

## Effect of a topical corticosteroid, a retinoid and a vitamin D<sub>3</sub> derivative on sodium dodecyl sulphate induced skin irritation

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Exposure of the skin to sodium dodecyl sulfate (SDS) leads to disruption of barrier and skin irritation. We used repetitive short exposure to a low molarity SDS solution as an *in vivo* model to mimic the development of irritant contact dermatitis. In this model, we studied clinical (erythema), functional (transepidermal water loss (TEWL)) and cell biological changes. 24 healthy volunteers were patch tested with SDS (0.2%) for 4 h a day for 5 consecutive days. After removal of the patches, the exposed sites were treated 1× daily either with a topical corticosteroid (triamcinolon acetone cream 0.05%), a retinoid (tretinoin cream 0.025%), or a vitamin D<sub>3</sub> derivative (calcipotriol ointment 50 microgram/g). Irritant reactions were assessed by erythema scoring and measurement of barrier function with TEWL up to 14 days after the first challenge. Skin biopsies were taken for cell biological changes at day 4. Vehicle-treated sites served as controls. Repetitive exposure of human skin to SDS resulted in a gradual increase in erythema scoring and TEWL associated with the upregulation of proliferative cells as measured by the expression of Ki-67-antigen and of differentiation markers, visualized by increased expression of involucrin and epidermal-fatty-acid binding protein (E-FABP). Skin irritation as assessed by erythema scoring and TEWL was not significantly suppressed by triamcinolone cream. However, a significant reduction of the number of cycling keratinocytes and a decrease in involucrin positive cell layers was observed in this group. Neither treatment with calcipotriol ointment nor with tretinoin cream induced improvement of skin irritation as judged by visual scoring and TEWL. In contrast to steroid treatment, no significant effect of calcipotriol ointment or tretinoin cream treatment was observed with regard to the number of cycling cells and differentiation markers. Further studies are needed to assess whether treatment with topical corticosteroids is an effective modality in skin irritation and irritant contact dermatitis.

**Key words:** sodium dodecyl sulfate; irritant contact dermatitis; corticosteroids; retinoids; vitamin D<sub>3</sub> derivatives; treatment; skin irritation. © Munksgaard, 1997.

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Irritant contact dermatitis (ICD) is characterized by mild erythema, hyperkeratosis or dyskeratosis, chapping and fissures (1) and is pathogenetically thought to be the results of cumulative exposure to irritants which may be of various origin (2). Daily exposure to these irritants cannot be standardized. To study the pathogenesis of ICD, experimental models in men are needed. *In vivo* models have been used to investigate the effects of the cumulative exposure of human skin to sodium dodecyl sulfate (SDS) in terms of erythema, skin dryness and transepidermal water loss (TEWL) (3). Reports on cell biological changes in SDS exposed skin are scarce. Recently, we demonstrated that the development of erythema and TEWL was associated with the upregulation of the number of cyc-

ling cells and the epidermal differentiation markers (involucrin, epidermal fatty acid binding protein (E-FABP)) in the irritant skin reaction after a single 4-h exposure to SDS (5%) (w/v) (4). An SDS concentration of 0.2% caused a significant increase in TEWL, whereas no erythema or any change in epidermal proliferation and differentiation as compared to the normal skin was seen (5). A 0.2% concentration of SDS was chosen in this cumulative model to avoid severe damage to the skin and to mimic daily life exposure. In the model presented, we studied the time course of the erythematous response, TEWL and epidermal growth in the irritant human skin induced by repeated daily 4-h exposures to SDS (0.2%) for 5 consecutive days.

