Transepidermal water vapour loss is not increased during and following dithranol irritation

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Summary
Dithranol is established as a very successful treatment for psoriasis. Its main disadvantages are irritation and staining at sites of application. The aim of the present study was to elucidate further the mechanism of dithranol-induced irritation, in particular to what extent this is related to an impairment of the skin barrier.

Dithranol 3% in cream, paste and petrolatum was applied to the forearm skin of 20 volunteers and left in situ for 1 h. Transepidermal water loss (TEWL) was measured during a period of 2 weeks following dithranol application. In addition, a visual scoring system and colorimetry were used to assess erythema.

The study showed conclusively that TEWL was not affected by the application of dithranol, even though pronounced erythema occurred.

Dithranol has been used for more than 75 years in the treatment of psoriasis, but although this treatment has been very successful, irritation and staining at the sites of application remain a limitation. In order to counteract dithranol-induced irritation, it is important to understand its mechanism. As the psoriatic lesion itself is far less susceptible to dithranol irritation than normal-appearing skin, studies on normal skin are indicated, to elucidate the mechanism of dithranol irritation.

Irritation following a single application of dithranol is characterized by delayed inflammation. After 3–5 days, several markers for the inflammatory response reach maximum levels. The response has been characterized by visual assessment, laser Doppler flowmetry, contact thermometry and skin-fold thickness. At the cellular level, dithranol application to normal skin has been shown to induce endothelial cell activation, a mixed inflammatory infiltrate, epidermal hyperproliferation and expression of the hyperproliferation-associated keratin.

The aim of the present investigation was to discover whether increased epidermal proliferation and abnormal keratinization, which have been reported as components of dithranol-induced irritation, are reflected in terms of skin barrier function.

Following single applications for 1 h of dithranol 3% in petrolatum, dithranol 3% in cream, and dithranol 3% in paste, transepidermal water loss (TEWL), which is a reflection of the barrier function of the skin, was assessed using evaporimetry. Impairment of the barrier function, and an inflammatory reaction, may both occur after exposure of the skin to an irritant, but they do not always occur together. Therefore, the irritant reaction was also assessed visually, and by colorimetry. Assessment of erythema by colorimetry has been used in the past for quantification of UV-induced erythema, and erythema induced by sodium lauryl sulphate application.

Methods

Subjects
Twenty healthy volunteers, without signs or a history of skin disease, participated in the study (10 females, 10 males; age range 19–44 years). They all gave written informed consent. The experiments were conducted from November 1993 to March 1994.

Test agents
Dithranol 3% was formulated in cream, paste and petrolatum bases. The composition of the vehicles is shown in Table 1. These vehicles were also tested with regard to irritancy without dithranol.
Table 1. Vehicles for dithranol

<table>
<thead>
<tr>
<th></th>
<th>Amount</th>
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<tbody>
<tr>
<td>Cetiol</td>
<td>205 g</td>
</tr>
<tr>
<td>Cera cetomacrogolis emulsificans</td>
<td>150 g</td>
</tr>
<tr>
<td>Paraffinum subliquidum</td>
<td>150 g</td>
</tr>
<tr>
<td>Acidum salicylicum</td>
<td>10 g</td>
</tr>
<tr>
<td>Acidum sorbicum</td>
<td>1-5 g</td>
</tr>
<tr>
<td>Acidum asorbicum</td>
<td>0-5 g</td>
</tr>
<tr>
<td>Aqua demi filtrata ad</td>
<td>1000 g</td>
</tr>
<tr>
<td>Paste</td>
<td></td>
</tr>
<tr>
<td>Acidum salicylicum 2%</td>
<td></td>
</tr>
<tr>
<td>Pasta zincli oxidii</td>
<td></td>
</tr>
<tr>
<td>Petrolatum</td>
<td></td>
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<tr>
<td>Vaseline album</td>
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</table>

In preliminary experiments, it was shown that a 1 h application of this concentration of dithranol induced substantial erythema and oedema, but this was tolerated by the subjects.

Exposure

Each substance (0-1 ml) was applied within a marked circle (diameter 1.5 cm) on the volar aspect of the forearm. As there is a site variation in dithranol inflammation on forearm skin, applications were performed in a randomized way, according to the Latin square principle. After 1 h the substances were removed with arachis oil and the skin was washed with soap and cold running water.

Two unexposed sites served as controls. Only one of these two test sites was treated with the washing procedure (control 2), the other control site was kept dry (control 1). For practical reasons it was not possible to rotate the dry control site according to the Latin square principle.

Evaluation

Visual scoring. The clinical changes at the exposure sites were determined, for all subjects, after 1, 2, 3 and 4 days, and rated as follows: 0 = no erythema; 1 = hardly perceptible erythema; 2 = weak but definite erythema; 3 = marked erythema; 4 = marked erythema with minimal oedema; 5 = marked erythema with marked oedema.

Colorimetric quantification of erythema. A Minolta Chromameter, model CR-200, was used to measure erythema. A colour is expressed in a three-dimensional coordinate system with an a*-axis (green–red), a b*-axis (yellow–blue), and an L-axis (brightness), according to the CIE system (Commission Internationale de l’Eclairage). Erythema caused by dithranol application is expected to result in an increase of the value on the a*-axis. For all subjects, measurements were performed in duplicate before application on day 0, and on the following 4 consecutive days, with an interval of 24 h between measurements. The mean of the two measurements was used in the analysis.

