

## The calcipotriol dose–irritation relationship: 48 hour occlusive testing in healthy volunteers using Finn Chambers<sup>®</sup>

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Accepted for publication 16 September 1997

### Summary

Calcipotriol is widely used in the treatment of psoriasis. Adverse lesional and perilesional irritation may occur. Allergy may occasionally be suspected. Allergy patch testing with calcipotriol may be difficult or impossible because calcipotriol is a local irritant. The aim of the present study was to assess the calcipotriol dose–irritation relationship, and establish a non-irritant patch test concentration for calcipotriol allergy patch testing. The study was a prospective, double-blind, randomized, dose titration evaluation in 180 healthy volunteers never previously exposed to calcipotriol. All individuals were patch tested with a calcipotriol dilution series (range 0.016–250 µg/mL). Clinical reading of test sites and measurement of erythema using a Minolta ChromaMeter were performed on days 2 and 3. Laser Doppler perfusion imaging of cutaneous blood flow was performed on day 3.

Doubtful reactions (score ½) and weak reactions (score 1) were frequent and observed even at low dose exposure. Reactions declined in strength between the readings on day 2 and day 3. Only score 2 reactions with moderate erythema and some infiltration showed a threshold of no irritation. This threshold was confirmed by colorimetry and flowmetry. Cases of suspected allergy to calcipotriol may, to avoid irritant reaction and false positive readings, be patch tested with calcipotriol 2 µg/mL citrate-buffered isopropanol solution applied under occlusion for 48 h using small Finn Chambers<sup>®</sup>. Score ½ and 1 reactions are likely to reflect irritation. A positive test should be repeated after a minimum period of 3 months to ensure its consistency over time. A repeated open application test may be indicated.

Calcipotriol (Dovonex<sup>®</sup>, Daivonex<sup>®</sup>) is a vitamin D analogue widely used for topical treatment of psoriasis. The drug is usually well tolerated and safe. However, treatment may cause lesional and perilesional irritation and ectopic facial irritation.<sup>1–4</sup> The skin reactions are mostly mild and in only few cases are the reason for withdrawal of calcipotriol treatment. It is a general experience that patients who are intolerant of calcipotriol treatment may tolerate the drug later. The adverse dermatitis to calcipotriol is therefore typically considered to be irritant in nature. In the literature there are reports of possible allergic contact dermatitis during treatment with Daivonex<sup>®</sup> ointment<sup>5–10</sup> and cream.<sup>11</sup> However, cases of true allergic contact dermatitis to calcipotriol have hitherto been difficult to verify, as a valid standard test procedure has been lacking and as the non-irritant test concentration of calcipotriol has not been known. None of these reports therefore allows a definite conclusion.

Calcipotriol is a known irritant, and acute reactions to

calcipotriol including patch test reactions include redness, infiltration, papules and vesicles: the same clinical features on which the clinical grading of allergic patch test reactions is based. This makes differentiation between irritant and allergic reactions very difficult or impossible.

A standard allergy patch test procedure should be based on non-irritant doses of calcipotriol. The aim of the present study was to establish the non-irritant threshold of calcipotriol in an appropriate test vehicle. The study was performed in healthy volunteers never previously treated with calcipotriol, as a recent study showed that the irritant response to calcipotriol is the same in the skin of healthy volunteers and psoriatics.<sup>12</sup> Colorimetric measurement of erythema and laser Doppler image scanning of cutaneous blood flow were used as objective tools supplementary to clinical scoring. Recent studies have shown that the irritant profile of calcipotriol is dominated by vascular reactions and erythema in contrast to a normal transepidermal water loss and minor oedema.<sup>12,13</sup>

## Materials and methods

### *Study design*

The study was a prospective, randomized, double-blind, within-subject, placebo-controlled patch test study in healthy volunteers never previously exposed to calcipotriol. The trial was approved by the local Ethics Committee of Copenhagen County (reg. no. KA 95146 gs) and was reported to the National Board of Health in Denmark.