We postulate that drugs which can influence epi-

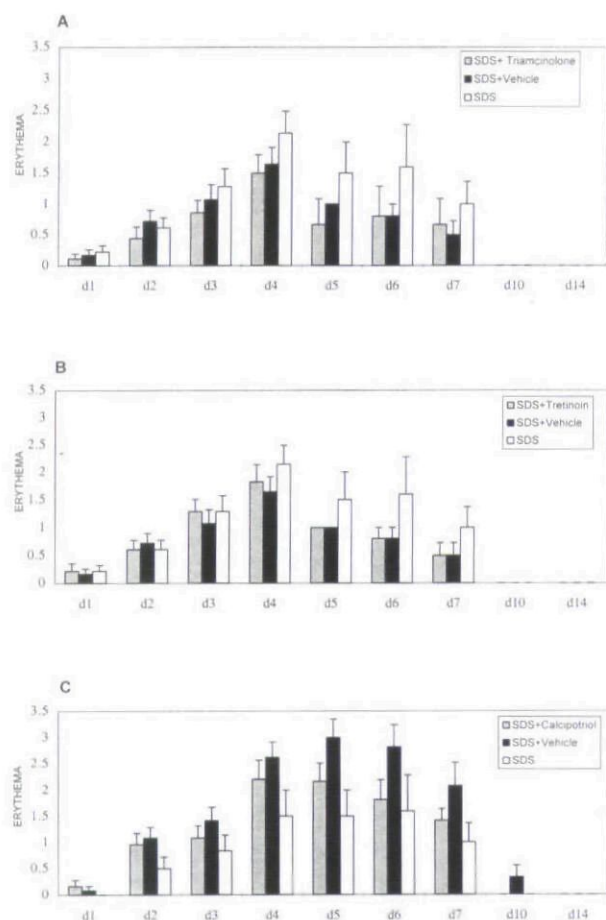


Fig. 1. Time course of erythema from day 0 to day 14. 0, no response; 1, slight, patchy redness; 2, diffuse mild redness; 3, moderate redness; 4, intense redness; 5, intense redness with edema; (A) of the SDS-exposed skin and the triamcinolone-treated group versus its vehicle; (B) of the SDS-exposed skin and the tretinoin-treated group versus its vehicle; (C) of the SDS-exposed skin and the calcipotriol-treated group versus its vehicle.

dermal growth resulting in the improvement of the skin barrier function, may be effective in the treatment of ICD. Topical steroid, retinoic acid and vitamin D<sub>3</sub> derivative are the therapeutic modalities used in dermatology to modulate epidermal growth and differentiation. They are often used in treatment of inflammatory skin disorder like psoriasis. Whether they can improve the disruption of skin barrier and irritation by irritants is not known.

Topical corticosteroids are frequently used in ICD (6) due to their anti-inflammatory effects. A previous study demonstrated a reduction of mitotic activity of the keratinocytes in psoriatic lesions associated with clinical improvement after treatment by topical steroid (7). Furthermore, the expression of skin-derived antileukoproteinase (SKALP), an elastase inhibitor which is induced in

inflammatory conditions (8, 9), is downregulated to the normal levels in the steroid-treated epidermis (7).

The vitamin A metabolite, retinoic acid, is essential to normal differentiation of many tissues including skin. Retinoic acid and synthetic retinoids are used in the treatment of acne (10), psoriasis (11) and certain malignancies (12). Several studies in mouse and man indicate that topical retinoic acid speeds repair of skin damage due to ultraviolet radiation (13, 14). Effects of retinoids on the epidermis include modification of keratin expression (15) and inhibition of cross-linked cornified envelope synthesis (16). Retinoids can either stimulate or inhibit cell growth (17).

Vitamin D<sub>3</sub> derivatives are the recent therapeutic modalities in psoriasis. They are known to affect the epidermal growth. Administration of vitamin D<sub>3</sub> to cultured keratinocytes causes suppression of cell proliferation and enhancement of cell differentiation (18). Vitamin D<sub>3</sub> derivatives can modulate inflammatory processes. When T-lymphocyte cul-

