**REVIEW**

Paracrine role for calcitriol in the immune system and skin creates new therapeutic possibilities for vitamin D analogs

R Bouillon, M Garmyn, A Verstuyf, S Segaert, K Casteels and C Mathieu

*Laboratory of Experimental Medicine and Endocrinology, and Division of Dermatology, Catholic University of Leuven, Belgium*

The role of vitamin D in calcium metabolism has been recognized and studied since the beginning of the century. After its production in the skin or its intestinal absorption, cholecalciferol needs metabolic activation in the liver and kidney. The production of the active hormone, calcitriol, is tightly feedback-regulated and is one of the key regulators of plasma calcium/phosphate and bone homeostasis through genomic (in)activation of a number of genes in its target tissues (intestine, bone, kidney, parathyroid gland). Indeed, calcitriol exerts its effects via a specific vitamin D receptor (VDR), a member of the superfamily of steroid/thyroid/vitamin A hormone receptors (1), leading to gene regulation mediating various biological responses. Recently, interactions between the VDR and one of the retinoid acid receptors, retinoid X receptor (RXR), also a member of the same superfamily of steroid receptors, have been described (2, 3). These interactions, namely the formation of heterodimers between activated VDRs and RXRs, is probably an important step in calcitriol-dependent gene activation. The presence of the natural ligand for RXR, 9-cis retinoic acid, appears not to be necessary to allow, and can even inhibit, this heterodimer formation or its transactivation activity (4). Receptors for calcitriol (i.e. VDRs) are not confined to these classical target tissues (intestine, bone, kidney, parathyroid glands) and other organs with known high calcium transport function (mammary glands, placenta, egg shell) but can also be found in a wide variety of “non-classical” target tissues such as several endocrine cells (e.g. endocrine pancreas), muscle cells, normal or malignant keratinocytes, breast cancer or prostatic cells and cells belonging to the immune system (circulating T lymphocytes (activated), circulating B lymphocytes and monocytes/macrophages).

Apart from the near-universal presence of vitamin D receptors, most cells are, in addition, capable of metabolizing calcidiol or its further metabolites. Although this widespread intracellular metabolism was initially thought of as part of the catabolism, it is now clear that several tissues are also able to convert calcidiol into calcitriol by a specific 1α-hydroxylase that may be different but is certainly regulated differently compared to the renal 1α-hydroxylase. These cells (keratinocytes, bone, placental and brain glia cells, monocytes/macrophages and pneumocytes) can all produce or be induced to synthesize calcitriol, probably only for regional distribution and not for systemic secretion. As these cells locally synthesize calcitriol, possess a specific receptor and are responsive to its action, a paracrine function for calcitriol can be postulated in these tissues. An unequivocal proof for a physiological paracrine/cytokine-like function of calcitriol is difficult to provide in vivo because cytokine functions nearly all have a large degree of redundancy.

A paracrine function creates the possibility of pharmacological manipulation of the humoral factor to enhance its activity or selectivity profile. Because the vitamin D hormone is an unusually flexible molecule in comparison with other steroid hormones (due to its open B ring and long side chain), a large number of analogs have been synthesized and indeed several superagonists (10 to 100-fold more potent than the natural hormone) have been detected. Moreover, several analogs show clear dissociation of their activity profile in that their effects on the immune system or on cell differentiation largely exceed their calcemic activity (10 to 100-fold difference in activity ratio) (5, 6). The molecular basis for this dissociated (super)agonist activity profile is still only partially understood but differences in pharmacokinetics, intracellular metabolism and intrinsic transactivation potency all contribute to their spectrum of activity (4, 6–8). The present review does not intend to describe fully the wide diversity of action of vitamin D as a calcitropic hormone or as a pluripotent factor, or to describe the wide spectrum of vitamin D analogs or its molecular mechanism of action, because for each topic excellent recent reviews are available (4, 6, 8–10). We, however, selected to discuss two aspects of the non-calcemic action profile of vitamin D that are closest to clinical applications: its immunomodulatory role and its actions in skin.

