Studies on the Time Course of Dithranol-induced Inflammation by Quantification of Alkaline Phosphatase

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An inflammatory response of the skin to dithranol-induced free radicals seems to be essential for its clinical efficacy. In normal volunteers this response was evaluated at the level of the microvasculature following 30 min, 2 h and 24 h applications, using a functional parameter (erythema) and a biochemical parameter (alkaline phosphatase). The results of 'short contact' and 24 h applications were similar. In all schedules a maximum erythema was seen 2–3 days after the application which had resolved totally after 6–8 days. A marked discrepancy was established between the duration of functional and biochemical abnormalities; the alkaline phosphatase activity reached a maximum 1 day after the culmination of the erythema and persisted up to at least 7 days after disappearance of the erythema. These findings are discussed in the light of the day-to-day management of psoriasis with dithranol. ***Key words: Anthralin; Endothelium; Psoriasis; Enzymology.***

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Dithranol is a well-established treatment for chronic plaque psoriasis (1, 2). Although its antipsoriatic working mechanism has not yet been clarified, the induction of free radicals is supposed to play a central role (3). In the management of psoriasis an adequate concentration adjustment is a *conditio sine qua non* for therapeutic success. Concentrations too low lack clinical efficacy, concentrations too high induce unpleasant irritation of the skin. Therefore, the irritative potential of dithranol is a crucial issue.

Studies on dithranol-induced irritancy have been carried out using several approaches: clinical assessment of erythema (4, 5), reflectance photometry (6), measurement of skin contact temperature by thermometry or by thermography (5, 7, 8), measurement of superficial blood flow by laser-Doppler flowmetry (5) and measurement of oedema by Harpender calipers (9). A direct assessment of inflammation of the skin is possible by fluorometric quantification of alkaline phosphatase (ALP) in biopsies (10, 11). ALP is a marker enzyme for the ascending capillary loops (12). In experimental inflammation of the skin and inflammatory dermatoses the expression of ALP in the endothelial cells is increased substantially above values observed in non-inflamed skin and infiltrate cells of different origin show a mild expression of the enzyme (13).

The aim of the present investigation is to investigate the time course of dithranol-induced irritation of the skin at the level of the microvasculature by visual assessment of erythema and by measuring the activity of the marker enzyme ALP after 24 h applications, 2 h applications and 30 min applications of the drug on normal skin.

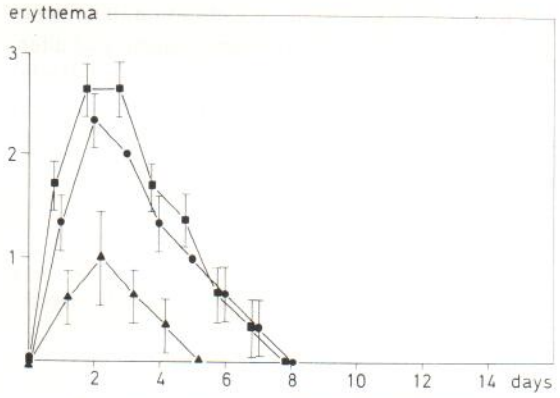

Fig. 1. Erythema scores at different time intervals following the application of dithranol in petrolatum (mean ± SEM). ■, 24 h application of dithranol 3%, ●, 2 h application of dithranol 10%, ▲, 1/2 h application of dithranol 10%.

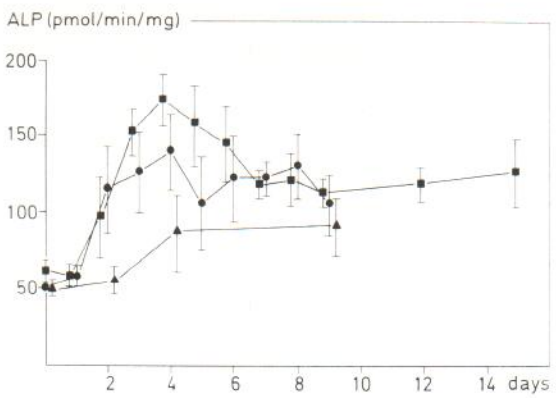

Fig. 2. Activity of ALP at different time intervals following the application of dithranol in petrolatum (mean ± SEM). ■, 24 h application of dithranol 3%, ●, 2 h application of dithranol 10%, ▲, 1/2 h application of dithranol 10%.

MATERIALS AND METHODS

Subjects

Altogether 9 subjects, 8 males and 1 female, aged 28 ± 4 years (mean ± SD), participated in this study. They had no signs or history of skin diseases. The subjects were allocated at random into 3 groups, each with a different schedule for the application of dithranol.

