Reduction of Epidermal Abnormalities and Inflammatory Changes in Psoriatic Plaques During Treatment With Vitamin D₃ Analogs

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Vitamin D₃ analogs interfere with various aspects of epidermal growth, inflammation, and cellular differentiation. Most data are derived from in vitro studies. In the present review, the in vivo effects of vitamin D₃ analogs on the psoriatic plaque are discussed. Calcipotriol, tacalcitol, and calcitriol in ointment modulate aspects of epidermal growth, differentiation, and inflammation. Immunohistochemical studies suggest that the inflammatory changes might be more expressed after treatment with calcitriol and tacalcitol. Flow cytometric quantification of the percentage of cells in S-G₂-M phase and of keratin 10-positive cells revealed that calcipotriol reduced both indices significantly during treatment of psoriatic plaques. Flow cytometric analysis of epidermal cell suspensions using triple labeling for epidermal proliferation, expression of keratin 10, and vimentin permits a quantitative assessment of DNA synthesis selectively in the basal cells of the epidermis, an estimation of the distribution of the basal and suprabasal compartments, and a quantification of the distribution of mesenchymal and nonmesenchymal cells. Using this approach, the interference of tacalcitol with growth control of basal cells was demonstrated. Remarkably, recumbentization of basal and suprabasal cells and mesenchymal and nonmesenchymal cells proved to be inconspicuous during this treatment. Key words: calcitriol/calcipotriol/tacalcitol.

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In the last decade, vitamin D₃ analogs have become an important approach in the treatment of psoriasis. Calcipotriol is available as a routine treatment in many countries (Binderup and Kragballe, 1992). In Japan, tacalcitol (la, 24-dihydroxyvitamin D₃) is a first line of treatment for psoriasis (Nishinura et al., 1993). Calcitriol (la,25-dihydroxyvitamin D₃) is the naturally occurring, active vitamin D₁ and has been investigated with respect to its antipsoriatic efficacy and safety (Langner et al., 1992).

The aim of the present review is to evaluate the actions of calcipotriol, tacalcitol, and calcitriol on psoriatic skin during topical treatment.

VITAMIN D₃ ANALOGS INHIBIT EPIDERMAL PROLIFERATION, ENHANCE NORMAL KERATINIZATION, AND MODULATE INFLAMMATION

Calcipotriol, calcitriol, and tacalcitol bind to the vitamin D receptor (VDR), and the complex binds to vitamin D response elements within promoter regions of vitamin D response genes (Ozone et al., 1991). VDR belongs to the steroid receptor superfamily together with the receptors for retinoids, estrogen, thyraxine, and glucocorticosteroids. Evidence is accumulating that the receptors within this family do not act in isolation, but interact. As such, the heterodimer formation of VDR and the RXR-α receptor is important because the heterodimer exerts a more potent binding with vitamin D response elements compared with VDR alone (Kliewer et al., 1992).

In addition to these nuclear mechanisms, vitamin D₃ analogs have a direct effect on calcium entry. Indeed at physiologic concentrations, calcitriol has been shown to induce calcium entry into keratinocytes (Brittener et al., 1991).

Either via nuclear mechanisms or by increased entry of calcium into the cell, vitamin D₃ enhances the production of inositol trisphosphate and 1,2-diacylglycerol (MacLaughlin et al., 1990). Another important effect of vitamin D₃ analogs in cell signaling is the translocation of protein kinase C from the cytosolic to the membrane position (Yada et al., 1989).

At the cellular level, vitamin D₃ analogs have been shown to inhibit proliferation of keratinocytes and to enhance cornified envelope formation (Binderup and Bramm, 1988; Kragballe and Wildfang, 1990). At the molecular level, vitamin D₃ analogs enhance transglutaminase activity, which is a crucial enzyme for cornified envelope formation (Hosomi et al., 1983; Lee et al., 1989; Matsunaga et al., 1990). In contrast to retinoids, vitamin D₃ analogs do not modulate the transcription of keratin genes. These compounds specifically interfere with the last step of differentiation (Regnier and Darmon, 1991).

Vitamin D₃ analogs have diverse effects on inflammation control. Interleukin (IL)-1-induced T-lymphocyte proliferation is inhibited by active vitamin D₃ (Tsoukas et al., 1984). Production of IL-2 and IL-6 by T lymphocytes and accumulation of mRNA for IL-2, interferon-gamma, and granulocyte-macrophage colony-stimulating factor increase in the T lymphocyte after incubation with calcitriol (Tsoukas et al., 1984; Gupta et al., 1989; Hustmyer et al., 1991; Lemire, 1992). Macrophages are activated by calcitriol, and...
### Table I. Monoclonal Antibodies Used for the Immunohistochemical Assessments

<table>
<thead>
<tr>
<th>Cell Biologic Feature</th>
<th>Antibody</th>
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<tr>
<td>Recruitment of cycling cells</td>
<td>Ki-67</td>
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<tr>
<td>Keratin 16 expression</td>
<td>K, 8.12</td>
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<tr>
<td>Involucrin</td>
<td>Mon-150</td>
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<tr>
<td>Filaggrin</td>
<td>Anti-filaggrin (BT 576)</td>
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<tr>
<td>Transglutaminase I</td>
<td>Anti-TG-ase I (IgG2 κ)</td>
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<td>Pan T cells</td>
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<tr>
<td>Polymorphonuclear leukocytes</td>
<td>Anti-elastase</td>
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<td>Monocytes (CD14)</td>
<td>WT 14</td>
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<tr>
<td>Langerhans cells</td>
<td>OKT 6</td>
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*The immunohistochemical procedures have been described before (de Jong and van de Kerkhof, 1991; Gerritsen et al, 1993, 1994, 1995).*

