Topical Calcitriol is Degraded by Ultraviolet Light

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Calcitriol ointment has been approved for the treatment of psoriasis in many countries around the world. It may be prescribed in conjunction with phototherapy. Three double-blind, randomized, vehicle-controlled trials have recently been reported (Langner et al, 2001). Calcipotriene, the only vitamin D analog already approved for psoriasis in the USA, has been used successfully in combination with ultraviolet B (UVB) and psoralen plus UVA (PUVA) (Hecker and Lebwohl, 1997; Speight & Farr, 1994). In those studies, calcipotriene ointment was applied after treatment with phototherapy, as UV A has been shown to degrade calcipotriene (Lebwohl et al, 1997). Several other studies have suggested that calcipotriene ointment blocks UV light (Marsico and Dijkstra, 1996; Youn et al, 1997; De Rie et al, 2000).

Work conducted with other vitamin D analogs has shown significant interactions with UV light (Lebwohl et al, 1997). UV A, at 15–22 J per cm², reduced calcipotriene concentrations by 2%–75%, with a mean reduction of 28%. UVB in doses of 100–150 mJ per cm² in the latter study did not reduce the concentration of calcipotriene, but, using an in vitro model, narrowband UVB in doses ranging from 250 to 1000 mJ per cm² resulted in dose-dependent decreases in the concentration of 25-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3 (Lehmann et al, 1995).

Calcipotriene ointment has been shown to absorb UV light in the UV C and UVB ranges (Kornreich et al, 1995). As psoriasis patients are treated with several forms of UV light as well as topical therapy, we decided to examine the impact of UV irradiation on topical calcitriol, and the effect of calcitriol ointment on the transmission of UV light.

MATERIALS AND METHODS

Two grams of calcitriol 3 μg per g ointment was applied over a 40 cm² area and irradiated with 100 mJ per cm² of broadband UVB, 3.0 J per cm² of narrowband UVB, or 10 J per cm² of UVA. The light source for broadband UVB consisted of eight FS72 T12-UVB-HO bulbs. The UVA light source contained 36 F72 T12-BLHO bulbs, and the narrowband UVB source was a Phototherapeutics phototherapy unit containing 32 Philips TL01 bulbs. Control samples were exposed for 10 min to ambient light and fluorescent light bulbs.

Following irradiation, ointment was removed with a wooden spatula and placed in vials protected from light.

Apparatus Calcitriol and its related substances were assayed on a high performance liquid chromatography system including a Merck-Hitachi (Tokyo, Japan) pump model L-6200A, an Alltech (Deerfield, IL) detector model 2487, an Alltech (Deerfield, IL) column heater model 530, and a PC terminal equipped with Waters Millennium 3051 acquisition software.

Chemicals Calcitriol analytical reference standard was supplied by Solvay Pharmaceuticals (Weesp, The Netherlands).

Dichloromethane (HPLC grade) and iso-octane (analytical grade) were purchased from Merck (Darmstadt, Germany) and 2-ethoxyethanol (analytical grade) was obtained from Fluka (Buchs, Switzerland).

Procedure The liquid chromatograph was equipped with a 265 nm detector and a 4.6 mm x 250 mm Hypersil Si 5 μm column (Supelco, Bellefonte, PA) with a 3 x 10 mm Si Aquapore 5 μm precolumn (Alttech). The column temperature was maintained at 30°C. The mobile phase consisted of a mixture of dichloromethane, 2-ethoxyethanol, and iso-octane (275:45:680). The flow rate was 1.5 ml per min.

Sample preparations with a final calcitriol concentration of 0.48 μg per ml were obtained by dispersing an accurately weighed amount of ointment
into the mobile phase at 70°C for 10 min. A portion of the dispersion was centrifuged at 4000 rpm for 20 min and the clear supernatant was used for chromatography.

Standard preparations with a final calcitriol concentration of 0.48 μg per ml were obtained by dissolving an accurately weighed amount of calcitriol analytical reference standard into the mobile phase.

Equal volumes (200 μL) of the standard and sample preparations were separately injected into the chromatograph and the heights and areas of the calcitriol, precalcitriol, and trans-calcitriol peaks were measured.

