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Effects of calcipotriol on stratum corneum barrier function, hydration and cell renewal in humans

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Summary Calcipotriol, a vitamin D analogue utilized for psoriasis, has irritation as its most frequent reported adverse event. However, studies on its irritant properties in humans have produced conflicting data. This study evaluates the effect of calcipotriol on stratum corneum barrier function, hydration and cell turnover in healthy volunteers, compared with sodium lauryl sulphate (SLS) as a model irritant. Calcipotriol 0·005% ointment and 1% aqueous SLS solution were applied for 60 min once daily for 2 weeks (5 consecutive days weekly) on untreated and on dansyl-chloride-labelled skin. Irritant responses were documented by visual scoring and by measurement of the transepidermal water loss (TEWL) and stratum corneum hydration (electrical capacitance), until day 18. Stratum corneum turnover time (SCTT) was the time in days between staining (day 0) and the disappearance of dansyl fluorescence. SLS caused more erythema, scaling, and a significant TEWL increase for 18 days. In contrast, calcipotriol induced erythema, and slightly but significantly increased TEWL on day 11 only, as compared with the vehicle control ($P < 0·05$). SLS, but not calcipotriol, caused skin dryness from day 4 to day 18. The shortest SCTT was obtained at SLS-exposed sites ($11·2 \pm 0·7$ days: mean ± SD). Calcipotriol significantly shortened SCTT ($16·3 \pm 1·1$ days) when compared with its vehicle. Compared with the skin irritation induced by SLS, under these test conditions, calcipotriol is a far weaker irritant on normal human skin. In addition, calcipotriol accelerates stratum corneum turnover to a significantly greater extent than its vehicle.

The mode of action of calcipotriol in psoriasis has not been conclusively established.[1,2] *In vitro* studies have revealed that calcipotriol inhibits proliferation and enhances differentiation of epidermal keratinocytes which may be responsible for its beneficial effect.[2,3] Its most frequent adverse event is lesional/perilesional skin irritation whose incidence is approximately 9–20%. Calcipotriol has, however, recently been reported to be a relatively weak irritant, in healthy subjects, with occlusive patch testing.[4,5] The reaction to calcipotriol was dominated by an increase in redness, compared with a normal transepidermal water loss (TEWL).[5] On the other hand, another experiment showed that open application of calcipotriol once daily for 5 days to normal skin, resulted in a two to threefold increase in TEWL.[6] Moreover, in contrast to the earlier reported *in vitro* and *in vivo* effects of calcipotriol,[2,3,7,8] the authors demonstrated that calcipotriol increased both epidermal proliferation and thickness in normal mouse skin, which may be due to its irritation property.[6] The differing findings led us to study further the biological effect of calcipotriol on stratum corneum of human skin compared with sodium lauryl sulphate (SLS), a widely used model irritant.[9]

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Patients and methods

Chemicals

Calcipotriene (calcipotriol) 0.005% ointment (Dovonex®, Westwood-Squibb Pharm. Inc., Buffallo, NY, U.S.A.) was purchased; its pH was 7·0. The pH of the ointment base was 6·4. Dansyl chloride (5-dimethylamino-1-naphthalene sulphonyl chloride) and SLS (purity 99%) were obtained from Sigma Chemical Co. (St Louis, MO, U.S.A.).

Subjects and test procedure

Six healthy volunteers (three females and three males, age 28–40 years) provided informed consent. The stratum corneum was labelled with fluorescent dansyl

chloride according to the method of Jansen *et al.*[10] Clearance of the fluorescence was examined daily under UV illumination.

Fifty microlitres (approximately 45 mg) of calcipotriol ointment, and 100 μl 1% aqueous solution of SLS were applied to both dansyl-chloride-labelled and untreated volar forearms using polypropylene chambers (19 mm diameter, Hilltop Laboratories, Cincinnati, OH, U.S.A.) on paper adhesive tape (Scanpor) for 60 min once daily (5 consecutive days weekly for 2 weeks). Occlusive application was necessary to avoid the spread of SLS solution from the test site to adjacent skin. Deionized water served as the vehicle control. The distribution of the chambers was randomized between panelists. Untreated skin served as the control site.