**Immunomodulatory role of calcitriol**

The immune system consists of a complex network of cells, communicating through different pathways (e.g. cell-to-cell contact and cytokines). This network ensures the protection of the body (self) against all foreign invasions (non-self) (e.g. infectious agents, cancer cells, transplanted organs, autoantigens in autoimmunity). In the last decade it has been well established that calcitriol may play a role in the
communication within this immune system and may therefore be a cytokine-like molecule (11–13). Indeed, activated macrophages, the cells with the most central role in the immune system, possess the 1a-hydroxylase enzyme that allows for the production of calcitriol (14). This is seen in the hypercalcemia and elevated calcitriol levels that can be found in granulomatous diseases (e.g., sarcoidosis) (15–16). On the other hand, receptors for calcitriol can be found on almost all cell types of the immune system, and calcitriol affects the function of most of these cell types in vitro (11, 14, 17). The VDR present in immune cells is identical to the intestinal receptor and up to now there is no evidence for a second VDR or alternative gene splicing. Finally, severe immune disturbances are seen in rickets or other vitamin D-deficient situations, such as hemodialysis; in particular, defective antimicrobial monocyte/macrophage function is evident and can be corrected by treatment with calcitriol (18).

**In vitro immune effects of calcitriol**

Monocytes/macrophages express VDRs constitutively. Studies on monocytes (normal human monocytes, as well as monocytic leukemic lineages) have demonstrated first of all a clear stimulation by calcitriol of the differentiation of immature monocytes towards mature macrophages (12, 19, 20). Whereas monocytes derived from rachitic subjects show impaired antimicrobial function, addition of calcitriol can restore this defect (18). The antimicrobial function of macrophages taken from normal subjects is also stimulated by calcitriol: adherence, chemotaxis, phagocytosis and killing of bacteria are enhanced (21–23). Moreover, calcitriol increases heat shock protein production and protects cells from thermal injury (24). Cytotoxicity, H$_2$O$_2$ and oxygen radical production are stimulated, as is the fusion of alveolar macrophages (11). Interestingly new data demonstrate that the defective migration and killing of mycobacteria by monocytes in AIDS patients is also enhanced by calcitriol, but data on intracellular killing of the HIV virus itself in the monocytes are inconclusive (25–28).

Findings on the effects of calcitriol on the accessory cell function of macrophages are more diverse (31–34). This confusion is partly due to the fact that some papers reporting enhanced antigen-presenting capacity, HLA Class II expression or monokine production (i.e. IL-1), mostly studied monocytic leukemia lines, where calcitriol induces differentiation of the very immature leukemic cells towards mature monocytes/macrophages (34). Through this differentiation, accessory cell function is increased but remains low compared to normal monocytes. Papers dealing with normal peripheral monocytes instead indicate an inhibition of accessory cell function, with down-regulation of antigen-presenting capacity, lower levels of HLA Class II antigen expression and inhibition of monokine production (IL-1, IL-6, TNF-α) (33, 35). Of particular interest are the inhibitory effects of calcitriol on IL-12 production by monocytes (and B lymphocytes), because this cytokine plays an important role as a co-stimulatory signal in lymphocyte activation (36).

In contrast to monocytes, resting T lymphocytes do not express VDRs, although at a certain maturational stage lymphocytes residing in the thymus (thymocytes) do possess VDRs (14). Upon activation, all T lymphocytes express VDRs and become sensitive to calcitriol. Although it has been well established that T cells can be direct targets for calcitriol (37, 38), it is clear that in reality the effects on T lymphocytes are largely mediated through its effects on monocytes/macrophages as antigen-presenting cells (39–42). Calcitriol inhibits T lymphocyte proliferation induced by different stimulatory agents, such as antigen (mixed lymphocyte reaction), anti-CD3 or lectin stimulation, by preventing progression from early to late G1 phase in the cell cycle (11). Inhibition of cell proliferation is at least partially mediated by inhibition of the production of the autocrine lymphokine IL-2 by the T lymphocytes themselves, because the effects of calcitriol on T-cell proliferation can be abrogated partly by the addition of IL-2 (12). Also, the production of other lymphokines (IFN-γ, TNF-α) is down-regulated by calcitriol (43, 44). Recently, we have demonstrated that additive inhibition of T-cell proliferation and cytokine production is induced by a combination of calcitriol with more classical inhibitors of T-cell function, such as cyclosporin A (CyA), FK506 and rapamycin (45). These cooperative effects between calcitriol (or its analogs) and classical immunosuppressants could be confirmed by us and others in several in vivo models of autoimmunity and tissue transplantation (46–48) (Bouillon, personal data on experimental allergic encephalitis).

