

Placebo-controlled evaluation of the irritant potential of tacalcitol (1 α , 24-dihydroxyvitamin D₃) in healthy volunteers

K. SCHLOTMANN¹, C. ORTLAND², N. J. NEUMANN¹, T. RUZICKA¹ AND P. LEHMANN¹

¹Department of Dermatology, Heinrich-Heine-University, Duesseldorf, Germany

²Hermal, 21465 Reinbek, Germany

In the treatment of psoriasis with topical vitamin D₃ analogues, lesional and perilesional irritation is the main side-effect. The aim of this study was to investigate whether local side-effects generated by tacalcitol, a vitamin D₃ analogue, show concentration dependence. 3 different concentrations of tacalcitol (0.4; 4; 40 μ g/g ointment) and the vehicle were applied on normal skin of the back of 25 healthy volunteers under occlusive conditions for 5 days. Assessment of erythema, infiltration and scaling as well as measurement of transepidermal water loss (TEWL) was performed on days 1 to 5. On day 5, additional skin barrier tests (DMSO test, alkali resistance test) were performed. Erythema and slight infiltration, but no scaling, were observed in a number of subjects without significant differences. TEWL also did not show significant differences for the test formulations, though there was a tendency towards lower values in the untreated areas. In the skin barrier tests, a tendency towards higher alkali resistance in the test areas treated with 40 μ g tacalcitol/g ointment was detected. Thus, under occlusive conditions, the irritant potential of tacalcitol is very low. There is no convincing evidence of concentration dependence in irritation generated by tacalcitol when applied under occlusive conditions.

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Topical application of vitamin D₃ analogues is a widely used treatment for psoriasis. The effects are presumed mediated via inhibition of keratinocyte proliferation and induction of their differentiation. With the development of calcipotriol ointment, a synthetic vitamin D₃ analogue showing good efficacy (1) was provided. However, lesional and/or perilesional skin irritation was reported to occur in up to 20% of patients treated with calcipotriol ointment 50 μ g/g (2, 3).

Tacalcitol, 1 α ,24-dihydroxyvitamin D₃, is a recently-introduced synthetic vitamin D₃ analogue that is efficacious at a concentration of 4 μ g/g (4). While therapy of Japanese psoriatics with 2 μ g/g tacalcitol b.i.d. (5–7) resulted in the occurrence of skin irritation in only 1% of the patients (8), the 1 $\times$ daily application of 4 μ g/g in European patients showed locally-irritating symptoms in 12.3% (4).

Until now, it has not been clear whether vitamin D₃ analogues cause skin irritation in a dose-dependent manner. Arafat et al. (9) investigated the occlusive application of different concentrations of

calcipotriol on test areas on the back in 12 volunteers. They reported a linear relationship between calcipotriol concentration and skin irritation assessed by TEWL measurement and erythema scoring. The concentrations tested were at or below the therapeutic strength of 50 μ g/g. Furthermore, animal experiments resulted in the hypothesis that all vitamin D₃ analogues cause irritation in a concentration-dependent way (10, 11).

The aim of this study was to investigate the correlation between various concentrations of tacalcitol and skin irritation. The assessment was based on visual scoring, TEWL measurements and 2 irritation models. Because of the suspected low irritancy of tacalcitol, 1 of the strengths tested was 10 $\times$ higher than the therapeutic concentration.

Materials and Methods

Study design

The study was designed as a monocentre, double-blind, placebo-controlled, randomized, intraindivi-

dual comparison. The procedures were approved by the Ethical Committee of Human Experimentation at the Heinrich Heine University, Duesseldorf. The study was conducted according to GCP and the Declaration of Helsinki. Written informed consent was obtained from all subjects prior to conducting any study-related procedure.

Subjects

25 healthy volunteers (11 female, 14 male, aged 20 to 46 years) with skin type II–III according to the classification of Fitzpatrick (12), were included into the study. The subjects had to show no acute or chronic skin disease, no need for regular systemic or topical medical treatment and no exposure to sunlight or sunbed within the preceding 2 months. A laboratory test at visit 1 had to reveal no abnormalities.

Materials

Tacalcitol (0; 0.4; 4; 40 $\mu\text{g/g}$ ointment) was obtained from Hermal, Reinbek, Germany, in a blinded application form.