Transepidermal water loss. TEWL measurements were performed using an evaporimeter (Tewameter TM 210, Courage and Khazaka, Köln, Germany). The operating principles are described in detail by Nilsson. Measurements were performed according to the guidelines described by the Standardization Group of the European Society of Contact Dermatitis. Room temperature was kept between 20 and 22°C, and relative humidity between 38 and 42%.

In nine subjects, TEWL measurements were performed before exposure on day 0, and on the following 4 consecutive days, with intervals of 24 h between measurements. Measurements were also performed for a longer period in a group of five subjects, before exposure on day 0, and on days 2, 4, 8 and 15.

Statistical analysis

To compare the group means for TEWL and the group means for colorimetry, Student’s t-test for paired observations was used. The Wilcoxon ranking test for matched pairs was used to compare the visual scores. The association between the visual score and the colorimetric values was estimated by using Spearman’s rank correlation coefficient. Statistical significance of a variable was assumed when the corresponding P-value was <0.05.

Results

Visual assessment

Figure 1 summarizes the visual scores (mean ± SEM) following dithranol applications. No significant difference was noted between days 0 and 4 for the control sites and the vehicles alone. All 3% dithranol formulations produced a significant increase of erythema scores during the experimental period. The 3% dithranol
cream showed a significantly higher erythema score on days 1–4, in comparison with the 3% dithranol paste and 3% dithranol in petrolatum.

**Colorimetric assessments**

Figure 2 shows that all dithranol test agents caused a significant increase in erythema with time. No significant increase was noted between days 0 and 4 for the control sites and the vehicles. The cream formulation of dithranol induced a significantly more pronounced erythema than the other two formulations. The difference between 3% dithranol cream and 3% dithranol paste was significant on days 1 ($P < 0.01$), 2 ($P < 0.001$), 3 ($P < 0.001$) and 4 ($P < 0.001$). The difference between 3% dithranol cream and 3% dithranol in petrolatum was also significant on days 1 ($P < 0.01$), 2 ($P < 0.001$), 3 ($P < 0.001$) and 4 ($P < 0.001$). There was no significant difference between 3% dithranol paste and 3% dithranol in petrolatum.

**Transepidermal water loss**

Table 2 summarizes the TEWL measurements ($n = 9$) following dithranol application. The control sites, and the sites of application of the vehicles and the 3% dithranol formulations did not show any significant increase in TEWL during the experimental period of 4 days.

There was no significant increase in TEWL with any of the test agents during the prolonged experiment, at any time interval, when treatment and pretreatment values were compared (Table 3).

**Discussion**

From the present study, it can be concluded that TEWL did not increase during and following a single application of dithranol 3% in various vehicles. Follow-up: 4 days.
application of dithranol to normal skin, despite pronounced erythema, assessed by subjective visual estimation and quantitative colorimetry. The observation that the application of dithranol in the cream formulation induced consistently higher erythema scores suggests that the cream base formulation might enhance the percutaneous penetration of dithranol. Such a penetration-enhancing effect might counterbalance the decreased bioavailability of dithranol in cream formulations.

In previous studies on the response of normal skin to sodium lauryl sulphate, it was demonstrated that concentrations producing 'subclinical irritation' induced a substantial increase of TEWL.\(^1\)\(^5\) Hence, the absence of modulation of TEWL by dithranol, which was observed in the present study, cannot be attributed to insufficient sensitivity of the TEWL methodology.

A single application of dithranol to normal skin has been shown to stimulate epidermal proliferation (recruitment of cycling epidermal cells), and to induce keratin 16 expression in the suprabasal compartment.\(^6\) The present study, however, demonstrates conclusively that TEWL is not modulated by dithranol, which implies that the skin barrier is not affected by dithranol. From this observation, it can be concluded that severe inflammation of the dermoepidermal junction, and substantial epidermal proliferation, may occur without any effect on the integrity of the skin barrier.

During treatment of psoriatic plaques with daily dithranol applications, TEWL measurements have been carried out and compared with TEWL measurements during PUVA treatment.\(^1\)\(^6\) TEWL is increased in untreated psoriatic plaques, and reverts to normal following restoration of the skin barrier. Normalization of TEWL occurred after PUVA treatment. Following dithranol treatment, clinical clearing occurred, but in contrast with PUVA treatment, TEWL did not normalize.\(^1\)\(^6\) The authors hypothesized 'it is possible that the persistently increased water vapour loss was due to the local effect of dithranol on the permeability of the stratum corneum in the plaque, occurring in parallel with its staining'. The response of normal skin to the application of dithranol, as described in the present study, suggests that dithranol irritation does not compromise the skin barrier. However, it is possible that repeated applications of dithranol might modulate the keratinization process directly, aside from its irritation potential. Further studies using an experimental approach of repeated application of dithranol are indicated.

**References**

3. Mustakallio KK. Irritation and staining by dithranol (anthralin) and related compounds. I. Estimation with chamber testing and contact thermography. *Acta Derm Venereol (Stockh)* 1979; 59: 125–32.