Instrumental measurements

Before and during the exposure period, each site was examined prior to the reapplication of test substances on days 0, 2, 4, 9 and 11, and after completion of treatment on days 16 and 18. TEWL, as an indicator of stratum corneum integrity, was measured with an evaporimeter (Servo Med, Stockholm, Sweden). TEWL measurements were conducted at ambient conditions (45–65% relative humidity; 20–22°C); volunteers rested at least 15 min before measurements were taken. Electrical capacitance as an indicator of stratum corneum hydration was measured in duplicate with a capacitance meter (Corneometer CM820 PC, Courage & Khazaka, Cologne, Germany).

Clinical scoring

With the same time schedule as noted above, each test site on unstained forearm was examined and graded for erythema and scaling by the same investigator according to a visual scoring system.[11] *Erythema:* 0, no erythema; 0·5, equivocal reaction; 1, slight erythema, either spotty or diffuse; 2, moderate, uniform erythema; 3, intense erythema; and 4, fiery redness with oedema. *Scaling:* 0, no scale; 1, minimal, fine; 2, moderate; and 3, large flakes, intense peeling.

The level of fluorescence on stained forearm was assessed daily in the dark under ultraviolet (UV) illumination, using an arbitrary scale of 0–10, where 10 is the brightest fluorescence subsequently after staining (100%) and 0 is no longer fluorescent visibly (0%). SCTT describes the time, in days between staining (day 0) and the disappearance of fluorescence.

Statistical analysis

Differences in visual scores, TEWL, electrical capacitance, and SCTT between the treatments were examined for statistical significance using the non-parametric Friedman test. This test affords a two-way analysis of variance by ranks for matched samples. When the Friedman test revealed significant differences between the treatments, multiple comparisons of all groups were conducted by the Wilcoxon–Wilcox test.

Results

Erythema and scaling

SLS produced a significant increase in erythema as compared with its vehicle and calcipotriol at almost all time points ($P < 0{\cdot}05$) (Fig. 1). The difference in erythema score between calcipotriol and ointment base (control vehicle) was significant on day 11 only ($P < 0{\cdot}05$). However, calcipotriol did not cause erythema in two of six volunteers. The vehicle controls induced no significant changes of skin redness. SLS, but neither calcipotriol nor both vehicle controls, caused significant scaling (data not shown).

Transepidermal water loss

The difference in TEWL between SLS-exposed and calcipotriol-exposed sites was statistically significant from days 4 to 18 ($P < 0{\cdot}005$) (Fig. 2). At day 18, TEWL at the SLS-treated sites was still markedly

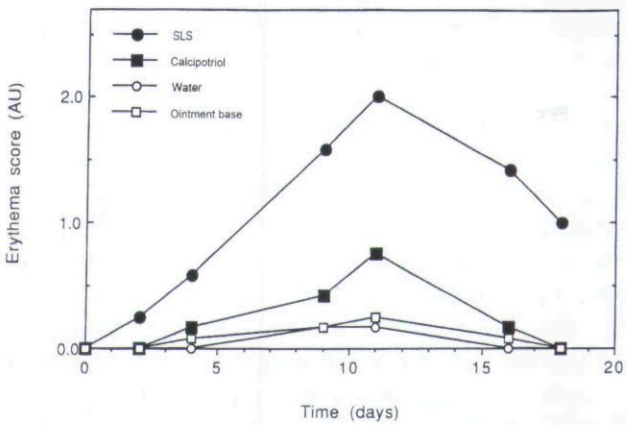

Figure 1. Erythema score induced by a 10-day cumulative application of the test substances. Sodium lauryl sulphate (SLS) caused a significant increase in erythema from day 4 to day 18 as compared with its vehicle control (*$P < 0{\cdot}05$). Calcipotriol significantly increased erythema on day 11 only (*$P < 0{\cdot}05$). Each point represents the mean, SD and the sign for significant P values (*) are not shown for graphic clarity.

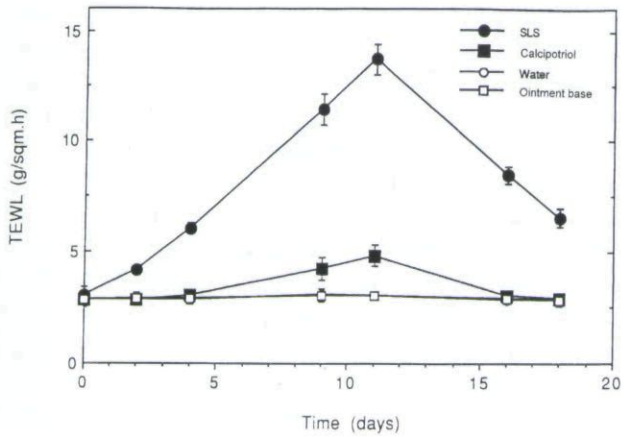

Figure 2. The transepidermal water loss (TEWL) as an indicator of stratum corneum integrity. Sodium lauryl sulphate (SLS) significantly increased the TEWL from day 4 to day 18 (**$P < 0{\cdot}005$), whereas calcipotriol slightly but significantly elevated TEWL on day 11 only (*$P < 0{\cdot}05$) when compared with the vehicles. Signs for significant P values (* and **) are not shown for graphic clarity.