Procedures

Test formulations were applied on 4 consecutive days after transepidermal water loss (TEWL) measurement and visual scoring. The ointments were applied sparingly to 4 test areas (ca. 25 cm²) on the back of the subjects. A minimum distance of 5 cm separated the areas to avoid spreading artefacts. The treated areas and an untreated control area were covered with plastic foil and an adhesive tape (Mefix®). On day (D) 5, skin barrier tests were additionally performed after removing the occlusive patch. The occlusive application became necessary after a preliminary experiment with 4 volunteers with an open application of tacalcitol up to 40 $\mu\text{g/g}$ showed no skin irritation at all (data not shown).

Clinical scoring and instrumental measurements

Before and during the exposure period, each side was examined prior to the re-application of the test

substances on D1–D5. Each test area was graded for erythema, infiltration and scaling according to a visual scoring system (Table 1) and the irritation score was documented. The TEWL, an indicator of skin barrier properties, was measured with the Tewameter® TM 210 (Fa. Courage & Khazaka, Cologne, Germany) at ambient conditions (45–65% relative humidity, 20–22°C); volunteers rested for at least 15 min before measurements were taken.

Alkali resistance test

On D5, the alkali resistance test was additionally performed on all test sites. The time required to induce 10 tiny erosions by application of 0.1N NaOH under the constant pressure of a glass block (3.5×2×1.5 cm) was measured as described previously (13–15).

Dimethylsulfoxide (DMSO) test

On D5, the DMSO test was additionally performed on all test areas. By 5 min of exposure to 100%, 95% and 90% DMSO wheals were induced. The DMSO solution was applied with the use of a plastic block (15×3×0.5 cm) in which holes, 8 mm diameter each, were drilled. Skin reaction was evaluated on a 1+ to 4+ scale (13, 16).

Statistical tests

For the individual data, means and standard deviations of the mean were calculated. Due to the suspected influence of occlusion on the untreated control area also, no pre-post comparison was performed. The statistical analysis uses the data obtained on D5. Irritation score and DMSO test were analyzed by the use of the rank sum test for

Table 1. Irritation score

Score	Erythema	Infiltration	Scaling
0	no erythema	no infiltration	no scaling
1	faint erythema	slight infiltration	slight scaling
2	moderate erythema	moderate infiltration	moderate scaling
3	severe erythema	severe infiltration	severe scaling

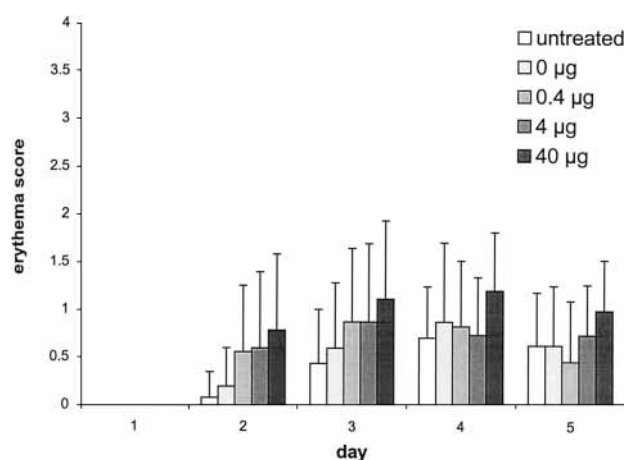


Fig. 1. Erythema (day 1–5).

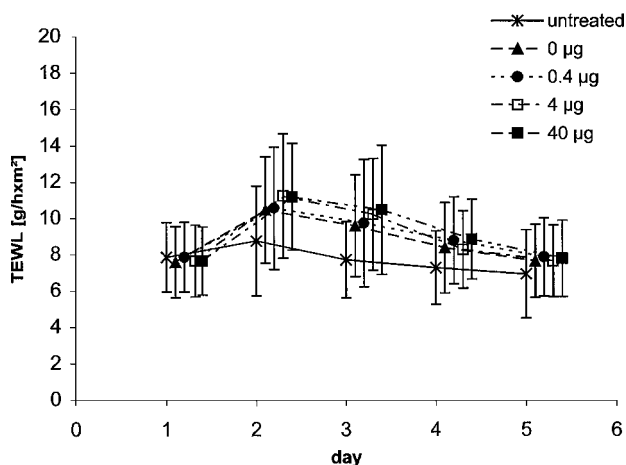


Fig. 2. TEWL (day 1–5).