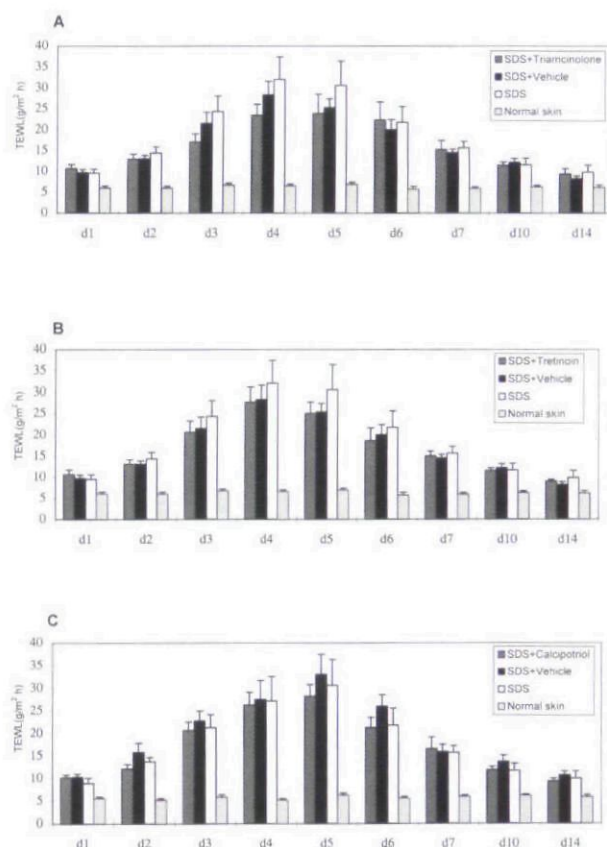


Fig. 2. Time course of transepidermal water loss (TEWL) from day 0 to day 14: (A) of the SDS-exposed skin and the triamcinolone-treated group versus its vehicle; (B) of the SDS-exposed skin and the tretinoin-treated group versus its vehicle; (C) of the SDS-exposed skin and the calcipotriol-treated group versus its vehicle.

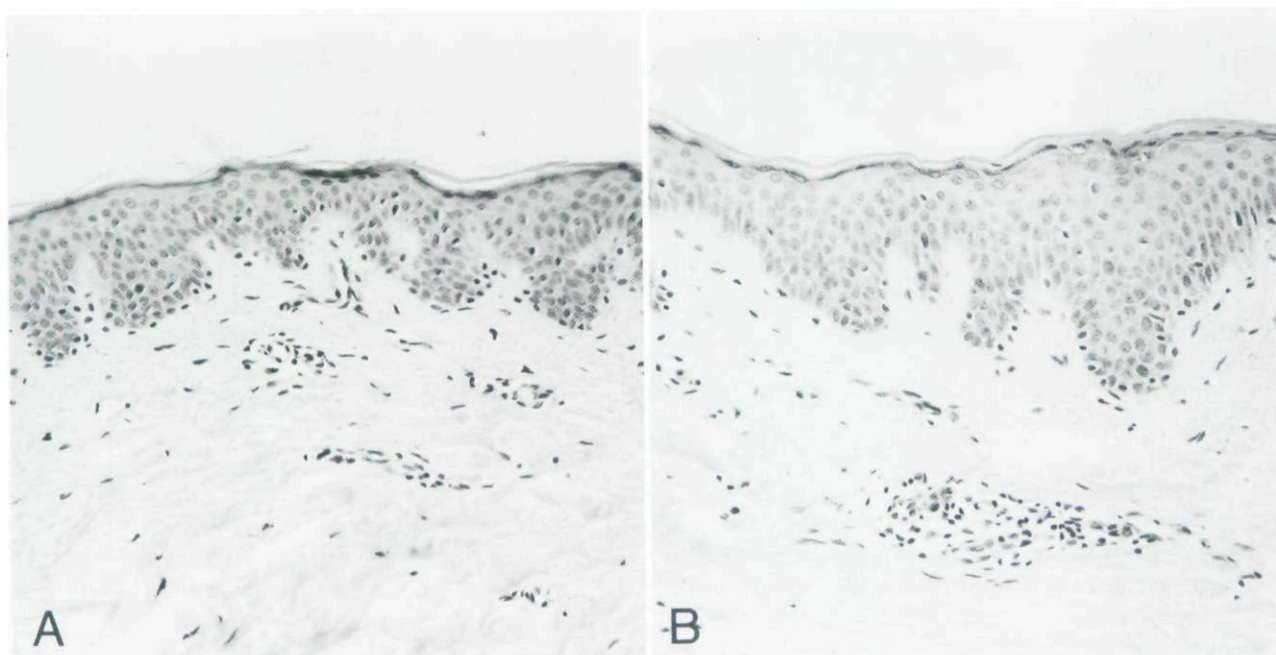


Fig. 3. H&E sections showing the difference between the triamcinolone-treated group versus its vehicle with respect to acanthosis, hypergranulosis and perivascular infiltrate. (A) with triamcinolone treated; (B) with vehicle treated.

tures were incubated with  $1\alpha,25\text{-(OH)}_2\text{-D}_3$ , proliferation of T-lymphocytes was reduced (19). Calcipotriol is the first vitamin  $\text{D}_3$ -derivative registered in several European countries. Calcipotriol suppresses cell proliferation and upregulates differentiation of epidermal cells, resulting in improvement of psoriatic skin lesions (20, 21). A side-effect of psoriasis treatment with topical calcipotriol is skin irritation. A study of Serup et al. (22) demonstrated that calcipotriol ointment was a mild irritant of the non-corrosive type, i.e., with no influence on the skin barrier. The irritant effect of calcipotriol ointment was also noted in some clinical studies (23, 24). The vehicle of calcipotriol ointment may be at least in part responsible for this skin irritation.