### *Study subjects*

A total of 180 healthy volunteers (120 women and 60 men) participated. The mean age was 36.2 years (range 18–70). Of the subjects, 179 qualified for analysis of dose–response. Subjects participated only following verbal and written information about the study and their signed consent. Subjects were not included if they had been treated with systemic corticosteroids, immunosuppressive medicines, ultraviolet B or sunbathing (including tanning beds) within the last 4 weeks. Subjects previously exposed to calcipotriol were also excluded. Treatment of the test region was restricted to personal care products for 2 weeks prior to testing.

A cyclic variation in relation to menstrual phase with increased sensitivity of the skin to the standard irritant sodium lauryl sulphate (SLS) before and during menstruation has been reported.<sup>14,15</sup> To study phase-related susceptibility to calcipotriol, women participating in the study and with a regular menstrual cycle were grouped as: (i) females in the menstrual period, defined as the period of any menstrual bleeding, and (ii) females in the intermenstrual period.

### *Occlusive chamber application*

Occlusive testing on the upper back was performed with calcipotriol solutions (250, 50, 10, 2, 0.4, 0.08, 0.016 and 0 µg/mL) in an isopropanol/citrate buffer solution (isopropanol 417 mg, sodium citrate dihydrate 1 mg, purified water to 1 mL). The pH was about 8.5. Patch testing was performed in duplicate using large (12 mm diameter) and small (8 mm diameter) Finn Chambers® (Epitest, Helsinki, Finland) on Scanpor® tape. Test solutions were applied on a filter disc fitted into the test chamber. Test chambers were placed in four vertical rows with large chambers in two rows and small chambers in two rows. Application of test solutions in large and small chambers, respectively, was randomized separately.

### *Clinical assessment*

Clinical assessment was performed on day 2 (30 min after removal of the patches) and again on day 3. Visual scoring of irritation was performed according to the following scale: 0, no reaction (negative); ½, scaling or very weak erythema only; 1, weak erythema, slight infiltration; 2, marked erythema, infiltration, possible vesicles; and 3, marked erythema, strong infiltration, papules, vesicles. Note that the clinical features of irritation scores ½, 1, 2 and 3 in this irritation scale are in accordance with those of scores ?+, 1+, 2+ and 3+ in the International Contact Dermatitis Research Group classification, although the two grading schemes are not identically phrased.

### *Non-invasive bioengineering measurements*

Measurement of skin colour/erythema was performed both on days 2 and 3. Skin colour was measured only at large chamber test sites. Measurement of cutaneous blood perfusion was done on day 3 at both large and small chamber test sites. Before initiation of measurements, the subjects rested in a horizontal position with the back uncovered for 15 min. The temperature in the room was kept constant at 23–25 °C.

### *Laser Doppler perfusion imaging*

The skin perfusion at individual sites was mapped using a Laser Doppler Perfusion Imager® (LDPI, Lisca® Development, Sweden). A detailed description of the equipment has been published elsewhere.<sup>16</sup> The equipment uses a laser diode to scan the tissue. LDI version 2.5 software was utilized. Ambient light was switched off during scanning to avoid optical interference. One scan was made of each site. The parameter settings during measurements were: image format: 32 × 32 measurement points for large chambers and 60 × 60 measurement points for small chambers (scanning four small chamber sites at a time); resolution, high; spatial resolution, 0.74 mm<sup>2</sup>; threshold background, 6.1; distance from scanner head to test site, 17.5 cm. In addition, an unexposed area (60 × 60 measurement points) was scanned.

Evaluation of skin perfusion was done by framing a square area centrally within each circular test site. For large chamber sites an area based on 9 × 9 measurement points covering 60 mm<sup>2</sup> was used. For small chambers an area based on 8 × 7 measurement points covering 41 mm<sup>2</sup> was used. Evaluation of basal blood

perfusion of the unexposed skin area was based on all  $60 \times 60$  measurement points scanned. As calcipotriol irritancy has been found primarily to affect the vasculature of the skin, differences in susceptibility to calcipotriol might depend on the basal blood flow.