### Table II. Reduction of Epidermal Proliferation and Modulation of Keratinization and Inflammation During Treatment With Vitamin D₃ Analogs

<table>
<thead>
<tr>
<th></th>
<th>Calcipotriol</th>
<th>Calcitriol</th>
<th>Tacalcitol</th>
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<tr>
<td></td>
<td>(50 µg/g)</td>
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<th>Ki-67</th>
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<th>Monocytes</th>
<th>Langerhans cells</th>
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*Quantification of the responses has been described before (de Jong and van de Kerkhof, 1991; Gerritsen et al, 1993, 1994, 1995). Abbreviations: ↓, small decrease after treatment; ↓↓, slight decrease after treatment; ↓↓↓, marked decrease after treatment; ↑, slight increase after treatment; ND, not done.*
FLOW CYTOMETRIC QUANTIFICATION OF EPIDERMAL CHANGES DURING TREATMENT

To quantify epidermal hyperproliferation in the psoriatic plaque before and during treatment with calcipotriol and betamethasone, flow cytometric assessment was carried out of DNA distribution and keratin 16 expression (de Mare et al., 1990). This study, a group of 20 patients with chronic plaque psoriasis were treated twice daily with calcipotriol (50 μg/g) ointment and betamethasone valerate (0.05%) ointment in a double-blind trial with a left-right comparison. Before treatment and after a treatment period of 6 wk, razor-blade biopsy specimens (0.5 mm thick, 4 mm in diameter) were taken from two lesions at both sides. Epidermal cell suspensions were prepared using a trypsinization procedure, as described previously (Bauer et al., 1980). To assess keratin 16 expression, we incubated the cell suspensions with the monoclonal antibody Ks 8.12 and fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG as second antibody (de Mare et al., 1990). Propidium iodide was added to measure relative DNA content per cell (de Mare et al., 1990). Five thousand cells of each sample were analyzed using an Ortho SEH flow cytometer equipped with a 5-W argon laser tuned at 488 nm. Both treatments induced similar reductions of these indices. The values, however, were still well above the normal ranges (percentage Ks 8.12 cells: 1.8 ± 1.0% [mean ± SEM] [van Erp et al., 1989] and percentage cells in SG2M phase: 4.0 ± 0.7 [Bauer et al., 1981]).

Although this approach permits quantification of proliferation characteristics in epidermal cells, the methodology is complicated by the fact that suprabasal cells and mesenchymal cells are not excluded from the analysis.

Recently, a new methodology was developed using a triple labeling approach. Per-cell DNA content was measured using the new DNA stain TO-PRO-3 iodide (TP3); anti-vimentin was used to identify all nonkeratinocytes (infiltrate cells and other mesenchymal cells); RKSE 60 staining was carried out to assess keratin 10 expression, which indicates whether a cell belongs to the suprabasal cell population. Cell suspensions were prepared as described before, and the staining procedures have been described previously (Bauer and Boezeman, 1983; van Hooijdonk et al., 1995). The second step of the indirect immunofluorescent staining was performed with monoclonal goat antibodies against mouse IgG2a and IgG2b, conjugated to phycoerythrin and fluorescein-isothiocyanate (FITC), to assess RKSE 60 binding and anti-vimentin binding, respectively. Phycoerythrin and FITC were excited with an air-cooled argon laser (633 nm), and TP3 was excited with an HeNe laser (488 nm). After electron compensation for spectral overlap, which was minimal in the case of TP3, fluorescence was measured using band-pass filters at 525 nm (green FITC), 575 nm (orange, phycoerythrin), and 675 nm (red, TP3). The ratio of area to peak of IgG2a and IgG2b was calculated. In total, 20 patients participated in a left-right comparative study between calcipotriol (4 μg/g) in ointment and the ointment base only (Glade et al., 1995). Applications were done once daily. Razor-blade biopsy specimens were taken before treatment and after 8 wk of treatment from two lesions at both sides. Cell suspensions were prepared, and triple-label flow cytometric assessment was carried out according to the methods described above. The sides treated with tacalcitol showed a mean reduction of the psoriasis area severity score of 48%. The psoriasis area severity score of the lesions treated with placebo ointment was decreased by 28%.

In the psoriatic lesions before tacalcitol treatment, the percentage of basal cells in SG2M phase was 20.0 ± 1.9% (mean ± SEM); after 8 wk of treatment, the percentage was reduced to 13.2 ± 1.1%. This reduction was statistically significant (p < 0.01). At the placebo-treated sides, the pretreatment value was 17.8 ± 1.8%, and the value after 8 wk of placebo treatment was 15.1 ± 1.2%.

The pronounced reduction of this cell-cycle kinetic marker during tacalcitol treatment illustrates the potent effect of tacalcitol on epidermal hyperproliferation in vitro. Triple-label flow cytometry has proved to be an adequate tool to restrict the analysis of indices for epidermal proliferation to the basal cells, even within a population as heterogeneous and complex as the inflamed epidermis of the psoriatic lesion.

I would like to acknowledge the secretarial support of Mrs. T. van San-Verschoten.

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VITAMIN D3 AND THE PSORIATIC LESION

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