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Biological activity of vitamin D analogues in the skin, with special reference to antipsoriatic mechanisms

P.C.M. VAN DE KERKHOF
Department of Dermatology, University Hospital Nijmegen, the Netherlands
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Summary
Active vitamin D₃ modulates epidermal growth, keratinization and inflammation, and various vitamin D₃ analogues have been shown to be effective in the treatment of psoriasis. These analogues now provide a useful addition to the therapeutic modalities available for the treatment of psoriasis. Epidermal hyperproliferation, abnormal keratinization and inflammation are the well-established hallmarks of the psoriatic plaque. The aim of this review is to provide an update of information on the cell biological effects of vitamin D₃, and the influence of vitamin D analogues on the pathomechanisms of psoriasis.

Vitamin D₃ is absorbed from the gut, and is also synthesized in the skin from 7-dehydrocholesterol following exposure to ultraviolet light (290–300 nm). It is activated by hydroxylation. The classic target organs of vitamin D₃ are the gut, bones and kidneys.

Several vitamin D₃ analogues have been shown to be effective in the management of psoriasis.

General cell biological effects
Vitamin D₃ can be 'activated' by hydroxylation at the 1α and 25 positions. Various vitamin D₃ derivatives modulate the biological functions of many cell types. Two basic mechanisms are responsible for the action of these hormones: a nuclear mechanism involving modification of gene transcription, and a non-nuclear mechanism involving signalling.

Nuclear mechanisms
Active vitamin D₃ binds to a nuclear receptor, the vitamin D₃ receptor (VDR). This receptor is a ligand-inducible transcriptional regulatory protein. The complex of VDR and active vitamin D₃ modulates the transcription of target genes by binding to specific DNA binding sites designated 'vitamin D response elements'. Vitamin D response elements are present in the promoter region of target genes of 1α,25-dihydroxyvitamin D₃ (1α,25-(OH)₂D₃).

The vitamin D receptor has been cloned and sequenced. This receptor demonstrates structural homology with known steroid receptors: glucocorticoid receptor, oestrogen receptor, thyroid receptor and retinoic acid receptors. These receptors belong to the nuclear receptor superfamily. The receptor has a domain which binds to the steroid, another which interacts with other members of the nuclear receptor superfamily, and a DNA binding domain. The vitamin D receptor is a high-affinity receptor for 1α,25-(OH)₂D₃, and many cell types possess this high-affinity receptor. The DNA binding domain of the nuclear receptor superfamily is characterized by two zinc finger-like motifs. The DNA response elements to which these receptors bind are similar in sequence and organization. Evidence is accumulating that the vitamin D₃ is communicating vitamin D₃-mediated signals in concerted action with the other members of the nuclear receptor superfamily. For this interaction between members of the steroid receptor superfamily, a novel structural domain has been identified. This is conserved within the members of the nuclear receptor superfamily, and consists of a helix-turn-zipper which may function in transactivation of cognate genes. Interaction with the retinoic-X receptor-alpha (RXR-α) has been reported by several authors. RXR-α interacts directly with, and enhances the binding of, nuclear receptors conferring responsiveness to vitamin D₃ and thyroid hormone. RXR-α is a dimerization partner for the vitamin D₃ receptor. It has been postulated that RXR-α enhances tight binding of VDR with vitamin D response elements. Via this principle, cross-talks are possible between the ligands of the steroid receptor superfamily. The expression of the high-affinity vitamin D receptor is susceptible to various control
mechanisms. It has been demonstrated in mouse fibroblasts that the vitamin D$_1$ receptor is upregulated by calcipotriol and 1$\alpha$,25(OH)$_2$D$_3$.

The expression of the vitamin D receptor has been shown to be 4-5-fold increased in rapidly proliferating endothelial cells, compared with density arrested cells, which suggests that the expression of the receptor can be modulated by factors involved in growth control. The vitamin D receptor has been shown to be upregulated by protein kinase C activation, and this phenomenon was prevented by sphingosine.