Both subpopulations of T lymphocytes (CD4 and CD8 cells) carry VDRs, but CD4 cells appear to be the preferential target for calcitriol (37, 44, 49, 50). In recent years, two distinct functional CD4 cell types have been identified: TH1 and TH2 cells (51). While TH1 cells represent cells producing preferentially IL-2 and IFN-γ and stimulating the cellular immune system, TH2 cells preferentially secrete IL-4 and IL-10 and inhibit TH1 function, while they stimulate the humoral immune system (i.e. IgE production). TH1 cells are considered the main deleterious effector cells in many autoimmune diseases and organ rejection, while TH2 cells behave more as regulatory cells because they inhibit TH1 cells. Lemire demonstrated recently that calcitriol inhibits TH1 cells but stimulates TH2 function (11).

Specific T-cell functions, such as cytotoxicity and T-suppressor cell function, are also influenced by calcitriol. It has been established both in vitro and in vivo that calcitriol is a very potent stimulator of suppressor cell function (52, 53). Data on cytotoxicity
are less clear, but most authors report an inhibition of T cytotoxic activity (5, 52).

B Lymphocyte function. Cell proliferation and Ig production are inhibited by calcitriol (54). Here, again, these effects are only partly mediated through direct action on the B lymphocytes. In a mixed immune cell population, down-regulation of the accessory cell function of the monocytes/macrophages and inhibition of the T-helper function will by themselves greatly impair B-cell function (55).

Finally, calcitriol also affects natural killer (NK) cell activity. Most authors report a stimulation of killer activity in vitro (11). Personal data show a marked stimulation of NK activity already after 1.5 h of preincubation of the NK cells with 10^{-8} \text{ mol/l} \text{ calcitriol. This stimulation of NK activity may be an important asset in the anti-cancer activity of calcitriol in vivo because NK cells are the main immune attackers of the cancer cells.}

As calcitriol can be produced by activated macrophages and it exerts important modulatory effects on most cells of the immune system, it is obvious to suggest a physiological role for calcitriol as a regulatory messenger or cytokine-like molecule between the cells of the immune system. A schematic proposal for this paracrine role of calcitriol in the immune system is depicted in Fig. 1.

**In vivo immune effects of calcitriol**

Based on the discovery of major effects of calcitriol on immune cells in vitro it became clear that calcitriol might be an interesting molecule for immunomodulation in vivo. The calcemic effects of calcitriol, however, make the use of high doses in vivo impossible and limit its applications. In recent years new synthetic structural analogs of calcitriol have become available (6, 7, 9, 56) and several of these substances share the immunoregulatory capacity of calcitriol but have substantially lower calcemic effects (5, 57). Because no laboratory has yet reported comparative studies of the superagonists synthesized by different pharmaceutical companies or academic laboratories using the same calibration of concentration and dilution, media and cell lines, it remains difficult to define the accurate rank order of potency but it is undoubtedly possible to select structural modifications of calcitriol capable of enhancing their antiproliferative, differentiative and/or immunomodulatory potency 10–100(0)-fold.

Several groups have demonstrated impressive effects of calcitriol and its analogs in different animal models for autoimmunity and organ transplantation (Table 1). In experimental allergic encephalitis (EAE), the murine model for multiple sclerosis, Lemire and co-workers demonstrated that calcitriol itself not only prevented disease induction by myelin basic protein (MBP) in naive animals but also abrogated the transfer of the disease by MBP-specific T-cell clones isolated from presensitized animals (58, 59). Recently, the same group and others (60) have demonstrated prevention of EAE by several analogs of calcitriol. Experimental lupus erythematosus studied in MRL/lpr/lpr mice could be prevented by calcitriol and several of its analogs (61, 62). Clinical disease, proteinuria and even histological lesions were improved. Immunomodulation through restoration of the thymocyte phenotypes has been suggested as a possible mechanism. In two models of autoimmunity in the rat, experimentally induced Heymann nephritis, nephrotoxic serum nephritis and HgCl₂-induced nephritis in the BN rat, again a partial, but important protection against clinical and histological lesions of the disease by calcitriol (48, 63, 64).