higher than baseline value implying that repair of water barrier disruption was incomplete. The cumulative application of calcipotriol slightly but significantly elevated TEWL on day 11 only ($P < 0{\cdot}05$). The vehicle controls did not significantly alter TEWL.

Capacitance

SLS significantly decreased stratum corneum hydration from days 4 to 18 more than did water (vehicle control)

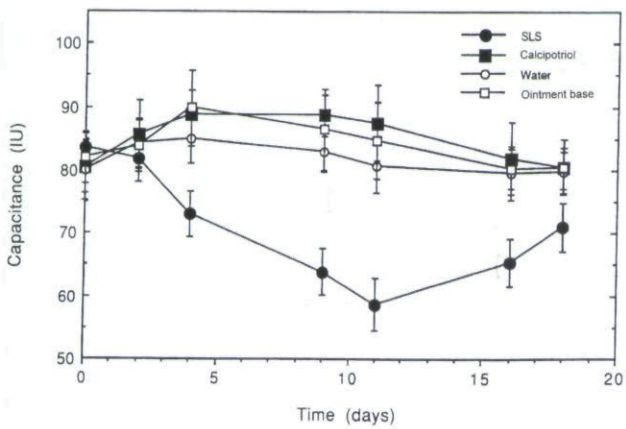

Figure 3. Electrical capacitance as an indicator of stratum corneum hydration. Sodium lauryl sulphate (SLS) significantly decreased stratum corneum hydration from day 4 (**$P < 0{\cdot}005$) to day 18 (*$P < 0{\cdot}05$) more so than the vehicle. The lowest values for capacitance were recorded at day 11, thereafter returning towards baseline. Calcipotriol visibly, but insignificantly, elevated stratum corneum hydration; there was no significant difference between calcipotriol and its vehicle control. Signs for significant P values (* and **) are not shown for graphic clarity.

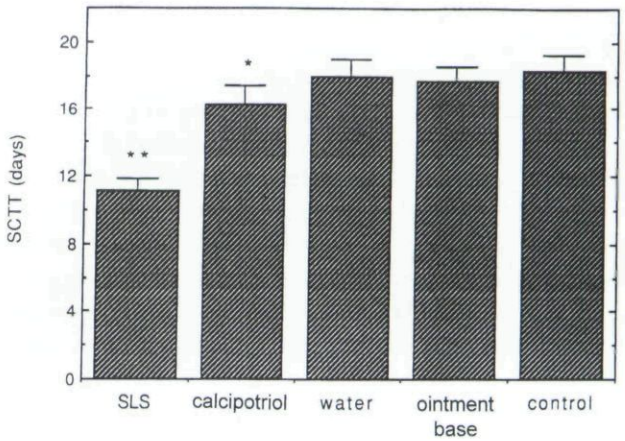

Figure 4. Stratum corneum turnover time (SCTT): **$P < 0{\cdot}005$ for sodium lauryl sulphate (SLS) < water, and *$P < 0{\cdot}05$ for calcipotriol < ointment base. Water and ointment base did not significantly change SCTT when compared with that of untreated skin (control).

($P < 0{\cdot}005$) (Fig. 3). The lowest values for capacitance were obtained after 10 days of exposure, thereafter returning towards baseline. Calcipotriol visibly, but insignificantly, elevated stratum corneum hydration. There was no significant difference between calcipotriol-treated and ointment base-treated sites.

Stratum corneum turnover time

SLS shortened SCTT ($11{\cdot}2 \pm 0{\cdot}7$ days: mean ± SD) significantly more than its vehicle (18 ± 1 days), and than did calcipotriol ($16{\cdot}3 \pm 1{\cdot}1$ days) ($P < 0{\cdot}005$) (Fig. 4). In sites treated with calcipotriol, SCTT was significantly less than that in ointment base-exposed areas ($17{\cdot}7 \pm 0{\cdot}9$ days) ($P < 0{\cdot}05$). The vehicle controls did not significantly change SCTT when compared with untreated skin (control site) ($18{\cdot}3 \pm 0{\cdot}9$ days).

