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Calcipotriol ointment and cream or their vehicles applied immediately before irradiation inhibit ultraviolet B-induced erythema

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Summary Results of ultraviolet (UV) B phototherapy can be improved by the application of calcipotriol, but studies are needed to decide how the two treatments should be combined. We studied the effect of UVB after application of calcipotriol ointment (50 μg g^{-1}) and calcipotriol cream (50 μg g^{-1}) and determined the optimal time of application of calcipotriol when combined with UVB phototherapy (280–350 nm), in a single-blinded randomized vehicle-controlled study of 37 healthy adult volunteers. Calcipotriol ointment or cream was applied randomly on five areas on the back at different time intervals from UVB irradiation. One area was left untreated as the control. Application times were the evening before, the morning before, 2 h before, immediately before, and immediately after irradiation. UVB irradiation was administered by TL20W/12 fluorescent tube lamps at increasing doses (20, 25, 32, 40, 50 and 64 mJ cm^{-2}) to six subunits of each test area. Clinical assessment was performed 24 h after UVB irradiation by a blinded investigator. Calcipotriol ointment and cream were applied in 19 and 18 subjects, respectively, and erythema was measured for each application time quantified. We found that erythemal reactions were significantly smaller when calcipotriol ointment or cream was applied immediately before irradiation compared with all other application times. To explain these findings, a vehicle control study was performed. No difference in erythema was seen between calcipotriol medication and the vehicle controls. Spectrophotometric analysis of the calcipotriol cream and ointment showed no UV absorbance in the UVB range. No signs of photosensitization were noted. In conclusion, the vehicles of the calcipotriol ointment and cream inhibit the induction of erythema by UVB irradiation if applied immediately before phototherapy. Consequently, calcipotriol ointment and cream should not be applied directly before UVB irradiation; however, they may be applied at any time up to 2 h prior to or immediately after UVB irradiation. Possible explanations for this sunscreen activity are discussed.

Key words: calcipotriol, erythema, psoriasis, ultraviolet B therapy

Calcipotriol is a safe and effective treatment for psoriasis.[1–4] Phototherapy with ultraviolet (UV) B is also effective in the second-line management of psoriasis, but both broad- and narrow-band UVB are associated with skin tumours in mice.[5] Combining UVB phototherapy with calcipotriol can provide a UVB-sparing effect, as the addition of broad- or narrow-band UVB to calcipotriol ointment monotherapy resulted in superior treatment outcomes than with calcipotriol ointment alone.[6–9] However, we have recently demonstrated that the combination of calcipotriol ointment with high-dose narrow-band UVB is as equally effective as high-dose narrow-band UVB alone.[10]

There is uncertainty about how calcipotriol ointment and UVB phototherapy should be combined to provide optimal therapeutic results and to achieve a UVB-sparing effect. Already, some studies have investigated possible interactions of calcipotriol ointment and UVB irradiation.[11–13] Marsico and Dijkstra[11] showed that minimal erythema doses (MEDs) were increased immediately following application of calcipotriol ointment; 2 h

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after application, MEDs returned to baseline. Lebwohl *et al.*[12] found MEDs to be unaffected unless calcipotriol ointment was applied in a thick layer. In the same study it was demonstrated that calcipotriol was not degraded in a clinical situation. Maier *et al.*[13] detected a UVB-blocking action of calcipotriol in comparison with an indifferent ointment base, but differences in MED were not statistically significant.

In this study we aimed to determine the optimal time for the application of calcipotriol ointment (50 $\mu g\ g^{-1}$) and cream (50 $\mu g\ g^{-1}$) related to UVB phototherapy, resulting in the highest penetration of UVB, i.e. the strongest erythemal reaction.

Materials and methods

Study population

The study was approved by the Academic Medical Centre ethics review committee. Forty healthy non-psoriatic volunteers of either sex with skin phototypes II and III were included in the period between July and September 1997. All volunteers were at least 18 years old and gave informed consent. Women of child-bearing potential had a negative urine pregnancy test and used an adequate method of contraception. Exclusion criteria were: uneven pigmentation of the back, sunbathing or artificial sun exposure 6 weeks prior to study entry, any use of concurrent medication other than oral contraception, concurrent participation in any other trial or within 3 months prior to inclusion, and known hypersensitivity to any of the components of calcipotriol ointment or cream.