In the NOD mouse, the murine model for type I or autoimmune juvenile diabetes, calcitriol reduced the incidence of the histopathological lesion (insulitis) and the clinical disease (diabetes) itself (53, 65). Extensive immunological screening of these mice revealed a restoration of the suppressor cell function in the otherwise suppressor-deficient NOD mice. Other immune anomalies, such as thymocyte anergy or absent NK cell function, were not restored. An important point in this study was the fact that no real immunosuppression was present. This potential to prevent insulitis and/or diabetes was confirmed for several analogs (60). A remarkable finding common to most of these models is the relatively high dose needed.
to achieve these effects. This treatment was, nevertheless, well tolerated by the animals when given on alternate days. When, however, calcium metabolism or bone turnover was closely studied, it was obvious that in most treated mice important bone remodeling was taking place and slight elevations of serum calcium were induced, thus raising the question of whether these in vivo immunomodulatory effects of calcitriol and its analogs were not due to sustained mild hypercalcemia. Recently, we could demonstrate insulitis and diabetes prevention in the NOD mouse by low doses of a 20-epi-analog, KH 1060 (0.2 μg/kg every 2 days), without interfering with calcium or bone metabolism as measured by several techniques (66).

Another common feature in these prevention studies was that treatment was more effective if started before the onset of autoimmunity. When, in the NOD model, mice were treated from weaning until 100 days of age and followed until 200 days of age, a significantly lower insulitis (46% (7 of 15), p < 0.025 versus the control group) and diabetes incidence (30% (5 of 15), p < 0.001) was observed (Fig. 2). When therapy was only initiated at day 100 (when control mice have insulitis in about 75%), insulitis was present in 90% (18 of 20) and 80% (16 of 20) of these mice developed diabetes by 200. These values are not significantly different from the control values. When, however, not the endpoints but the timing of diabetes onset was compared, these treated animals showed a delayed onset of diabetes compared to the control group (p < 0.01) (Log Rank statistics). This study confirms previous observations by us and others that calcitriol can prevent autoimmunity, especially when treatment is started before or at the initial stage of the autoimmune attack. At this early moment a relatively short-term treatment may induce a long-term protection by creating a new state of tolerance, through restoration of a defective immune balance (e.g. restoration of suppressor function). When treatment is started after the autoimmune attack has begun, only a delay in disease presentation can be obtained.

In several models of autoimmunity an additive and even synergistic effect on disease prevention was demonstrated between calcitriol and CyA (46, 48). This synergy was also observed in a model of secondary autoimmunity prevention: recurrence of type I diabetes after syngeneic islet transplantation (47). Type I diabetes is characterized by the existence of an autoimmune memory, causing the destruction of newly transplanted β cells (in the form of islets or a whole pancreas) even when these are perfectly HLA matched or syngeneic, thus raising a major problem for