Dithranol applications

Dithranol in petrolatum was applied as open patch tests on areas (diameter 5 cm) on the upper arms. Following the applications, test areas were covered with permeable gauzes. At the end of the application period the ointment was removed with arachis oil, water and soap.

Studies on the response to 24 h applications of dithranol over a concentration range between 0.5 and 10% showed that 3% of the drug resulted in a marked erythema with some oedema and a slight to moderate burning sensation without blistering. Using 2 h application, the concentration of dithranol had to be increased to 10% in order to achieve a similar irritation.

The dynamics of the following schedules was analysed: (i) 24 h applications of dithranol in 3% concentration, (ii) 2 h applications of dithranol in 10% concentration and (iii) 30 min applications of dithranol in 10% concentration. At regular time intervals after the applications the erythema within the test areas was scored using a 4-point scale and biopsies were taken using a razor blade in conjunction with a metal guard (hole 4 mm, biopsy-weight about 3 mg). No anesthetic was used.

Analytical procedures

Biopsies were homogenized in 1 ml of bovine serum albumin (1 mg/ml) using an all glass homogenizer and the homogenate centrifuged. The ALP assay was as described previously (10). In brief, 20 µl samples of supernatant were incubated with 20 µl of a solution of 0.5 mM 4-methylumbelliferylphosphate at pH 9.8, containing 5 mM NaF. After 1 h at 37°C the reaction was stopped by adding 1 ml carbonate buffer (pH 10.5) and the 4-methylumbelliferone release was determined by fluorescence.

RESULTS

The scores for erythema at different time intervals after the dithranol applications are summarized in Fig. 1. The activities of ALP at different intervals following the application of dithranol are shown in Fig. 2.

The application of dithranol (3%) during 24 h resulted in a marked erythema, which was already significant after 1 day, with a maximum after 2–3 days. By 7–8 days the erythema had resolved. A mild brownish staining of the skin was seen the first 2 days after the applications. All 3 volunteers showed a similar response and experienced a slight to moderate burning sensation. The induction of ALP after the 24 h application was marginal after 2 days. A maximum induction was reached after 4 days. In contrast to the recovery of the erythema, the ALP induction remained increased for at least 15 days after the application.

The erythema scores after 2 h and 30 min applications of dithranol (10%) showed the same time course as observed following the 24 h application. At the time of maximum erythema a slight to moderate burning was noticed in the 3 volunteers treated with the 2 h application. Only a mild erythema was seen and no visible response at all occurred in 1 of the 3 volunteers treated with the 30 min application. These volunteers did not experience any burning sensation. Staining of the skin following these applications was

inconspicuous. The time course of the ALP induction after 2 h and 30 min applications showed a similar pattern to that seen following the 24 h application. The maximal ALP induction was lower in case of 30 min applications compared to the 2 h and 24 h applications.

DISCUSSION

The quantification of dithranol-induced irritation of the skin by assessment of erythema is limited by the fact that dithranol causes staining which complicates a reliable estimation (5). The first 2 days following 24 h applications of dithranol the erythema scores might have been underestimated for this reason. A second drawback of estimating erythema is the limited observation range (4 points only). ALP has been used as a marker for inflammation of the skin in psorasis (11, 14–16). In contrast to functional parameters for irritation such as erythema, skin temperature, or blood flow, ALP represents a direct biochemical marker. An increased ALP activity in biopsies from inflamed skin represents mainly increased metabolic activity of the endothelium and/or angioneogenesis (13).

The time course of dithranol-induced irritation assessed by erythema scores is in line with other reports (4, 5, 7–9). In contrast to UVB-induced erythema, which reaches a maximum 12–24 h after irradiation (17), dithranol erythema reaches a maximum 2–3 days after the application. The length of the application period of dithranol did not modify the shape of the curve.

In contrast to erythema, the ALP activity showed a later onset and a maximum after 3–4 days which persisted 15 days, although the erythema had already faded after 7 days. This indicates that dithranol-induced irritation has a prolonged metabolic effect on the endothelium. Again the application period of dithranol did not alter the time course of irritation assessed by ALP.

The penetration of dithranol through the defective skin barrier of the psoriatic lesion is enhanced compared to the situation in normal skin. Therefore relatively high concentrations have to be applied on normal skin in order to reach the level of irritancy as induced during treatment of the psoriatic plaque with this drug. Further studies on the response of lesional skin at the level of the endothelium are worthwhile.

The dynamics of dithranol-induced inflammation has a clear impact on the management of this treatment modality. As the maximum irritation occurs 2–3 days after application, concentration increments should be limited to a maximum frequency of alternate days. In the light of the prolonged endothelial changes induced by dithranol, it seems worth-while to study intermittent treatment schedules.

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