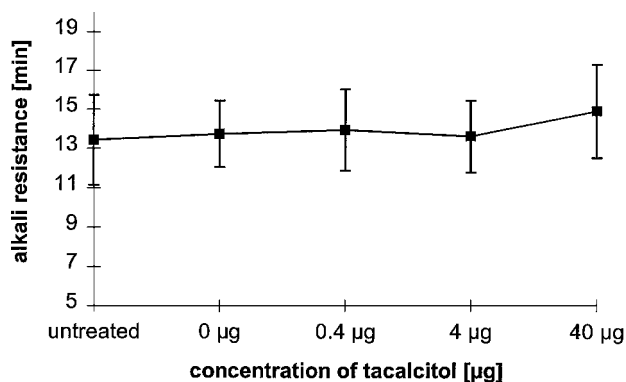


Fig. 3. Alkali resistance (day 5).

ordinal scale data for 3 or more samples. TEWL and alkali resistance test were analyzed with the randomization test for 3 or more samples.

Results

Clinical irritation score

No test area showed signs of scaling. In most cases, only erythema and – to a lesser extent – slight infiltration were observed. No significant differences between the treatments were observed for erythema scoring (Fig. 1). A tendency towards lower values was seen in the untreated area. The decrease in erythema score after D4, which followed an initial transitory increase in the first 3 days, did not reach statistical significance.

TEWL

No significant differences between treatments was found (Fig. 2). There was a tendency towards lower values in the untreated areas. It was observed that, after an initial increase in TEWL, the values de-

creased again between D2 and D5. This effect was more pronounced for all tacalcitol treated areas than for the untreated control area.

Skin barrier tests

There was no difference in intensity of response to DMSO between the areas on D5. For the alkali resistance test, however, there was a tendency towards a higher resistance on the test areas treated with 40 µg/g tacalcitol ointment (Fig. 3). These differences did not reach statistical significance.

Discussion

The aim of the study presented here was to show a concentration dependence in the generation of skin irritation by tacalcitol ointment. Based on the results of a study with different concentrations of calcipotriol in healthy volunteers (9), and animal experiments which investigated calcipotriol, tacalcitol and calcitriol (10, 11), a dose-dependent irritant potential of vitamin D₃ analogues seemed to be nearly established. While Arafat et al. (9) could already observe a dose dependence for erythema and TEWL under therapeutic concentrations (0; 5; 10; 20; 50 µg/g calcipotriol ointment), Fullerton & Serup (10, 11) investigated a range of concentrations between 5 and 500 µg/ml solvent. In their experiments, TEWL remained unchanged for all preparations tested, while erythema developed depending on the concentration applied. Nevertheless, another study with calcipotriol ointment in humans lead to the hypothesis that not the substance itself but the vehicle was responsible for skin irritation (17).

The results of our study do not show any significant concentration dependence of skin irritation after the occlusive application of tacalcitol. Our preliminary experiments had indicated that the irritant potential of tacalcitol even at a concentration of 40 µg/g was too low to produce skin irritation when applied to healthy skin for 5 days without occlusion. The visual scoring that was done for erythema, infiltration and desquamation showed, in accordance with the results of Effendy et al. (18), that erythema was the main skin reaction after topical application of vitamin D₃ analogues. The tendency to generate an elevated TEWL, which could be found for all preparations as compared with the untreated control area, was not as pronounced as the effect reported for calcipotriol (9). Nevertheless, this effect on TEWL was also observed for the vehicle used in our study, which is in accordance with the results reported by Fullerton et al. (17).

Due to the low irritability of tacalcitol, no dose-

dependent relationship could be seen. This was also the case for the skin barrier tests: although there was a tendency towards elevated resistance to 0.1 N NaOH in the areas tested with the highest tacalcitol concentration, this result did not reach statistical significance. Further work, preferably with higher concentrations of different vitamin D analogues in their vehicles, needs to be performed in patients and healthy volunteers to elucidate this question further.

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Address:

K. Schlotmann
Department of Dermatology
Heinrich-Heine-University
Moorenstr. 5
40225 Duesseldorf
Germany