#### Minolta ChromaMeter CR-300 measurements

For measurement of erythema the Minolta ChromaMeter CR-300<sup>®</sup> (Minolta, Osaka, Japan) was used. The apparatus measures skin colour by tristimulus analysis of reflected xenon flash light according to the CIE system. The colour is expressed in a tridimensional system with a green–red ( $a^*$ ), a yellow–blue ( $b^*$ ) and an  $L^*$  axis expressing brightness. Measurements were done according to the guidelines from the standardization group of the European Society of Contact Dermatitis.<sup>17</sup> Measurements were based on recording  $a^*$  values. Every site was measured three times with measurements repeated for the second and third times in the same order of operation. The mean  $a^*$  value was used. In addition, an unexposed site on the right flank of each participant was measured three times and the mean  $L^*$  value was calculated. Increased light reflection from the skin indicating a 'fair' skin has been found to be associated with increased susceptibility to the standard irritant SLS.<sup>18</sup> Subjects with a 'fair' skin might also show increased susceptibility to calcipotriol.

#### Statistical analysis

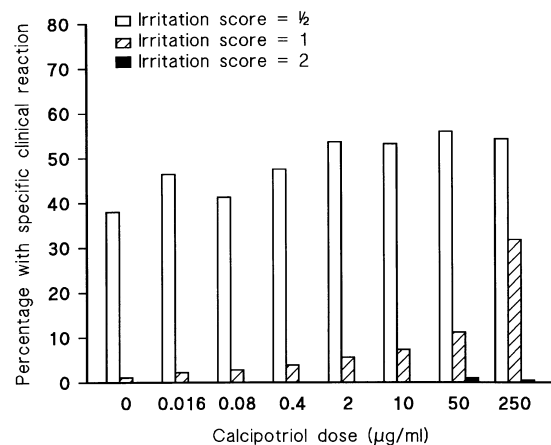
Differences between means of skin colour and cutaneous blood perfusion measurements, respectively, were assessed as appropriate by the unpaired and paired Student's *t*-tests. Analysis of variance was used when more than two means were considered (validation of non-invasive measurements vs. clinical irritation scores). The blood perfusion measurements were logarithmically transformed to normalize variations. The proportion of subjects having a positive clinical irritation was compared between women in the menstrual period and women in the intermenstrual period by Fisher's exact test. All tests were two-tailed and  $P < 0.05$  was considered statistically significant.

## Results

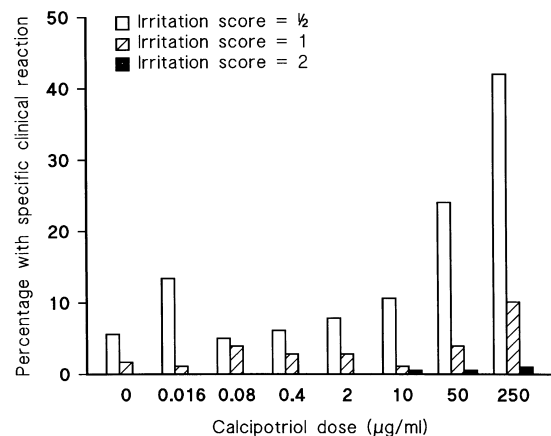
#### Clinical assessment

The distributions of day 2 and day 3 clinical readings of the small chamber series are given in Fig. 1(A,B). The percentage of irritative reactions in any group increased