Various DNA response elements are available for the members of the nuclear receptor superfamily. The vitamin D$_3$ response elements differ considerably in their homology to each other. Vitamin D$_3$ response elements are characterized by a tandem repeating oligonucleotide sequence of six base pairs (bp). Selectivity for vitamin D$_3$ within the nuclear receptor superfamily is determined by half-site spacing of the response elements. Decreasing the half-site spacing of the response element for thyroid hormone will convert it to a vitamin D$_3$ response element, whereas increasing the half-site spacing will convert it into a retinoic acid response element. Recently, two classes of vitamin D response elements have been identified which are activated by the vitamin D receptor alone or by heterodimers of the vitamin D receptor and RXR-$\alpha$.

**Transmembrane signalling**

Active vitamin D$_3$ has been shown to induce an increased influx of calcium into the cell via a non-nuclear mechanism. The rise of the calcium concentration within the keratinocytes has been shown to occur at physiological concentrations of 1$\alpha$,25(OH)$_2$D$_3$ ($10^{-11} - 10^{-9}$ M). The accumulation of calcium in the keratinocyte occurs within 90s of the addition of 1$\alpha$,25(OH)$_2$D$_3$ to the culture medium. This timing is not compatible with a nuclear mechanism. The effect of 1$\alpha$,25(OH)$_2$D$_3$ on calcium metabolism is not inhibited by cycloheximide, which indicates that protein synthesis is not involved. In keratinocyte cultures, 1$\alpha$,25(OH)$_2$D$_3$ has been shown to enhance rapid hydrolysis of phosphatidyl inositol phosphate, which results in an increase of the intracellular concentration of calcium.

**Intracellular signalling**

The nuclear effects of active vitamin D$_3$, and the more immediate induction of a transmembrane calcium influx, result in a modulation of intracellular signalling. It has been shown that 1$\alpha$,25(OH)$_2$D$_3$ enhances the production of inositol trisphosphate and 1,2-diacylglycerol, and induces an increase of intracellular calcium. Vitamin D$_3$ promotes the translocation of protein kinase C from the cytosolic to the membrane position. It is difficult to define to what extent the nuclear mechanisms or the transmembrane signalling are responsible for these effects.

The physiological relevance of protein kinase C as an intermediary stage between vitamin D$_3$ action and epidermal differentiation is evident from the observation that inhibition of protein kinase C causes marked suppression of 1$\alpha$,25(OH)$_2$D$_3$-induced formation of cornified envelopes.

An increase of the intracellular calcium concentration has various biochemical effects. Increasing the extracellular calcium concentration in keratinocyte cultures results in keratinization. An increase of the intracellular calcium concentration occurs during differentiation of these cells.

Many cell types respond to vitamin D$_3$. In this review, the cell types involved in the pathogenesis of psoriasis are highlighted.

**Interference with keratinocytes**

Active vitamin D$_3$ inhibits proliferation of keratinocytes in culture. Incorporation of $^3$H-thymidine in DNA is inhibited by 1$\alpha$,25(OH)$_2$D$_3$ at concentrations equal to or higher than 1$\times$10$^{-10}$ M. 1$\alpha$,25(OH)$_2$D$_3$ has also been reported to inhibit growth of malignant cell lines. A 3-day incubation of NIH:OVCAR 3 cells with 100 nmol 1$\alpha$,25(OH)$_2$D$_3$ resulted in 49% inhibition of cell growth. An antitumour effect has also been shown in breast carcinoma cells, and squamous carcinoma and HL-60 cell lines. The 1-hydroxymethyl-25-hydroxyvitamin D$_3$ homologues proved to have anti-proliferative activity on murine keratinocytes similar to that of 1$\alpha$,25-dihydroxyvitamin D$_3$. However, these homologues were less than 0-1% as effective as vitamin D$_3$ in binding to the vitamin D$_3$ receptor.

