An investigation into correlations between onychonal antifungal flux and resulting fungal inhibition in *in vitro* assays.

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**Introduction**

Topical therapy of onychomycosis (e.g. Fig. 1 below) has advantages over systemic therapy, such as avoidance of adverse effects and drug interactions. For an effective topical antifungal preparation, the drug must partition out of the formulation into the nail plate, diffuse through the latter and reach the nail bed in sufficient quantities and kill the fungus (as schematically shown in Fig. 2 below).

Thus, in *in vitro* studies to predict the *in vivo* efficacy of topical products, the most common measurements are:

i) drug concentrations in the nail plate and/or a simulated nail bed, such as agar gel, or,

ii) the fungicidal activity of the permeated drug on fungi-seeded agar gels.

There have been few investigations in which both drug concentrations in the nail plate, simulated nail bed and the antifungal inhibition have been measured together, in the same experimental setup, and where correlations between drug concentrations and antifungal inhibition have been explored.

**Aim**

The aim of the study was, therefore, to study correlations between drug concentrations and antifungal inhibition in *in vitro* studies.

**Methods**

Formulations A, B and C of an anti-fungal drug were applied on to the dorsal surface of nail plates, which were then placed on agar gels seeded with *Trichophyton mentagrophytes* or *T. rubrum*, with the ventral nail surface contacting the agar gel (as shown in Fig. 3).

The set-up was incubated at 27°C for 7d, after which,

i) the diameter of the zone of fungal inhibition around the nail plate, and

ii) drug concentrations in the nail plate and in the agar gel were determined.

Correlations between fungal inhibition and drug concentrations were explored.

**Results and Discussion**

Figure 4 below shows an example of an agar gel plate setup, at the end of the incubation period.

The area of the zones of fungal inhibition, and drug concentrations in the nail plate and in the agar gel (measured by HPLC) are shown in the table below.

<table>
<thead>
<tr>
<th>Drug formulation</th>
<th>Drug-in-nail (inside and outside formulation application area) (mg/g)</th>
<th>Sum of Drug-in-gel (immediately underneath nail and 3-5 mm away) (mg/g)</th>
<th>Area of inhibition zone (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. mentagrophytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A: 10% lacquer</td>
<td>0.430 ± 0.414</td>
<td>0.007 ± 0.004</td>
<td>924 ± 7</td>
</tr>
<tr>
<td>B: 10% Gel</td>
<td>1.30 ± 0.25</td>
<td>0.076 ± 0.060</td>
<td>1288 ± 1</td>
</tr>
<tr>
<td>C: 20% lacquer</td>
<td>0.640 ± 0.414</td>
<td>0.027 ± 0.029</td>
<td>855 ± 10</td>
</tr>
<tr>
<td>T. rubrum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A: 10% lacquer</td>
<td>0.365 ± 0.089</td>
<td>0.009 ± 0.002</td>
<td>471 ± 0.4</td>
</tr>
<tr>
<td>B: 10% Gel</td>
<td>1.63 ± 0.24</td>
<td>0.081 ± 0.072</td>
<td>779 ± 0.4</td>
</tr>
<tr>
<td>C: 20% lacquer</td>
<td>0.403 ± 0.083</td>
<td>0.013 ± 0.012</td>
<td>572 ± 2</td>
</tr>
</tbody>
</table>

It can be seen that the greatest fungal inhibition zone and highest drug concentrations were achieved with formulation B.

Formulations A and C both showed lower drug concentrations and zones of inhibition compared to B, but were similar to each other.

The large standard deviations observed are assigned to the very low drug levels measured, and the inherent variability of the biological tissue.

**Conclusion**

In general, a positive correlation was found between concentration and activity (in terms of fungal inhibition) of drug that had permeated through the nail plate from 3 different formulations.

This shows that drug concentrations in the nail plate and in the agar gel may be used as indications of the anti-fungal activity of topical nail products.

However, the relationship between drug concentration and fungal zone of inhibition does not seem totally proportional. More work is required to investigate the predictivity of drug concentrations to anti-fungal activity.