A



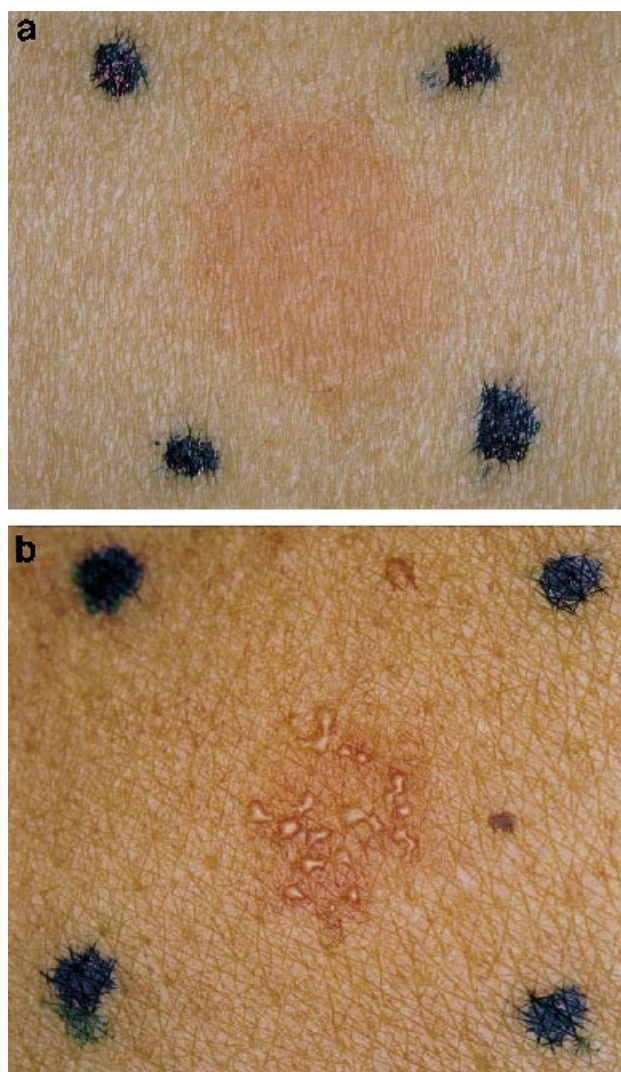
B



**Figure 1.** Clinical assessment of skin reactions after patch testing in small Finn Chambers<sup>®</sup>. Percentage of study subjects with a specific clinical reaction as a function of calcipotriol concentration at (A) day 2 and (B) day 3.

with calcipotriol concentration in a dose-dependent manner. Doubtful reactions (score 1/2) were common, regardless of concentration. Score 1 reactions (Fig. 2A) were found even at the lower test concentrations, whereas score 2 reactions (Fig. 2B) were seen in a few cases only, i.e. after occlusive testing with concentrations of calcipotriol in the range 50–250 µg/mL on day 2 and in the range 10–250 µg/mL on day 3. No strong reactions with score 3 were observed. Comparing results for days 2 and 3 showed that the number of irritant skin reactions decreased over time.

Occlusive testing with calcipotriol solutions in large Finn Chambers<sup>®</sup> resulted in more frequent and stronger reactions than when small chambers were used. However, the dose–response curve was more scattered after patch testing using the large chambers.