Based on these in vitro observations, it can be concluded that the growth inhibitory effect of active vitamin D$_3$ is not a direct reflection of affinity for the vitamin D$_3$ receptor. The bioavailability of the vitamin D$_3$ analogue is one explanation for this discrepancy. Some analogues may have different clearance rates in vivo. An alternative explanation is that some analogues act more specifically on the plasma membrane than on the nuclear pathway.

Epidermal differentiation is enhanced by active vitamin D$_3$ in keratinocyte culture. Cornified envelope
formation and transcription of transglutaminase are enhanced by 1α,25(OH)₂D₃ at concentrations of 10⁻¹⁰ M or higher. Human keratinocytes grown on de-epidermized dermis are able to reconstruct a morphologically normal stratified and keratinizing epidermis. It has also been shown that the stratum corneum is thicker under the influence of 1α,25(OH)₂D₃. A prominent reduction of the intermediate differentiation compartment was observed, and it was shown that this reduction is not due to a block of the progression of basal cells. In air–liquid interface culture, 1α,25(OH)₂D₃ was shown to increase the number of stratum corneum layers, and to reduce the water permeation. The interference with keratinization is different from the effect of retinoic acid. In contrast with the suppression of the transcription of keratins 3, 5, 10, 14 and 16 by retinoic acid, 1α,25(OH)₂D₃ does not induce such a suppression. 1α,25(OH)₂D₃ induced transglutaminase activity in transformed epidermal cells (PAM 212), and this effect was shown to be synergistic with the induction of transglutaminase by retinoic acid. 1α,25(OH)₂D₃ and retinoic acid induce transglutaminase via separate mechanisms.

1α,25(OH)₂D₃ increases entry of calcium into the keratinocyte at concentrations between 10⁻¹¹ and 10⁻⁹ M. Such a concentration is sufficient for the induction of modulation of keratinization. Indeed, increases of intracellular calcium are associated with keratinization. In this respect, it is of relevance that transglutaminase is calcium-dependent. Via the nuclear pathway, 1α,25(OH)₂D₃ enhances the production of inositol trisphosphate and 1,2-diacylglycerol, and also enhances the transcription of protein kinase C. Activation of protein kinase C is associated with enhanced keratinization. The molecular interphase between 1α,25(OH)₂D₃ activation and the antiproliferative action remains to be elucidated. A dissociation between antiproliferative action and affinity for the vitamin D₃ receptor exists for various new vitamin D₃ analogues.

Recently, it has been demonstrated in HL-60 cells that pretreatment with 1α,25(OH)₂D₃ renders these cells more resistant to apoptosis. Calcium influx into the cell plays an important role in events culminating in apoptosis, and a high level of expression of proteins which buffer fluxes of calcium can effectively block death in apoptosis-susceptible cells. Although still highly speculative, it is attractive to postulate that calcium entry into cells, as induced by vitamin D₃, might be a crucial factor in the induction of apoptosis.

As the process of keratinization might be regarded as apoptosis, further studies on the effect of vitamin D₃ on the modulation of apoptosis in keratinocytes are indicated.

Modulation of inflammation

Active vitamin D₃ interferes with various aspects of inflammation. Again, nuclear and non-nuclear mechanisms are involved.

1α,25(OH)₂D₃ inhibits interleukin-1-induced T-cell proliferation and the production of immunoglobulins by these cells. The production of interleukin 2 and interleukin 6 by T cells is also inhibited by 1α,25(OH)₂D₃. Both 1α,25(OH)₂D₃ and cyclosporin inhibit interleukin 2 production by CD4⁺ cells. These compounds have been shown to have a synergistic effect, which implies that immunomodulation by cyclosporin is different from immunomodulation by 1α,25(OH)₂D₃. At the molecular level it has been shown that 1α,25(OH)₂D₃ increases accumulation of mRNA for interleukin 2, interferon gamma (IFN-γ), and granulocyte-macrophage colony-stimulating factor. At a cellular level, suppressor cell activity is promoted, and the generation of cytotoxic and natural killer cells is inhibited. In mice, it has been shown that topical application of 1α,25(OH)₂D₃ increases contact hypersensitivity at the elicitation site.