In this study, we examined the effects of a topical corticosteroid (triamcinolone acetonide 0.05% cream), a retinoid (tretinoin 0.025% cream) and a vitamin  $\text{D}_3$  derivative (calcipotriol ointment) in irritant reactions induced by repeated exposures of the skin to SDS. The effects were studied clinically (erythema), functionally (TEWL) and immunohistochemically using markers of epidermal growth, known as Ki-67 antigen, present in cycling cells; involucrin and E-FABP, two terminal differentiation markers (25–27); skin-derived-anti leuko-proteinase (SKALP) and cytokeratin 16, two molecules associated with hyperproliferation and inflammation (8, 28)

### Materials and Methods

24 healthy volunteers with no history of skin diseases (14 male, 10 female, mean age 31, ranging from 19 to 57 years) participated in the study. After approval of Medical Ethic Committee, informed consent was obtained from all subjects.

#### *Skin exposure to sodium dodecyl sulfate and topical formulations*

200  $\mu\text{l}$  of SDS (0.2%) (w/v), pipetted on the patches consisting of a 1.5 cm piece of absorbent, non-woven fabric on a 4  $\text{cm}^2$  piece of impermeable plastic foil (4), which was daily fixed at the same site with tape (Fixomull<sup>®</sup> stretch, Beiersdorf AG, Hamburg) to the back of the subjects for 4 h for 5 consecutive days, from day 0 to day 4. After removal of the patches, 0.5 cm of the cream or ointment (about 0.35–0.40 g) was applied daily over the area of previously SDS-exposed skin, dried at room temperature for 30 min. The cream or ointment was applied for 7 consecutive days.

The following formulations were used: (i) triamcinolone acetonide (0.05%) cream; (ii) tretinoin (0.025%) cream; (iii) vehicle of triamcinolone and tretinoin cream consisted of cetomacrogol cream; (iv) calcipotriol ointment (Daivonex<sup>®</sup>, Leo, Denmark); (v) vehicle of calcipotriol ointment.

### Clinical scoring and transepidermal water loss (TEWL) measurements

Visual grading and TEWL were assessed at: day 1 to day 7, day 10 and day 14. TEWL was measured with a Tewameter™ 210 (Courage & Khazaka, Cologne), according to standard guidelines (29). Room temperature varied from 20°C to 25°C and relative humidity from 40% to 60%. Clinical scoring (erythema) was graded visually: 0, no response; 1, slight, patchy redness; 2, diffuse mild redness; 3, moderate redness; 4, intense redness; 5, intense redness with edema.

### Biopsy procedures

A total of 40 punch biopsies (3 mm diameter) were taken: 16 biopsies at day 2 to examine the histology of the triamcinolone-treated group, the tretinoin-treated group, the vehicle-treated group