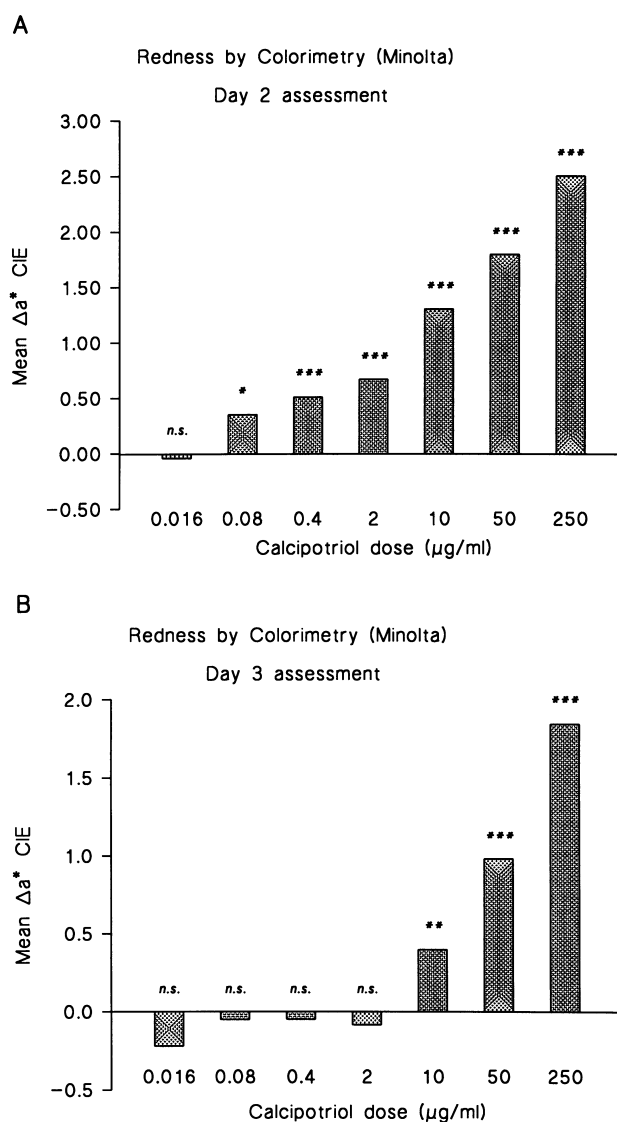


**Figure 2.** Clinical appearance at day 3 of (A) a typical score 1 reaction, with weak erythema and slight infiltration and (B) a score 2 reaction of the vesicular type.

#### *Comparison of subject susceptibility to calcipotriol*

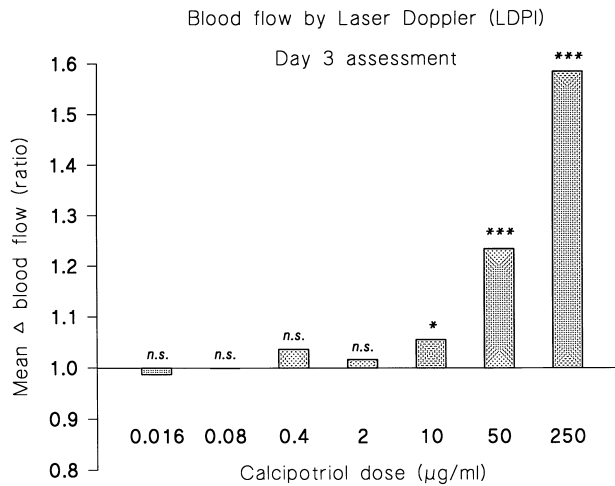
Subjects having a clinical score of  $\frac{1}{2}$  or more to a calcipotriol solution at any test site and a clinical score of 0 to the placebo solution were compared with the group of subjects having a clinical score of 0 at all test sites with respect to the mean  $L^*$  value found at the right dorsal flank and the mean blood perfusion of unexposed skin of the back. No significant differences were found between the two groups regarding the mean  $L^*$  and the mean basal blood flow.

Women in the menstrual period were compared with women in the intermenstrual period with



**Figure 3.** Difference in erythema value  $a^*$  between active and placebo-treated sites. *P*-values after paired *t*-tests comparing sites for concentrations 250, 50, 10, 2, 0.4, 0.08 and 0.016  $\mu\text{g/ml}$  were, respectively, (A) at day 2:  $P < 0.001$  (\*\*\*),  $P < 0.001$  (\*\*\*),  $P < 0.001$  (\*\*\*),  $P < 0.001$  (\*\*\*),  $P < 0.001$  (\*\*\*),  $P = 0.011$  (\*) and  $P = 0.75$  (n.s.); (B) at day 3:  $P < 0.001$  (\*\*\*),  $P < 0.001$  (\*\*\*),  $P = 0.001$  (\*\*),  $P = 0.47$  (n.s.),  $P = 0.70$  (n.s.),  $P = 0.68$  (n.s.) and  $P = 0.084$  (n.s.).