Forty appropriate subjects were recruited before the start of the study. One subject failed to attend after giving informed consent. Of the remaining 39 subjects, 21 were randomized to the ointment group and 18 to the cream group. The study population consisted of 26 men and 13 women, with an average age of 32·1 years (range 18–56); 12 subjects had skin phototype II, 27 subjects skin phototype III. By randomization, these population characteristics were equally divided among both groups.

One of the volunteers in the ointment group was lost to follow-up after randomization and the first application on day 1. In another subject the product under investigation was wrongly applied, and data derived from this patient were therefore not usable. Statistical analysis was performed with data from the remaining 37 volunteers.

Study procedure

After qualification, each subject was invited to visit the out-patient clinic on three consecutive days. During this period the subjects were asked not to wash their backs. In the evening of day 1, approximately 12 h before irradiation, each subject was randomized to either calcipotriol ointment or cream, and a nurse applied 0·5 g of the assigned substance to one of six demarcated areas of 3 × 24 cm (horizontally orientated) on the back of each subject. This amount is comparable with the quantity used by most psoriasis patients. During the whole study the calcipotriol was applied by one nurse in an even thin layer using a finger condom. The mid-central part of the back was

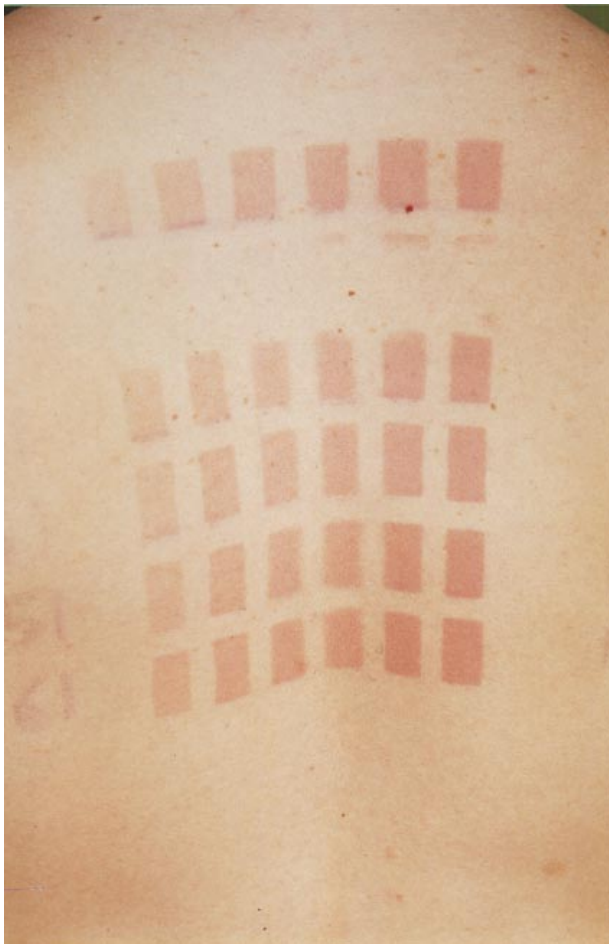

Figure 1. Erythemal reactions after ultraviolet (UV) B irradiation with increasing doses (columns) per application time (rows). For each application time erythemal reaction intensifies with increasing UVB dose, except for the area where calcipotriol ointment or cream was applied immediately before irradiation (second row).

used in all cases, to minimize intraindividual differences in erythemal reactions of the different regions of the back. For each individual subject, before the start of the study, the demarcated areas were randomized for the time of application of the ointment or cream. UVB irradiation was administered on day 2. The evening before, 4 h, 2 h and immediately before irradiation, 0·5 g calcipotriol ointment or cream was applied in four of the areas. After this, using a preprepared template, the following generally practised irradiation doses were administered to six sites within each demarcated area: 20, 25, 32, 40, 50 and 64 mJ cm^{-2} (Fig. 1). The distance from the UVB source to the subjects' back was kept constant. Immediately following irradiation, 0·5 g ointment or cream was applied in one of the two remaining areas. In the last (control) area no calcipotriol ointment or cream was applied. On day 3, approximately 24 h after UVB irradiation, single-blind clinical assessment was performed. Clinical assessment included assessment of the erythema, determination of the erythema indices, photography of the back of each subject, and enquiry about any adverse events during the study period.

