The use of Raman spectroscopy in the search for ungual enhancers

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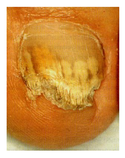
Introduction

Topical therapy of onychomycosis (e.g. Fig. 1) has advantages over systemic therapy, such as avoidance of adverse effects and drug interactions.



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Unfortunately, currently available topical nail medicines have limited efficacy, partly due to the poor permeability of the nail plate. To overcome the nail plate's poor permeability, a number of chemicals such as n-acetyl cysteine, thioglycolic acid, Urea Hydrogen peroxide have been investigated as ungual enhancers.

The mechanism of action of many of these enhancers is thought to be the reduction of the disulphide bonds of the nail keratin (1), which decreases the integrity of the nail plate and thereby increases nail plate permeability.

Nail-S-S-Nail + Enhancer → Nail-SH + HS-Nail + modified Enhancer (1)

We hypothesised that Raman spectroscopy might be useful to establish the mode of action of such enhancers. Raman spectroscopy may enable changes in –S-S- and –SH bonds in the nail plate following treatment with an ungual enhancer to be quantified. Accordingly, if correlations with the extent of such changes and that of ungual permeation enhancement are found, Raman spectroscopy could be used to rapidly screen chemicals for their potential as ungual enhancers.

Aim

The aim was thus to test this hypothesis, i.e. determine whether correlations exist between:

i) the ability of chemicals to alter the nail plate's SS and SH bonds (as determined by Raman spectroscopy) and
ii) their ability to increase ungual permeation of a model drug.

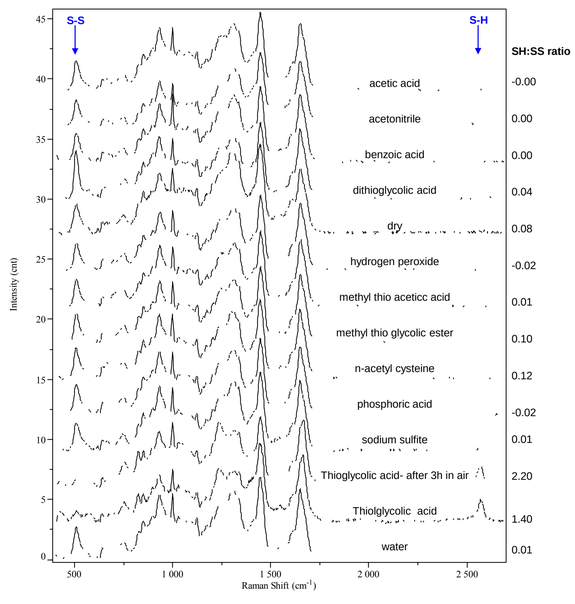
Methods

The influence of a number of chemicals - methyl thioglycolic ester, benzoic acid, acetic acid, acetonitrile dithioglycolic acid, hydrogen peroxide 30%, methylthio acetic acid; N-acetyl cysteine; phosphoric acid; sodium sulphite; thioglycolic acid - on the nail plate's SH and SS groups were investigated by incubating nail clippings in their aqueous solutions (except for methyl thioglycolic ester which did not dissolve in water) for 3 days. After 3 days, the nail clippings were removed from the liquids, rinsed in distilled water briefly, patted dry with a paper towel, and left for 2-3 minutes to air-dry. One sample – nail incubated in thioglycolic acid was air-dried for 3h to investigate the possibility of re-oxidation. Raman spectroscopy was then conducted using an XploRA™ Raman microscope (HORIBA Scientific, UK) at 785 nm (infra-red). Measurements were made at two positions for each sample (dorsal and ventral sides), and averaged. The spectra underwent a two step processing routine - baseline correction and normalisation (so that the area of the Amide I band, 1628-1679 cm^{-1} = 100). Each spectrum was treated in exactly the same way.

To determine the influence of the chemicals on ungual drug permeation, modified Franz diffusion cells were used. So far, N-acetyl cysteine, sodium sulphite and methyl thioglycolate have been tested. Nail clippings (incubated for 3 days in the chemicals were sandwiched between the donor and acceptor compartments of the modified Franz diffusion cells. A solution of 5,6-carboxyfluorescein (CF) – used as a model drug – was placed in the donor compartment and the ungual permeation of CF was monitored over time.

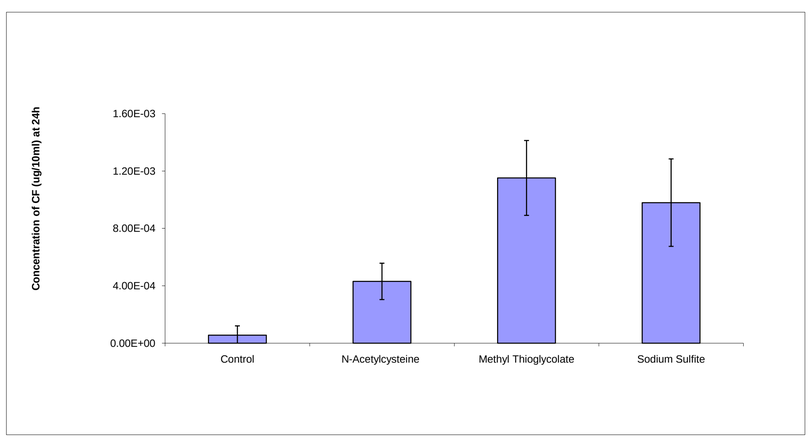
Results and Discussion

Raman spectrospcopy



The figure above shows the change in the SS and SH peaks in the nail plate following 3-day incubation in the different chemicals, and the SH:SS peak ratios. A few negative values for SH:SS ratio indicate the need to optimise the analysis of the spectra. The greatest changes in nail plate SH and SS bonds were due to thioglycolic acid.

Permeation studies



N-acetyl cysteine, sodium sulphite and methyl thioglycolate increased the ungual permeation of the model drug CF (figure above). It was not easy to test the influence of thioglycolic acid as nail clippings incubated in the latter for 3 days were extremely soft and were 'too damaged'. Experiments are underway to test the other chemicals.

Conclusion

Raman spectroscopy may give an indication of the ability of chemicals to damage the nail plate's disulphide bonds and to act as ungual enhancers. The correlation between Raman spectroscopy and permeability data is not, so far, straightforward. Further work is underway to optimise the analysis of Raman spectra and confirm the strength of correlation between Raman spectra and ungual permeability data.