The content in μg per g of calcitriol, including precalcitriol, was calculated using the formula

\[ I_{oa} \pm (f_1 I_{o2}) m_a V_a \times 1000 \]
\[ I_{oa} m_a V_a \]

in which \( I_{oa} \) and \( I_{o2} \) are the peak heights of calcitriol and precalcitriol, respectively, in the sample chromatogram, \( f_1 \) is the conversion factor of precalcitrol (2.23 × the ratio of the retention times of precalcitrol and calcitriol), \( I_o \) is the peak height of calcitriol in the standard chromatogram, \( m_w \) is the weighed amount of ointment in grams, \( m_o \) is the weighed amount of calcitriol analytical reference standard in milligrams, \( V_o \) and \( V_a \) are the dilution volumes in milliliters of the sample preparation and of the standard preparation, respectively, and 1000 is a factor to convert milligrams into micrograms.

The content in μg per g of trans-calcitriol was calculated using the formula

\[ I_{oa} m_o V_o \times 1000 \]
\[ I_{oa} m_o V_o \times f_2 \]

in which \( I_{oa} \) is the peak area of trans-calcitriol in the sample chromatogram, \( I_{oa} \) is the peak area of calcitriol in the standard chromatogram, and \( f_2 \) is the relative response factor of trans-calcitriol (1.25).

The effect of calcitriol on transmission of UVA and UVB was also assessed. Two grams of calcitriol 3 μg per g ointment was applied over a 40 cm² area of a clear film that allows transmission of UV (Saran Wrap) to create a thin film of calcitriol ointment. Four grams of calcitriol 3 μg per g ointment was applied over a 40 cm² area of the clear film to create a thick film of the ointment. The film with and without the two layers of calcitriol was held between UV bulbs and a spectroradiometer to quantify transmission of broadband UVB, narrowband UVB, and UVA. The light sources mentioned above were used. Transmission of UV light was recorded as a percentage of light transmitted through the film. According to previously published methods, transmission of UVB and UVA through thick film of the ointment. The film with and without the two layers of calcitriol, precalcitriol, and narrowband UVB reduced calcitriol to undetectable levels. In comparison, following exposure to broadband UVB, narrowband UVB, and PUV A are warranted, the results of this study suggest that calcitriol ointment should be applied following phototherapy, not immediately before.

At the time this study was performed, an application for an investigational new drug had not been filed for calcitriol ointment, and clinical studies could therefore not be performed. Applying in vitro data to clinical situations has significant limitations. Although calcitriol is degraded, it is always possible that degradation products might be active against psoriasis, and only clinical testing can absolutely refute or support the latter possibility. Moreover, other factors such as pH of the skin or skin surface temperature might affect degradation of calcitriol upon exposure to UV light. Again, these studies could not be performed because an application for an investigational new drug had not been filed. Both the observed instability of calcitriol when exposed to UV light sources and the reduction of UV transmission due to the vehicle would indicate that, if used in conjunction with phototherapy, this product should be applied after irradiation, not before. These observations should be confirmed by clinical trials, however.

### RESULTS

All three forms of UV light tested, which are used to treat psoriasis patients, resulted in degradation of over 90% of calcitriol (Table I).

UVA irradiation resulted in degradation of more than 98% of calcitriol. Broadband UVB irradiation resulted in degradation of 93% of calcitriol, and narrowband UVB reduced calcitriol to undetectable levels. In comparison, following exposure to broadband UVB, narrowband UVB, and PUV A are warranted, the results of this study suggest that calcitriol ointment should be applied following phototherapy, not immediately before.

### DISCUSSION

This study demonstrates similar effects of UVA, UVB, and narrowband UVB on calcitriol. All three light sources resulted in significant degradation of calcitriol. In addition, thin layers of calcitriol ointment do not significantly block transmission of UVA; application of thick layers of the ointment, however, may block both forms of UV light. Although studies examining the efficacy of calcitriol ointment in combination with UVB, narrowband UVB, and PUVA are warranted, the results of this study suggest that calcitriol ointment should be applied following phototherapy, not immediately before.

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### REFERENCES


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