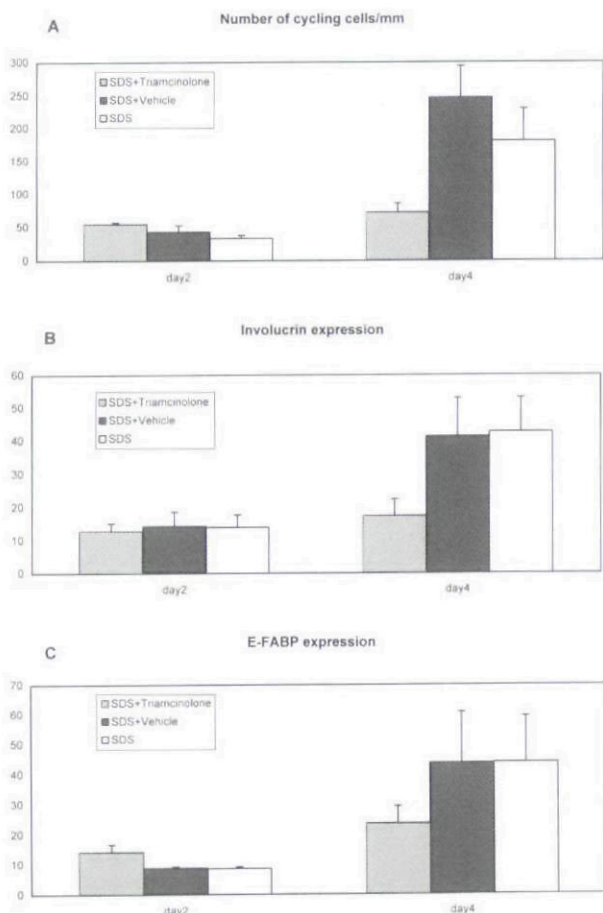


Fig. 4. Immunohistochemical stainings of the triamcinolone-treated group versus its vehicle. The results of (B) and (C) are expressed as a % of the positive stained-cell layers of the total epidermal cell layers. The values are mean  $\pm$  SEM: (A) number of proliferative cells per mm length section; (B) involucrin expression; (C) E-FABP expression.

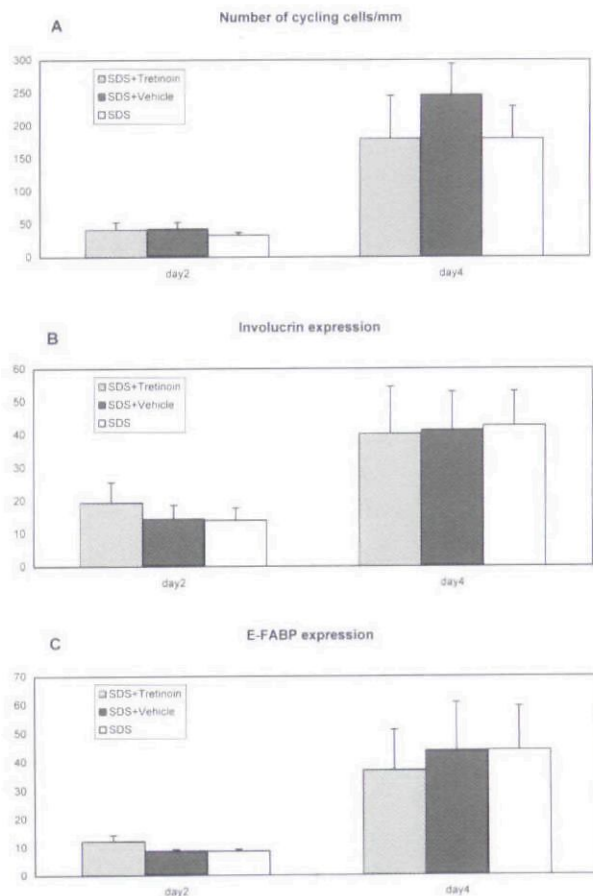


Fig. 5. Immunohistochemical stainings of the tretinoin-treated group versus its vehicle. The results of (B) and (C) are expressed as a % of the positive stained-cell layers of the total epidermal cell layers. The values are mean  $\pm$  SEM: (A) number of proliferative cells per mm length section; (B) involucrin expression; (C) E-FABP expression.

and the SDS-exposed group; 24 biopsies at day 4 for these 4 groups and the calcipotriol-treated group and its vehicle. Thus, 4 biopsies per treatment/per timepoint. After 4 h fixation in formalin, the samples were embedded in paraffin, and sectioned at 6  $\mu$ m for immunohistochemical staining.

### Immunohistochemical methods

The biopsies were stained with the following antibodies according to standard procedures, described in previous studies (4): (i) MIB-1 (Immunotech, S.A. Marseilles, France) which recognizes the cycling-associated Ki-67 antigen on formalin fixed material; (ii) MON-150, which binds to involucrin, a structural precursor protein for the formation of the cornified envelope, is described and prepared previously (30); (iii) Anti E-FABP, a polyclonal rabbit antiserum against the epidermal-fatty acid binding protein which is associ-

ated with terminal differentiation (kindly provided by Siegenthaler (26, 27); (iv) Anti SKALP/elafin, a polyclonal antiserum directed against recombinant SKALP/elafin is prepared as we described previously (8, 9); (v) Ks8.12 (Sigma, St Louis, MO, USA) was used to detect cytokeratin 16 which is expressed in suprabasal layers in the hyperproliferative tissues (28); (vi) H&E according to Mayer.