Vehicle control

A vehicle control study ($n = 5$) was performed later to check whether the inhibition of erythema resulting from the application of cream/ointment immediately before UVB irradiation was dependent on calcipotriol. Therefore, the cream and ointment vehicles were tested simultaneously with the active medication as described above for one time point: application directly before irradiation. UVB irradiation was given (20, 25, 32, 40, 50 and 64 mJ cm^{-2}) immediately after application (0·5 g per test area).

Study medication

The calcipotriol ointment containing 50 μg g^{-1} of calcipotriol was in the following vehicle: disodium hydrogen phosphate, POE stearylether, propylene glycol, tetracemine disodium, DL-alpha-tocopherol, petrolatum, paraffin liquid and purified water. The vehicle of the calcipotriol 50 μg g^{-1} cream was composed of cetomacrogol, cetostearyl alcohol, chloroallylhexaminium chloride, disodium edetate, disodium phosphate dihydrate, glycerol 85%, liquid paraffin, white soft paraffin and purified water. The medication was packaged in 30 g tubes.

Ultraviolet B source and dosimetry

UVB irradiation was administered with TL20W/12 fluorescent tube lamps with a spectrum ranging from 280 to 350 nm (Philips, Netherlands). Energy output, mean reading 2·2 mW cm^{-2}, was measured with a calibrated spectrometer before and after the study.

Clinical assessment

Minimal administered erythema dose. For each area, the lowest administered UVB dose eliciting uniform redness with sharp borders 24 h after irradiation was determined.

Erythema index. The skin reflectance of each subunit was measured using a Dermaspectrometer (Cortex Technology, Hadsund, Denmark). The erythema of the skin is expressed using the erythema index. This index is calculated as 100 (log $R_{660\ nm}/G_{570\ nm}$), where $R_{660\ nm}$ = (red reflectance from skin)/(red reflectance from white normal) and $G_{570\ nm}$ = (green reflectance from skin)/(green reflectance from white normal).[14]

Spectrophotometric analysis

The UV absorption spectra of the cream and ointment samples were analysed by a double-beam UV/VIS spectrophotometer (Perkin-Elmer Lambda 40). Samples were sandwiched between two quartz plates, using spacers of 10 μm, as described previously.[15] The UV absorbance in the range of 240–400 nm was recorded and corrected for light scattering levels.

Statistical analysis

Before inclusion it was estimated that 18 subjects were needed in both groups in order to provide 80% power. The volunteers were divided into two groups approximately of equal size; one group would eventually receive calcipotriol ointment, the other group calcipotriol cream.

The minimal administered erythema doses (MAEDs) of all application times were compared in pairs by Wilcoxon signed rank tests with a two-sided Bonferroni-corrected significance level; $P < 0{\cdot}05$ was considered statistically significant. Regarding the secondary response criterion, erythemal reaction data were collected and no statistical analysis was performed. For the erythema index, descriptive statistics,

i.e. mean ± SD, were performed for each application time per UVB dose.

Results

Clinical assessment

Clinical assessment was performed on every irradiated subunit of each area (Fig. 1). No differences were found between UV-related properties of the calcipotriol cream and ointment.

Minimal administered erythema dose. MAEDs of all application times of calcipotriol ointment/cream were compared in pairs. The distribution of the MAEDs in the study population (Table 1) was statistically significantly different for application immediately before UVB irradiation compared with all other application times and the control area where no ointment/cream was applied. MAEDs were significantly higher in areas where calcipotriol ointment/cream was applied immediately before UVB irradiation; $P < 0{\cdot}001$. Note that in Table 1 for application of calcipotriol ointment immediately before irradiation, 18 of 19 subjects had MAEDs > 64 mJ cm^{-2}. In contrast, in any other combination of application times, including comparisons with the control area, no significant differences could be detected. Calcipotriol cream gave similar results.

