

In vivo measurement of the keratolytic effect of salicylic acid in three ointment formulations

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SUMMARY

The release and activity of salicylic acid from different ointment formulations was studied *in vivo* by staining the horny layer with silver nitrate and measuring the light reflectance profile over 56 h, after application of the ointment for 4 h. The peeling effect of an ointment containing 5% salicylic acid and 10% urea was tested and compared with 5% and 10% salicylic acid in petrolatum. It was shown that shedding from the stained sites was faster with the urea-containing preparation than with the classic petrolatum ointments. The method described can be used to assess the bioavailability of keratolytic agents incorporated in topical formulations.

Salicylic acid is a widely used keratolytic agent in the treatment of hyperkeratotic conditions such as psoriasis, seborrhoeic eczema and neurodermatitis. Petrolatum containing 10% salicylic acid is a commonly used ointment for this purpose. Unfortunately, while the mechanism of action of the drug itself has been studied quite extensively,¹⁻³ its bioavailability in different vehicles has never been assessed.

Various methods have been proposed for the measurement of the rate of cell loss from the epidermal surface, including analysis of released proteins,⁴ disappearance of dansyl chloride fluorescent staining, and forced and passive desquamation techniques.⁵ However, the silver nitrate staining technique,^{5,6} offers several practical advantages when large series of experiments are to be performed. This method was therefore used in the present study to compare the relative keratolytic efficacy of three ointments containing salicylic acid.

METHODS

The bioavailability of salicylic acid in the ointments tested was defined as the quantity of drug released from the ointment base *in vivo*, measured by its keratolytic effect on normal human skin. The three products tested were 5% and 10% salicylic acid in petrolatum and a water-soluble ointment containing 5% salicylic acid and 10% urea (Kerasal® ointment, Spirig AG, Switzerland).

TABLE 1. Bioavailability indices of three salicylic acid-containing ointments

Ointment	Bioavailability index (<i>F</i>) mean \pm SD	<i>P</i> *
Kerasal®	1.088 \pm 0.151	
Salicylic acid 10%	1.023 \pm 0.065	NS
Salicylic acid 5%	1.012 \pm 0.087	< 0.05

* Compared with Kerasal®; Student's *t*-test.
NS: not significant.

The method used was based on those of Aschheim,⁶ Roberts and Marks⁵ and Roberts, Marshall and Marks³. Squares 4 \times 4 cm were marked out on the backs of six male subjects, with adhesive tape. These squares were stained with 100 μ l of a 1% silver nitrate solution, which was left to dry on the skin, followed by the application of the same volume of Dektol® (Kodak). The light reflectance of the stained areas was then measured with a light reflection photometer equipped with a 550 nm filter (Dr Lange, UME 3, F.R.G.), and the adhesive tape removed. The ointments were applied on 22 squares (24 for Kerasal®) on one side of the back, with contralateral sites on the other side of the spine treated with the ointment base (Kerasal® base without salicylic acid and urea, and pure petrolatum, respectively). The treated areas were covered non-occlusively with Telfa® compresses (Flawa, Switzerland), secured with Fixomull® tape (Beiersdorf, F.R.G.). The products were left in place for 4 h; the excess was gently wiped away and the reflectance of the skin areas was measured 2 h later (6 h) and then at 24, 32, 48 and 56 h after application.

The area under the reflectance/time curve (AUC_0^{56}) was calculated for each square, using the trapezoidal method. The bioavailability index *F* was defined as:

$$F = \frac{AUC_0^{56} \text{ of product A}}{AUC_0^{56} \text{ of ointment base A}}$$

for each pair of symmetrically-positioned squares. This value expresses the amount of salicylic acid or urea, or both, delivered to the horny layer during the application time. Thus, each pair of squares represents an experiment, and average bioavailability indices can be calculated for each product tested.

RESULTS

Reflectance was measured up to 56 h. After this time only small changes could be measured.

Due to the large standard deviations, direct comparisons among the products were difficult. The bioavailability index for each pair of squares was calculated and, assuming a normal distribution, Student's *t*-test was applied to compare the bioavailability indices of the different products. A non-parametric Wilcoxon test gave the same results as the *t*-test. Kerasal® had a significantly higher mean bioavailability index than did the 5% salicylic acid ointment ($P < 0.05$). Kerasal® also had a higher bioavailability than 10% salicylic acid ointment, but this difference was not statistically significant.

DISCUSSION

This study indicates that after a single 4-h application, Kerasal® ointment has greater *in vivo* keratolytic activity than 10% salicylic acid in petrolatum, the latter being commonly used for treatment of hyperkeratotic skin diseases. The combination of 10% urea and 5% salicylic acid in Kerasal® might exert a stronger peeling effect through a greater release of the acid, as previously demonstrated *in vitro*,⁷ or by a synergistic pharmacological effect of the two substances. Any additional effects caused by the respective ointment bases were excluded by the study design. The clinical relevance of the observed differences is currently under investigation.

The study also shows the applicability of the silver nitrate staining method to the assessment of *in vivo* activity of salicylic acid ointment formulations. It enables a large series of experiments to be performed over a relatively short period of time, in contrast to the technique using dansyl chloride as a staining agent.⁵ It also circumvents the cumbersome protein analysis described by Christensen *et al.*⁴ and the difficulty of counting shed corneocytes.⁵

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