### Histological examination

We quantified the number proliferative cells by counting the number of MIB-1-positive nuclei per

mm length of section. The expression of involucrin and E-FABP was similarly scored: at a representative interpapillary area, the ratio of positively stained cell layers and the total number of cell layers, were calculated.

### Statistical methods

The Student *t*-test and the Wilcoxon test were used when appropriate.

## Results

### Clinical grading and TEWL measurements

Repetitive exposure of human skin to SDS (0.2%) induced an increase in erythematous reaction (Fig. 1) and in TEWL measurement (Fig. 2), enhancing in intensity up to day 4, then gradually decreasing to day 14. No significant difference in erythema or in TEWL was found between the triamcinolon acetonide, tretinoin or calcipotriol treated groups versus their vehicles.

### Histology and immunohistochemistry

**H&E staining.** The exposed skin of day 4 showed mild to moderate parakeratosis, hypergranulosis, acanthosis, exocytosis and moderate perivascular infiltrate composed of mostly mononuclear cells and some eosinophils. The sections of the triamcinolon acetonide cream treated skin demonstrated a slightly milder perivascular infiltrate, less parakeratosis and less acanthosis as compared to its vehicle (Fig. 3). No difference between the tretinoin- or calcipotriol-treated skin and their vehicles was observed with respect to hypergranulosis, parakeratosis, spongiosis, acanthosis and perivascular infiltrate.

**Epidermal proliferation.** The response of untreated skin, repetitively exposed to SDS, was characterized by an increase in the number of MIB-1-positive nuclei from  $78 \pm 22$  per mm length of section, to a maximum of  $180 \pm 50$  (mean  $\pm$  SEM) at day 4 after stimulation. The number of proliferative cells of all treated skin groups was also enhanced up to day 4. A marked, significant decrease ( $p=0.02$ ) in the number of cycling cells/mm ( $72 \pm 14$ ) of the triamcinolon acetonide treated sections was observed as compared to the other exposed sites (Fig 4a). No significant difference in the number of cycling cells/mm between the tretinoin- or calcipotriol-treated groups versus that with their vehicles, was seen (Figs. 5a, 6a).

**Epidermal differentiation.** To study the effect of the verum drugs on epidermal differentiation in our model, we investigated the expression of invol-

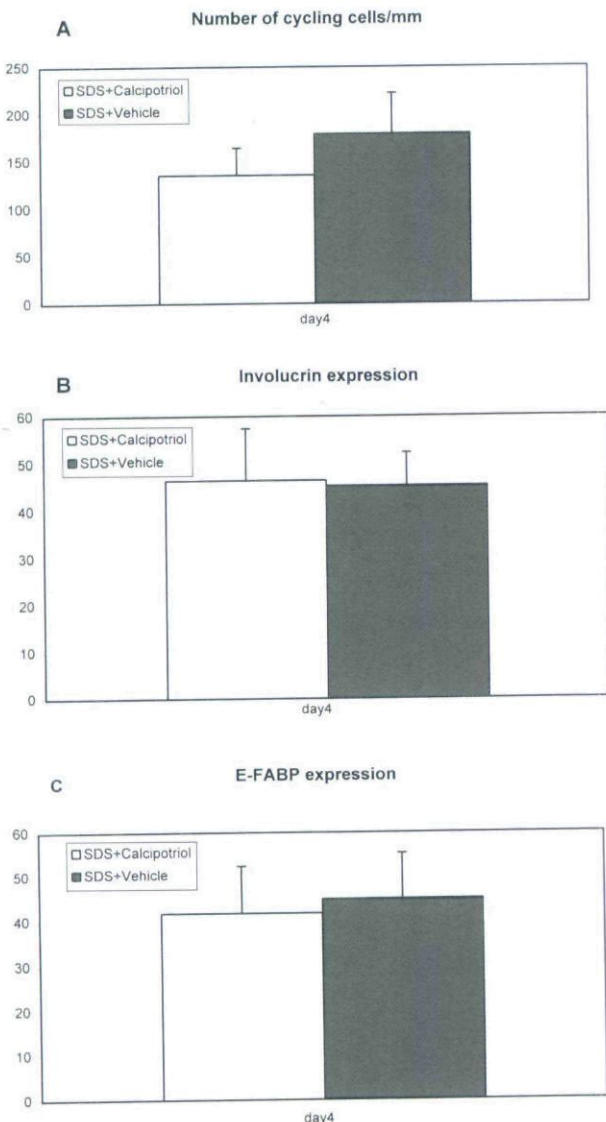


Fig. 6. Immunohistochemical stainings of the calcipotriol-treated group versus its vehicle. The results of (B) and (C) are expressed as a % of the positive stained-cell layers of the total epidermal cell layers. The values are mean  $\pm$  SEM: (A) number of proliferative cells per mm length section; (B) involucrin expression; (C) E-FABP expression.