Erythema index. Mean erythema indices regarding application times of calcipotriol ointment/cream are graphically presented for each UVB dose in Figures 2 and 3. These graphs demonstrate that erythema indices increase for all application times and control areas in parallel to increasing UVB doses, except for the application of calcipotriol ointment/cream immediately before irradiation.

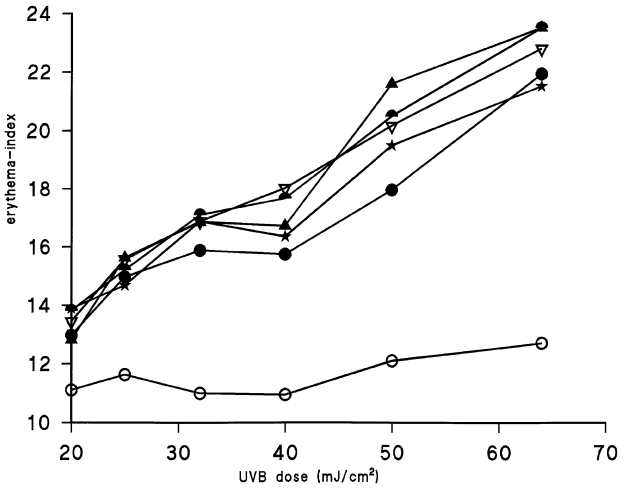

Figure 2. Erythema indices for each irradiation dose per application time of calcipotriol ointment. Erythema indices rise with increasing ultraviolet (UV) B doses, except for application immediately before irradiation. ▲, No application; ★, application evening before UVB; ▽, application in the morning; ●, application 2 h before UVB; ○, application immediately before UVB; —●—, application immediately after UVB.

Vehicle control

A vehicle control study was performed in five volunteers. As inhibition of erythema was only seen when ointment/cream was applied directly before irradiation, only this time point was studied. No significant difference was seen between the vehicles and active cream or ointment. Even after giving the highest UVB dose (64 mJ cm^{-2}), no differences were

Table 1. Distribution of minimal administered erythema doses (MAEDs) in the study population

	Application of calcipotriol ointment					
MAED (mJ cm^{-2})	No application	Evening before	4 h before	2 h before	Immediately before	Immediately after
≤ 20	5	6	4	5	1	4
25	6	2	5	3	0	6
32	4	6	5	4	0	4
40	2	3	3	3	0	5
50	1	1	1	2	0	0
64	1	1	1	0	0	0
> 64	0	0	0	2	18	0
Total	19	19	19	19	19	19

Figures indicate number of subjects with uniform redness with sharp borders per administered ultraviolet (UV) B dose for each application time. Six UVB doses were given: 20, 25, 32, 40, 50 and 64 mJ cm^{-2}. MAED: minimal administered dose eliciting uniform redness.

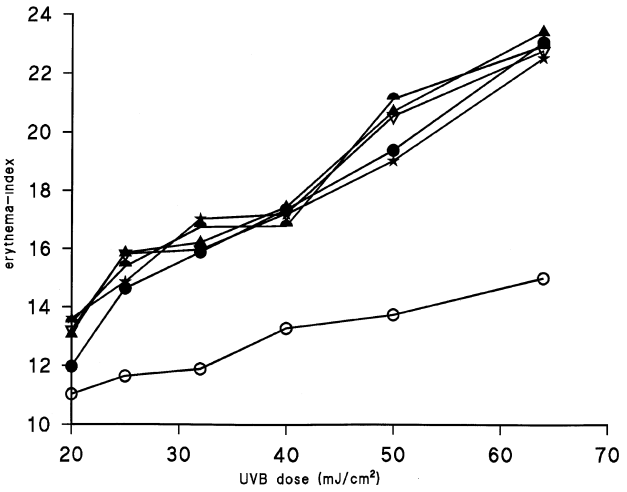

Figure 3. Erythema indices for each irradiation dose per application time of calcipotriol cream. Erythema indices rise with increasing ultraviolet (UV) B doses, except for application immediately before irradiation. ▲, No application; ★, application evening before UVB; ▽, application in the morning; ●, application 2 h before UVB; ○, application immediately before UVB; —●—, application immediately after UVB.

seen in the erythema indices between the vehicle and active cream (13·1 vs. 13·6) or vehicle and active ointment (12·7 vs. 14·6). This indicates that the vehicles, and not calcipotriol, are responsible for the inhibition of erythema.