1α,25(OH)₂D₃ increases cytotoxicity of macrophages. Interferon alpha (IFN-α) and 1α,25(OH)₂D₃ have a synergistic effect on the proliferation of monocytes–macrophages (U 937 cells). IFN-α increases the expression of the vitamin D₃ receptor in U 937 cells. 1α,25(OH)₂D₃ down-regulates HLA-DR expression on human peripheral blood monocytes. 1α,25(OH)₂D₃ promotes increased expression of CD14 but decreased expression of CD23. Retinoic acid has the opposite effect. IFN-γ augments functional and phenotypic characteristics of vitamin D₃-induced monocytoid differentiation in the U 937 human leukemic cell line. 1α,25(OH)₂D₃ down-regulates transferrin receptor expression and up-regulates the mannose–glucose receptor.

In myeloid cells, calcium-binding proteins (MRP₈ and MRP₁₄) are synthesized under specific conditions of myeloid cell differentiation. 1α,25(OH)₂D₃ enhances the expression of the genes encoding for these calcium-binding proteins. Platelet-activating factor is a potent lipid mediator of inflammation. In HL-60 cells, platelet-activating factor receptor gene expression is enhanced by 1α,25(OH)₂D₃. 1α,25(OH)₂D₃ has been demon-
stratified to inhibit proliferation and enhance differentiation in the HL-60 line.\(^{31,53-55}\)

In keratinocytes, interferon gamma induces the expression of the HLA-DR antigen, and 1α,25-(OH)\(_2\)D\(_3\) significantly decreases the IFN-γ-induced HLA-DR expression.\(^{56}\)

The release of arachidonic acid by polymorphonuclear leucocytes is inhibited by 1α,25-(OH)\(_2\)D\(_3\),\(^{57}\) which also inhibits migration of these cells.\(^{58}\)

**Vitamin D\(_3\) analogues**

**Calcipotriol**

Calcipotriol and 1α,25-(OH)\(_2\)D\(_3\) are comparable with respect to receptor binding, inhibition of epidermal proliferation, and modulation of keratinization and inflammation. However, there is a striking difference between calcipotriol and 1α,25-(OH)\(_2\)D\(_3\) with regard to interference with systemic calcium metabolism. Compared with 1α,25-(OH)\(_2\)D\(_3\), calcipotriol is rapidly metabolized into inactive compounds. From animal experiments it has been concluded that calcipotriol is 100–200 times less active than 1α,25-(OH)\(_2\)D\(_3\) with regard to induction of hypercalcaemia.\(^{58}\)

1α,24-dihydroxyvitamin D\(_3\) (tacalcitol)

1α,24-dihydroxyvitamin D\(_3\) [1α,24-(OH)\(_2\)D\(_3\)] has an increased receptor affinity compared with 1α,25-(OH)\(_2\)D\(_3\).\(^{59}\) The inhibition of proliferation of keratinocytes by 1α,24-(OH)\(_2\)D\(_3\) has been reported to be slightly greater than 1α,25-(OH)\(_2\)D\(_3\), whereas the enhancement of cornified envelope formation proved to be comparable.\(^{60}\) Although 1α,24-(OH)\(_2\)D\(_3\) has been reported to have no effect on the polymorphonuclear leucocyte,\(^{60}\) 1α,24-(OH)\(_2\)D\(_3\) and 1α,25-(OH)\(_2\)D\(_3\) proved to be equipotent with respect to the inhibition of the antibody response to the T-cell-dependent antigen.\(^{61}\) 1α,24-(OH)\(_2\)D\(_3\) proved to have less effect on systemic calcium metabolism than 1α,25-(OH)\(_2\)D\(_3\).\(^{62}\)

1(S), 3(R)-dihydroxy-20(R)-(4\(^{1}\)-hydroxy-4\(^{1}\)-ethyl-1\(^{1}\)-hexyloxy-9, 10-secopregna 5 (2), 10(1g)-triene (KH 1060)