Discussion

This study investigated the irritation potential of calcipotriol. The concentration of calcipotriol (0·005%) tested corresponds to the usual therapeutic level.[1,2] The SLS concentrations used in experimental cumulative irritant dermatitis range from 0·5 to 7·5%, depending on the exposure conditions.[12,13] A 1% aqueous solution of SLS, as a 24 h occlusive application, is widely used in experimental acute dermatitis, as this dose induces perceptible skin irritation without significant inconvenience to exposed subjects.[14–19] An ultimate study design would, however, compare equimolar doses and analogous vehicles of test compounds. As the goal of the study was primarily to define possible irritant

properties of calcipotriol, the presented comparison with SLS, a standard model irritant, gives us only a point of reference of the calcipotriol irritancy.

The SLS-induced functional changes in the stratum corneum are consistent with earlier studies. Skin irritation caused by SLS is characterized by intense erythema and scaling (inflammatory reaction) and, specifically, by a significant increase in TEWL (impairment of water barrier function) and a marked decrease in stratum corneum hydration (skin dryness).[12,13,19–21] As irritant responses to calcipotriol were significantly less than those to SLS, calcipotriol seems to be a relatively weak irritant when applied to normal human skin. Comparable findings have recently been reported by Serup and Fullerton.[5] However, our data are not consistent with the study by von Brenken and Proksch,[6] who reported that a 5-day open application of calcipotriol to human skin caused a two- to threefold increase in TEWL. This discordance is even more remarkable considering the longer cumulative application time in the assay presented here. Furthermore, these authors,[6] as well as others,[22] showed that calcipotriol increased skin thickness, possibly as a result of irritation. Von Brenken and Proksch[6] have, nevertheless, stated that an impairment of barrier function was not related in a linear fashion to the therapeutic effect of calcipotriol. The irritant reaction may thus be substance-specific. However, as calcipotriol studies reveal differing data, it is not possible to exclude that its irritancy might vary depending on the application site, skin type, atopic background, and climatic or other factors.[4]

The dansyl chloride fluorescence test (DCT), which estimates stratum corneum renewal, has proved useful.[23–26] As the transit of cells through the horny layers is a function of the rate of cell production by dividing cells, SCTT is an indirect and non-invasive measure of relatively mitotic activity.[10] However, Piérard[27] recently emphasized that the DCT may not be an appropriate tool for the analysis of epidermal cell kinetic induced by irritant surfactants. Considering the data obtained in his study, the suggestion may rather apply to a single occlusive test or to frequent patch tests, rather than to usual repeated open application tests, as the former may rapidly extract dansyl chloride from the skin. A recent study revealed that dansyl chloride could be instantly eliminated from the skin by some surfactants when the latter was applied frequently using patch tests within a short period.[28]

The SCTT of untreated skin presented here is in accordance with earlier studies.[10,23–26] Compared with calcipotriol, SLS possessed, a far higher potential to accelerate stratum corneum cell renewal. It is assumed that irritant properties may have, in part, a significant influence on stratum corneum turnover.[26,29] Using a conventional design of DCT, it seems that the stronger the irritancy potential, the shorter the SCTT.

Calcipotriol slightly but significantly accelerated epidermal cell renewal when compared with its vehicle. These data may support the findings by von Brenken and Proksch,[6] who reported that epidermal proliferation was increased in normal mouse skin after topical application of calcipotriol in isopropanol (175%) or in an ointment base (153%). On the other hand, the present data conflict with earlier results that calcipotriol stimulates cell differentiation and inhibits cell proliferation under *in vitro* conditions[2,3] as well as in psoriatics.[1,8] It is possible that despite differences between *in vitro* and *in vivo* effects, calcipotriol may act in normal human skin in a different manner from in psoriatic plaques. In fact, it has been reported that 1,25-dihydroxyvitamin D_3, a natural active vitamin D_3 metabolite with a comparable therapeutic effect for psoriasis, stimulates proliferation of normal epidermis after topical application *in vivo*;[30] yet the substance inhibits epidermal proliferation in psoriasis.[31,32] Further studies on the underlying pathogenic mechanisms of this phenomenon are indicated. However, we emphasize that we do not extrapolate these data to other test conditions (e.g. anatomical site, concentration or vehicle). This model study aimed at minimizing confounding variables, and should not be taken out of context.

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