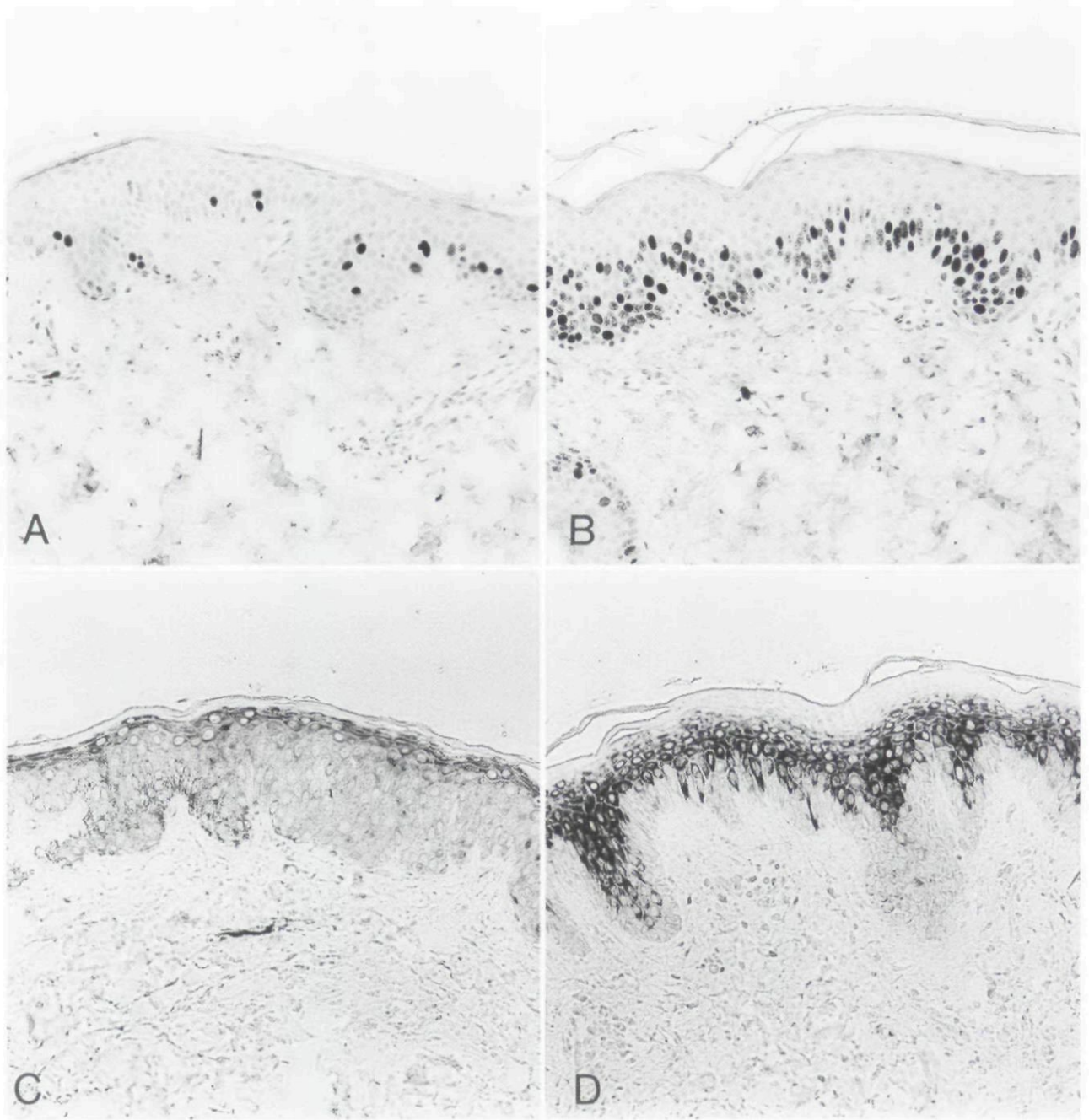


Fig. 7. Histological series demonstrating the difference in the number of cycling cells/mm (A and B) and in the involucrin expression (C and D) between the triamcinolone-treated group versus its vehicle. (A, C) with triamcinolone treated. (B, D) with vehicle treated, respectively.