This derivative has a pronounced immunosuppressive effect. In mice, KH 1060 suppressed skin-graft rejection,\(^{62}\) and also proved to be a potent immunosuppressive agent in HgCl\(_2\)-induced autoimmune nephritis in rats.\(^{63}\)

**Derivatives with a preferential interference**

Recently, new analogues have become available with a predilection for either non-nuclear or nuclear mechanisms.\(^{64-66}\)

Some analogues bind poorly to the vitamin D receptor but strongly stimulate the influx of calcium into the cell.\(^{57}\) Pertussis toxin, which interferes with coupling of certain ligand-gated receptors to ion channels, failed to block activation of calcium channels by vitamin D\(_3\). Selectivity, with only limited binding to the vitamin D\(_3\) receptor, has been demonstrated for the following analogues: 25-hydroxy-16 ene-23 yne D\(_3\) and 25-hydroxy-23 yne D\(_3\).

1,25-dihydroxy-16 ene-23 yne-vitamin D\(_3\) is four times more effective than 1,25-(OH)\(_2\)D\(_3\) with regard to inhibition of clonal proliferation of HL-60, EM-2, and U 937 myeloid cell lines, and induction of differentiation of HL-60 promyelocytes. This analogue has been shown to decrease the expression of the c-myc oncogene by 50% within 10 h. The analogue is 60% as effective in binding to the receptor of HL-60 cells, and is markedly less calcipotropic.\(^{55}\) This analogue markedly prolongs the survival of leukaemic mice. 1-hydroxymethyl-25-hydroxy vitamin D\(_3\) analogues have an antiproliferative effect comparable with 1α,25-(OH)\(_2\)D\(_3\). However, these analogues have less than 0.1% affinity for the vitamin D receptor than 1α,25-(OH)\(_2\)D\(_3\).\(^{30}\) 22-oxa-1,25-(OH)\(_2\)D\(_3\) has a decreased vitamin-D-receptor affinity compared with 1α,25-(OH)\(_2\)D\(_3\).\(^{68}\) This derivative has a more marked antiproliferative and differentiation enhancing potential than 1α,25-(OH)\(_2\)D\(_3\).\(^{25}\) The 22-oxa derivative has been shown to have a potent immunosuppressive effect.\(^{67}\)

Some analogues bind well to the vitamin D receptor, but display little or no activity in opening the transmembrane calcium channels.\(^{67}\) Such selectivity is a characteristic of the analogues 1,24-dihydroxy-22-ene-24 cyclopropyl D\(_3\) and 1,25-16 ene-23 yne, 26, 27F6-D\(_3\). The latter compound has been shown to have an 80-fold increased inhibition of HL-60 cells compared with 1α,25-(OH)\(_2\)D\(_3\).\(^{31}\) In HL-60 cell lines, it has been shown that the differentiation induced by the 26, 27-dialkyl analogues correlates well with receptor binding.\(^{53}\)

It has been shown that specific side-chain modifications affect the differentiation-inducing capacity without affecting anti-proliferative potency.\(^{66}\) Therefore, it may be possible to dissociate the antiproliferative activity from the differentiation-inducing activity by modification of the 1α,25-(OH)\(_2\)D\(_3\) molecule.
**In vivo effects**

Epidermal proliferation, abnormal keratinization, and cutaneous inflammation are key features of the psoriatic lesion. Vitamin D₃ analogues have been shown to modulate these processes *in vitro*. However, the extent to which this is relevant to the antipsoriatic efficacy remains to be elucidated. Several studies have been carried out to assess changes of epidermal growth, differentiation and inflammation during treatment of psoriatic plaques with vitamin D₃ analogues.