Ultraviolet absorbance

The UV absorbance (240–400 nm) of the calcipotriol cream, ointment and vehicles was studied. No specific absorbance in the UVC, UVB or UVA range was seen for calcipotriol cream/ointment or their vehicles. All showed identical UV absorbance curves (data not shown).

Adverse events

Two subjects in the calcipotriol cream group experienced an adverse event. One subject had a sunburn of the treated skin, and the other experienced moderately painful skin at the treated areas. Both events were recorded at the last visit on day 3 and were judged probably to be related to calcipotriol cream. Follow-up was considered unnecessary. No late adverse events were registered.

Discussion

In this study, we investigated the influence of the application time of calcipotriol ointment/cream on erythema induction by UVB in order to determine the optimal time for application of calcipotriol ointment/cream.

This is the first randomized, single-blind within subject and vehicle-controlled study in which UVB blocking properties of calcipotriol have been studied; in comparable studies, smaller sample sizes were used or they were not blinded.[11–13] Additionally, this is the first time UVB blocking effects of calcipotriol cream have been investigated.

For practical reasons, a standard sequence of UVB doses (20–64 mJ cm^{-2}) was given. As a consequence, the true MED was not measured in all subjects; in at least six patients the erythema reaction at 20 mJ cm^{-2} may not have been minimal; therefore, we used the term MAED.

The results found in our study show that calcipotriol ointment/cream inhibits the induction of erythema by UVB when applied immediately before UVB irradiation. There was no blocking effect when the substances were applied at any moment up to 2 h before irradiation. Although we did not measure the MED, our findings are in agreement with data found by Maier *et al.*,[13] who showed that application of calcipotriol ointment 20 min before UVB irradiation resulted in a mean increase of MED of 31%. Also, data found by Marsico and Dijkstra[11] support our results, as they showed that MEDs were increased immediately following application of calcipotriol ointment, but returned to baseline after 2 h.

Our vehicle control study shows that inhibition of erythema is not the result of the calcipotriol, as the same effect on the development of erythema was seen when only the vehicle was applied directly before UVB irradiation. In addition, the spectrophotometric analysis we performed showed that calcipotriol has no UV-absorbing properties in the UVB range. This is in agreement with Maier *et al.*, who showed that the maximal UV absorbance of purified calcipotriol is at a wavelength of 264 nm (UVC), and decreases with increasing wavelength and reaches zero at 360 nm.[13] The inhibition of UVB erythema, as seen when the calcipotriol cream/ointment is applied directly before UVB, must therefore be related to the vehicle. Alpha-tocopherol, which is an ingredient of the ointment, is a well-known photoprotective agent. This free radical scavenger is commonly used in cosmetics against harmful UVB. At this moment it is not clear whether other ingredients are also involved. Alternatively, just the fresh application immediately before UVB irradiation could also act as a

barrier. Several investigators have demonstrated in the past that emulsifying topicals containing, for instance, liquid paraffin (present in calcipotriol ointment/cream) have a sunscreen activity in clinically normal skin.[16,17]

The UVB dose needed to induce erythema shows considerable variation over the back, depending on the anatomical location.[18] In order to compensate for this, the horizontally distributed test sites on the subjects' backs were randomly allocated regarding timing of calcipotriol application to assure an even distribution of the application times in the study population as a whole. Furthermore, this assured the blinding of the investigator.

An additional clinically relevant outcome of this study is that no major photosensitivity reactions emerged. Photosensitization has been reported in patients treated with UVB phototherapy after addition of topical calcipotriol.[19] In our study, no such reactions were expected, as subjects who were included were not receiving any form of phototherapy. However, these results confirm that calcipotriol and UVB can be combined safely.

In conclusion, we have demonstrated a UVB-blocking effect of calcipotriol ointment/cream when applied immediately before irradiation. When combining UVB phototherapy with calcipotriol, neither calcipotriol ointment nor cream should be applied directly before irradiation as they will inhibit maximum UVB penetration. However, calcipotriol may be applied at any time up to 2 h prior to or immediately after UVB irradiation.

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