ucrin and E-FABP. Repetitive application of SDS caused an increase in the expression of involucrin, and an increase in the expression in E-FABP. At day 4, a small decrease in involucrin expression of borderline significance ( $p=0.09$ ) and a non-significant decrease in E-FABP expression ( $p=0.25$ ), were found in the triamcinolone acetate-treated group versus its vehicle (Fig. 4b, c). There was no difference in the expression of involucrin and E-

FABP in the tretinoin- and calcipotriol-treated groups versus their vehicles (Fig. 5b, c, 6b, c). The SKALP staining showed a large variation from one sample to another in the exposed treated and untreated skin (not expressed in normal skin). Cytokeratin 16 was not expressed in any section of the exposed treated and untreated skin (data not shown).

The number of cycling cells and the involucrin

expression of the triamcinolone-treated group versus its vehicle are illustrated in Fig. 7.

### Discussion

The induction of irritant reactions by repetitive exposure to SDS and the repair with or without treatment were studied using visual scoring, TEWL and markers of epidermal proliferation and differentiation. Repetitive exposure to SDS causes an increase in erythema and in TEWL to a maximum at day 4, then gradually decreases up to day 14 after the first challenge. The repetitive exposure to SDS not only induced histological changes to the epidermal cell compartment (parakeratosis, perivascular mononuclear infiltrates), but also upregulated the expression of markers of epidermal differentiation and proliferation, corresponding to a hyperproliferative skin. Treatment of skin irritation by triamcinolone acetonide, retinoic acid or calcipotriol did not improve the clinical scores in this model. Neither significant decrease in erythema scoring nor decrease in TEWL was observed. In contrast to this clinical finding, we found a statistically significant reduction of the number of cycling cells in the triamcinolone-treated skin sites versus the other exposed sites. In addition, the expression of involucrin and E-FABP was also downregulated in the triamcinolone-treated skin as compared to that treated with its vehicle.

The fact that triamcinolone acetonide cream induces a reduction of cycling cells may indicate that triamcinolone acetonide cream causes less irritation or delays repair. In contrast to other studies (31, 32) in which topical steroids are believed to be diminish the erythematous response of SDS exposed skin, we could neither demonstrate this effect in this model nor in a previous work (33).

Treatment with retinoic acid did not lead to any change in visual scoring, TEWL and modulation of the epidermal response to SDS, as compared to its vehicle. Adding retinoic acid to keratinocytes in vitro reduces transglutaminase activity and consequently may inhibit terminal differentiation and concomitant cornified envelope formation (34). Retinoic acid can induce epidermal hyperproliferation and increase in transglutaminase immunoreactivity when applied topically for 4 months to photoaged human skin (35). Furthermore, retinoic acid can increase the expression of transglutaminase, loricrin, involucrin, filaggrin when applied to normal human skin for 4 days under occlusion (36). Here, we found no alterations in involucrin and E-FABP-expression at the tretinoin-treated sites. The exact mechanism of retinoic acid in the SDS-induced skin irritation remains unknown.

Calcipotriol did not lead to modulation of the epidermal response after exposure to SDS with the exception of an increase in TEWL. This may be due to its vehicle, which is known to be irritant. At day 5 after challenge, a small, non-significant increase ( $p=0.18$ ) in TEWL of the vehicle of calcipotriol-treated sites versus the SDS exposed sites was found. In our present study, repetitive exposure to SDS for 5 days induced an increase in number of cycling cells and the expression of involucrin and E-FABP. Treatment with retinoic acid or calcipotriol in this irritant skin did not show any alteration with respect to these parameters.

Topical steroids may influence irritant reactions. Skin may deal with repetitive irritant stimuli with adaptation, regeneration or disease (irritant contact dermatitis). Intervention may influence the inflammatory response (7) and consequently may prevent tissue damage as caused by the inflammation, but may also hamper repair processes by its anti-mitotic activity. Therefore, it cannot be concluded from our data that topical steroids are beneficial to the skin.

Single exposure tests are inadequate for prediction of the effect of treatment modalities. Cumulative exposure models or clinical studies with standardized exposure are needed.

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