THE ACTIVITY IN VITRO OF FIVE DIFFERENT ANTIMYCOTICS AGAINST PITRYSPORUM 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.

Keywords: 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 was obtained from patients with tinea 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 performed.

Test medium
The medium (DST, Oxoid) used was compounded as follows: proteose peptone 30 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, thymine 0.01 g/l, aneurine 0.0002 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

<table>
<thead>
<tr>
<th>Strain no.</th>
<th>Miconazole (µg/ml)</th>
<th>Econazole</th>
<th>Clotrimazole</th>
<th>Sodium omadine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.4</td>
<td>0.2</td>
<td>0.1</td>
<td>0.75</td>
</tr>
<tr>
<td>2</td>
<td>0.75</td>
<td>0.4</td>
<td>0.4</td>
<td>0.75</td>
</tr>
<tr>
<td>3</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>0.75</td>
</tr>
<tr>
<td>4</td>
<td>0.75</td>
<td>0.4</td>
<td>0.4</td>
<td>0.75</td>
</tr>
<tr>
<td>5</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>0.75</td>
</tr>
<tr>
<td>6</td>
<td>0.75</td>
<td>0.4</td>
<td>0.4</td>
<td>1.5</td>
</tr>
<tr>
<td>7</td>
<td>0.75</td>
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<td>0.4</td>
<td>1.5</td>
</tr>
<tr>
<td>8</td>
<td>1.5</td>
<td>0.4</td>
<td>0.4</td>
<td>1.5</td>
</tr>
<tr>
<td>9*</td>
<td>50</td>
<td>12.5</td>
<td>12.5</td>
<td>3</td>
</tr>
<tr>
<td>10*</td>
<td>50</td>
<td>12.5</td>
<td>12.5</td>
<td>3</td>
</tr>
<tr>
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<td>0.75</td>
</tr>
<tr>
<td>12</td>
<td>0.4</td>
<td>0.2</td>
<td>0.1</td>
<td>0.75</td>
</tr>
<tr>
<td>13*</td>
<td>0.05</td>
<td>0.05</td>
<td>0.0125</td>
<td>0.2</td>
</tr>
<tr>
<td>14*</td>
<td>0.4</td>
<td>0.1</td>
<td>0.0125</td>
<td>0.2</td>
</tr>
<tr>
<td>15*</td>
<td>50</td>
<td>12.5</td>
<td>25</td>
<td>6</td>
</tr>
<tr>
<td>16</td>
<td>0.4</td>
<td>0.4</td>
<td>0.2</td>
<td>1.5</td>
</tr>
<tr>
<td>17</td>
<td>0.75</td>
<td>0.75</td>
<td>0.2</td>
<td>1.5</td>
</tr>
<tr>
<td>18</td>
<td>0.75</td>
<td>0.75</td>
<td>0.4</td>
<td>1.5</td>
</tr>
<tr>
<td>19</td>
<td>0.75</td>
<td>0.4</td>
<td>0.4</td>
<td>1.5</td>
</tr>
<tr>
<td>Mean value</td>
<td>0.63</td>
<td>0.38</td>
<td>0.26</td>
<td>1.13</td>
</tr>
</tbody>
</table>

* The MIC of these strains are not included in the mean value.

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 various concentrations of antimycotics was inoculated with 10⁶ viable units/ml of P. orbiculare (as determined in a counting chamber). As a control, plates were inoculated with 10⁶ and 10⁷ viable units/ml of C. albicans. In addition, DST medium, without additives, containing miconazole was inoculated with 10⁶ 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 1000 µg/l, 10% for econazole, 20% for miconazole, and 50% for clotrimazole. These solutions 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 10⁶ and 10⁷ 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 10⁶ and 10⁷ 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.
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 10⁵ viable units/ml, and by 1 000 µg/ml using 10⁶ 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 antifungal 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 effect 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 antifungal 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 10⁵ viable units/ml were inoculated the MIC was 1000 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**


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