respect to the proportion having a clinical score of  $\frac{1}{2}$  or more to a calcipotriol solution at any test site. The two groups were further compared with respect to the measurements of  $a^*$  value and blood perfusion at test sites using for each subject the mean value of all eight test sites. No significant difference was found between women in the menstrual period as compared with women in the intermenstrual period.



**Figure 4.** Mean ratio (geometric mean) between increasing calcipotriol concentrations and placebo at day 3 after occlusive testing in small Finn Chambers<sup>®</sup>. *P*-values after paired *t*-tests comparing active and placebo-treated sites for concentrations 250, 50, 10, 2, 0.4, 0.08 and 0.016 μg/mL were *P* < 0.001 (\*\*\*), *P* < 0.001 (\*\*\*), *P* = 0.034 (\*), *P* = 0.48 (n.s.), *P* = 0.16 (n.s.), *P* = 0.96 (n.s.) and *P* = 0.57 (n.s.), respectively.

#### Non-invasive bioengineering measurements

Figure 3(A,B) shows the mean difference in *a*\* values between calcipotriol exposure sites and placebo sites on days 2 and 3. The skin colour measured by the Minolta colorimeter showed a clear-cut dose–response relationship, with increased values after application of higher concentrations of calcipotriol. The highest calcipotriol concentrations that did not give a significant increase in *a*\* value compared with the placebo were 0.016 μg/mL at day 2 and 2 μg/mL at day 3.

Measurement of cutaneous blood flow was done using the laser Doppler image scanner. The LDPI

values were log transformed to normalize their distribution. Figure 4 shows the mean ratio (geometric mean) between increasing calcipotriol concentrations and placebo after occlusive testing in small Finn Chambers<sup>®</sup>. The results with large Finn Chambers<sup>®</sup> were in accordance with these results. A clear-cut dose–response relationship was found, with higher calcipotriol concentrations resulting in higher cutaneous blood flow. The highest calcipotriol concentration that did not give an increase in LDPI value compared with placebo (ratio < 1) at study day 3 was 2 μg/mL.

#### Validation of non-invasive measurements vs. clinical irritation scores

The mean *a*\* values and the mean blood perfusion values were related to the clinical irritation score (0, ½, 1, 2) for the calcipotriol concentration giving the most uniform distribution of the clinical scores. The results are shown in Table 1. With both non-invasive methods it was possible to differentiate between the different clinical scores (*P* < 0.001).

#### Discussion

In the present study, subjects never treated or otherwise exposed to calcipotriol were patch tested in order to define a non-irritant threshold of calcipotriol as a prerequisite for future calcipotriol allergy patch testing. It was demonstrated that doubtful and weak irritant reactions with erythema and possible slight infiltration are very common, and no lower threshold for these reactions exists. Score 1 reactions were, thus, found even at the lowest dose (0.016 μg/mL) of calcipotriol and after testing with placebo solution. A few subjects

**Table 1.** Comparison between clinical irritation scores and non-invasive measurements. Mean *a*\* values and clinical irritation score at day 2 after occlusive application of calcipotriol at 250 μg/mL using large Finn Chambers<sup>®</sup> and mean blood perfusion (LDPI) values and clinical irritation score at day 3 after occlusive application of calcipotriol at 250 μg/mL using small Finn Chambers<sup>®</sup>

