium omadine. This substance has a marked inhibitory effect on *P. orbiculare in vitro*. In contrast to the imidazole derivatives, it also had, in the DST medium with glycerol monostearate and Tween 80, an inhibitory activity against *C. albicans*. The *in vitro* activity of selenium sulphide, another drug with a good clinical effect in tinea versicolor, was not tested because of its low solubility in most solvents.

We found three strains of *P. orbiculare* which were less susceptible to miconazole, clotrimazole, and econazole. The patients from whom these strains were cultured were treated with miconazole cream and all were cured. With topical treatment a high concentration of the antimycotic can be achieved, and this may explain the beneficial clinical effect despite the high MIC.

In the *in vitro* study of the activity of sodium thiosulphate against *P. orbiculare*, the effect of the number of cells inoculated is shown. When 10^8 viable units/ml were inoculated the MIC was 1 000 μg/ml. This is a high value, but clinically sodium thiosulphate is used as a 10% solution, and this may explain its good clinical effect.

REFERENCES

1. Brauer, E. W., Opdyke, D. L. & Burnett, C. M.: The antiseborrheic qualities of zinc pyrithione (zinc pyridine-2-thiol-1-oxide) in a cream vehicle. J Invest Dermatol *47:* 174, 1966.
2. Brauer, E. W.: Antiseborrheic qualities of zinc pyrithione in a cream vehicle. III. Clinical experience in pityriasis capitis (dandruff, seborrheic dermatitis of the scalp). Cutis *5:* 723, 1969.
3. Burke, R. C.: Tinea versicolor: Susceptibility factors and experimental infection in human beings. J Invest Dermatol *36:* 389, 1961.
4. Faergemann, J. & Fredriksson, T.: An open trial of the effect of a zinc pyrithione shampoo in tinea versicolor. Accepted for publication in Cutis, 1979.
5. Fredriksson, T.: Treatment of dermatomycosis with topical miconazole (Daktar). Opuscula Med *21:* 80, 1977.

6. Gip, L.: The topical therapy of pityriasis versicolor with clotrimazole. Postgrad Med J *50:* 59, 1974.
7. Hoeprich, P. D. & Finn, P. D.: Obfuscation of the activity of antifungal antimicrobics by culture media. J Infect Dis *126:* 353, 1972.
8. Hoeprich, P. D. & Huston, A. C.: Effect of culture media on the antifungal activity of miconazole and amphotericin B methyl ester. J Infect Dis *134:* 336, 1976.
9. Iwata, K., Yamaguchi, H. & Hiratani, T.: Mode of action of clotrimazole. Sabouraudia *11:* 158, 1973.
10. Keddie, F. M. & Shadomy, S.: Etiological significance of *Pityrosporum orbiculare* in tinea versicolor. Sabouraudia *3:* 21, 1963.
11. Kuroda, S., Uno, J. & Arai, T.: Target substances of some antifungal agent in the cell membrane. Antimicrob Agents Chemother *3:* 454, 1978.
12. Lodder, J.: The Yeasts, second edition, 1167–1186. North-Holland Publishing Company, Amsterdam-Londan, 1971.
13. McClarin, M. & Knox, J. M.: Treatment of tinea versicolor. Cutis *4:* 619, 1968.
14. Olitzky, I.: Antimicrobial properties of a propylene glycol based topical therapeutic agent. J Pharm Sci *54:* 787, 1965.
15. Schwartz, K. J., Much, T. & Konzelmann, M.: Poliklinische Prüfung von Econazol bei 594 Fällen von Hautmykosen. Dtsch Med Wochenschr *100:* 1497, 1975.
16. Seidel, R., Kaben, U. & Heise, H.: Kulturelle Untersuchungen und Resistenzbestimmungen bei Pityriasis versicolor. Dermatol Monatsschr *160:* 483, 1974.
17. Swamy, K. H. S., Sirsi, M. & Rao, G. R.: Studies on the mechanism of action of miconazole: Effect of miconazole on respiration and cell permeability of *Candida albicans*. Antimicrob Agents Chemother *5:* 420, 1974.
18. Sternberg, T. H. & Keddie, F. M.: Immunofluorescence studies in tinea versicolor. Arch Dermatol *84:* 999, 1961.
19. Van Cutsem, J. M. & Thienpont, D.: Miconazole, a broad-spectrum antimycotic agent with antibacterial activity. Chemotherapy *17:* 392, 1972.
20. Van Den Bossche, H., Willemsens, G., Cools, W., Lauwers, W. F. J. & Le Jeune, L.: Biochemical effects of miconazole on fungi. II. Inhibition of ergosterol biosynthesis in *Candida albicans*. Chem Biol Interactions *21:* 59, 1978.
21. Yamguchi, H.: Protection by unsaturated lecithin against the imidazole antimycotics, clotrimazole and miconazole. Antimicrob Agents Chemother *13:* 423, 1978.

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