During treatment of psoriatic plaques with calcipotriol ointment (50 µg/g), it was shown that the accumulation of polymorphonuclear leucocytes decreases during the first week of treatment; the number of cycling epidermal cells showed a reduction after 2 weeks’ treatment; after 4 weeks' treatment the number of keratin-16-positive cells and the number of T lymphocytes decreased significantly.⁶⁹ The number of CD14 cells and Langerhans cells remained unaffected during treatment with calcipotriol.⁶⁹ A quantification of these changes by flow cytometry revealed a highly significant reduction of the percentage of cells in SG₂/M phase, and a highly significant reduction of the number of keratin-16-positive cells. However, even in those patients who showed complete clinical resolution of the psoriatic plaques, the percentages of keratin-16-positive cells and cells in SG₂/M phase remained well above the normal range.⁷⁰ During treatment of psoriatic plaques with calcipotriol, a reduction of the number of keratin-16- and keratin-17-positive cells, and a normalization of the number of keratin-5- and keratin-10-positive cells has been shown.⁷¹–⁷³ However, calcipotriol treatment of psoriatic plaques did not result in a modification of ornithine decarboxylase activity.⁷⁴ During treatment with calcipotriol a reduction of helper T cells and a preponderance of suppressor T cells was induced.⁷⁵ Most studies agree that calcipotriol induces pronounced changes in epidermal behaviour, leaving the mononuclear infiltrate largely unaffected.⁷⁶ However, one study suggested a relative persistence of the epidermal phenomena.⁷⁴ Compared with the effects of betamethasone valerate, calcipotriol induced a reduction of epidermal growth and differentiation characteristics to the same extent.⁷⁰ During treatment of psoriatic plaques with calcipotriol, a decline of staining intensity of interleukin 6, but not of tumour necrosis factor-α, was observed in lesional and clinically uninvolved skin.⁷⁷

Studies have been carried out during treatment with 1α,25-(OH)₂D₃ ointment (3 µg/g) and 1α,24-(OH)₂D₃ ointment (4 µg/g). Changes similar to those seen during calcipotriol application were observed during these treatments.⁷⁸–⁸¹ Remarkably, during treatment with 1α,25-(OH)₂D₃ and 1α,24-(OH)₂D₃, a reduction of the accumulation of T lymphocytes and monocytes was seen, in contrast with the inconspicuous changes induced by calcipotriol.⁷⁹,⁸⁰ Systemic treatment of psoriatic patients with 1α-hydroxyvitamin D₃ resulted in inhibition of keratinocyte proliferation and a reduction of keratin 16 expression.⁸²

It can be concluded that a reduction of epidermal proliferation and a decrease in the accumulation of polymorphonuclear leucocytes are the most prominent changes during treatment of psoriatic plaques with vitamin D₃ analogues.

**Conclusions and future prospects**

Vitamin D₃ analogues interfere, via nuclear and non-nuclear mechanisms, with epidermal proliferation, differentiation and cutaneous inflammation. Calcipotriol, 1α,25-(OH)₂D₃ and 1α,24-(OH)₂D₃ have been shown to be effective in the treatment of psoriasis. *In vitro* studies have revealed that the biological effects of vitamin D₃ analogues are substantial and diverse. *In vivo* studies on the mode of action of vitamin D₃ during treatment of psoriatic plaques have revealed that an influence on epidermal proliferation and accumulation of polymorphonuclear leucocytes is an early effect, whereas the modulation of the number of T cells, monocytes and macrophages are late effects. Following clinical clearance of psoriatic plaques by vitamin D₃ analogues (calcipotriol, 1α,25-(OH)₂D₃ and 1α,24-(OH)₂D₃) the cell biological markers are reduced, but are still abnormal. A limitation of treatment with calcipotriol is irritation of the skin in 20% of patients.

The future of vitamin D₃ analogues is bright. New analogues are available which have selective activity with regard to nuclear and non-nuclear mechanisms of action. Separation of antiproliferative and calcitropic potential has been achieved *in vitro* and in animal models.

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**References**

75 Verbergh CA, Nieboer C. Local application of vitamin D derivative MC903 in psoriasis: influence on cellular infiltrate, Langerhans