|                                     | Clinical irritation score |            |             |             | Between groups probability<br>(analysis of variance) |
|-------------------------------------|---------------------------|------------|-------------|-------------|--|
|                                     | 0                         | ½          | 1           | 2           |  |
| Mean <i>a</i> *                     | 12.33                     | 13.36      | 14.84       | 17.78–19.97 | <i>P</i> < 0.001                                     |
| Range                               | 9.00–15.92                | 8.15–18.47 | 10.84–19.31 | 15.67       |  |
| <i>n</i>                            | 22                        | 90         | 62          | 5           |  |
| Mean LDPI value<br>(geometric mean) | 0.83                      | 1.58       | 2.10        | 2.14–3.71   | <i>P</i> < 0.001                                     |
| Range                               | 0.29–2.46                 | 0.55–5.10  | 0.82–5.89   | 1.23        |  |
| <i>n</i>                            | 80                        | 71         | 18          | 2           |  |

had score 2 reactions. However, these moderate reactions were only seen after patch testing with calcipotriol at 10 µg/mL or more. The lower threshold of non-reactivity seems to be 2 µg/mL, but the number of individuals with such reactivity was limited. However, this threshold was verified by colorimetric measurement of erythema and by LDPI measurement of cutaneous blood flow on day 3. These two techniques give a more valid threshold value based on measurements in the whole group as compared with clinical scoring alone. With both methods, 2 µg/mL was found to be the highest dose not causing measurable effects on the vasculature.

Based on these results, allergy patch testing in suspected cases of allergy to calcipotriol should be conducted, to omit irritant reactions and false positive readings, with calcipotriol 2 µg/mL in isopropanol buffered with citrate. Occlusive testing should not be performed with calcipotriol in the ointment vehicle as this vehicle may itself cause irritation.<sup>12,19</sup> It is recommended that occlusive testing is done using 48 h exposure with small Finn Chambers®, as testing with large Finn Chambers® results in a more scattered dose-response curve. Type 2+ reactions (marked erythema and moderate oedema with papules and possible vesicles) at the day 3 reading might indicate allergic sensitization. Reading 1+ reactions as allergy may lead to an unacceptably high percentage of false positive readings. A positive test should always be repeated after a period of at least 3 months in order to establish that it is reproducible over time. Recently, Molin<sup>10</sup> presented a case where calcipotriol ointment caused severe dermatitis during treatment. Open testing indicated that an allergic contact reaction had to be considered. An open test after 3 months was negative. This case illustrates that variation in the threshold may render some patients more vulnerable to irritant events for limited periods of time. Weakening of reactions upon repeated occlusive testing after 3 months was observed by Steinkjer.<sup>7</sup> Thus, repetition of testing on some later occasion should be considered mandatory. In testing problem cases a ROAT may additionally be performed as outlined by Hannuksela and Salo.<sup>20</sup> The skin of the antecubital fossa should be avoided, as application at this site can easily result in irritant contact dermatitis.

The test procedures described merely serve as instruments in the search for the reason and mechanism behind the adverse dermatitis. Clinical manifestations, patient history and a ROAT with the product used will normally suffice for the practising dermatologist to decide whether a given patient can

continue with calcipotriol or not. In patients who are intolerant on one occasion, the treatment can often be reinstituted after a treatment-free period, as aggravating factors may have declined spontaneously in the mean time.

The present study was conducted in healthy volunteers. A reference group with truly allergic contact sensitization to calcipotriol needs to be studied in order to verify an allergy patch test design for this substance. However, no ultimate criteria, clinical or experimental, for calcipotriol allergy exist, and allergic sensitization to this substance seems to be rare or non-existent, with only a few published case reports of possible allergy, but no definite case with irritant reactions excluded. Calcipotriol is clearly a very difficult substance when tested under occlusion, and it is possible that allergy patch testing cannot be performed in a meaningful way using this compound.

## Acknowledgments

The skilful technical assistance of Lone Bergmann and Anna Grethe Nielsen, Department of Dermatology K, Gentofte Hospital, and of Birgitte Andersen, Tine Blaabjerg and Pavla Setina, Department of Dermatological Research, Leo Pharmaceutical Products, is gratefully acknowledged.

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