### Table 1. Effects of calcitriol and its analogs in autoimmunity and transplantation.

<table>
<thead>
<tr>
<th>Author and year</th>
<th>Model</th>
<th>Product</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jordan 1988</td>
<td>Skin grafts CBA→Balb/c</td>
<td>Calcitriol</td>
<td>Graft survival increased</td>
</tr>
<tr>
<td>Jordan 1988</td>
<td>Heart grafts Lew→Balb/c</td>
<td>Calcitriol</td>
<td>Graft survival increased</td>
</tr>
<tr>
<td>Fournier 1990</td>
<td>Thyroiditis in CBA mice</td>
<td>Calcitriol + CyA</td>
<td>Clinical improvement</td>
</tr>
<tr>
<td>Abe 1990</td>
<td>SLE in MRL/I mice</td>
<td>22-Oxa-calcitriol</td>
<td>Histological improvement</td>
</tr>
<tr>
<td>Lemire 1991</td>
<td>EAE in SJL/I mice</td>
<td>Calcitriol</td>
<td>Prevention of proteinuria</td>
</tr>
<tr>
<td>Inaba 1992</td>
<td>STZ-DM in CD-I mice</td>
<td>(1α)-Hydroxycholecalciferol</td>
<td>Disease prevention</td>
</tr>
<tr>
<td>Mathieu 1992</td>
<td>Type I diabetes in NOD mice</td>
<td>Calcitriol</td>
<td>Prevention of insulitis and diabetes</td>
</tr>
<tr>
<td>Lillevang 1992</td>
<td>HgCl₂ nephritis in BN rats</td>
<td>KH1060</td>
<td>Prevention of glomerulonephritis</td>
</tr>
<tr>
<td>Lemire 1992</td>
<td>Heart grafts C3H→Balb/c</td>
<td>1.25-Δ16-D₃</td>
<td>Graft survival increased</td>
</tr>
<tr>
<td>Veyron 1993</td>
<td>Skin grafts C57B1→CBA</td>
<td>KH1060</td>
<td>Graft survival increased</td>
</tr>
<tr>
<td>Branisteanu 1993</td>
<td>Heymann nephritis (BN rat)</td>
<td>Calcitriol</td>
<td>Reduction of proteinuria</td>
</tr>
<tr>
<td>Lemire 1993</td>
<td>SLE in MRL/I mice</td>
<td>Calcitriol</td>
<td>Prevention of skin lesions</td>
</tr>
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<td>Mathieu 1994</td>
<td>Type I diabetes in NOD mice</td>
<td>Calcitriol</td>
<td>Prevention of diabetes</td>
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<tr>
<td>Mathieu 1994</td>
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<td>MC903</td>
<td>Prevention of insulitis</td>
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<tr>
<td></td>
<td></td>
<td>RO 24-2637</td>
<td>Prevention of diabetes recurrence after islet transplantation</td>
</tr>
<tr>
<td>Mathieu 1994</td>
<td>Type I diabetes in NOD mice</td>
<td>KH1060</td>
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<td></td>
<td>KH1060 + CyA</td>
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<td>Bouillon 1994</td>
<td>EAE</td>
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<td>Johnsson 1994</td>
<td>Heart graft</td>
<td>MC1288</td>
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<tr>
<td>Mathieu 1995</td>
<td>Type I diabetes in NOD mice</td>
<td>KH1060</td>
<td>Prevention of insulitis and diabetes</td>
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*EAE: experimental allergic encephalitis; SLE: systemic lupus erythematosus; STZ-DM: streptozotocin-induced diabetes mellitus.*
transplantation of β cells in type I diabetic subjects (67, 68). We have demonstrated recently that KH 1060 prevents type I diabetes recurrence after syngeneic islet transplantation in spontaneously diabetic NOD mice, both alone and in synergy with CyA (47). In some animals treated with a combination of KH1060 and CyA, protection even lasted long after interruption of therapy, suggesting a restoration of tolerance in these mice.

While data on autoimmunity are very encouraging, data on graft rejection prevention in organ transplantation are less spectacular. Therapy with calcitriol itself succeeds in only slight prolongation of graft survival at near-toxic doses (69). However, when more potent, newer analogs were used, better results were obtained (70, 71). Some analogs succeed in prolonging graft survival to the same extent as CyA (72). Again, synergy with CyA could be demonstrated to the extent that some authors have suggested a role for calcitriol as a corticosteroid-replacing drug in transplantation immune therapy (73).

A striking feature when comparing data on autoimmunity and transplantation is the spectacular effect that can be achieved in autoimmunity as compared to the sometimes marginal effects obtained in graft survival. It could well be that the combined effects of calcitriol on monocytes/macrophages, T lymphocytes and B lymphocytes make calcitriol or its analogs the ideal drugs for the prevention of autoimmune diseases, because all of these immune cells play a more or less important role in the pathogenesis of these diseases. In transplant rejection, however, the main part is played by the T lymphocytes alone.

In conclusion, it has now been well established that calcitriol and its structural analogs have immunomodulatory capacities in vitro and in vivo, but many questions remain to be answered. We still have, for instance, only scarce information on the molecular actions of calcitriol in the immune cells. The road to clinical applications in the immune field is now opened by the very powerful structural analogs that have been, and still are being, developed (6). A place for these drugs in immunoprevention of autoimmune diseases or as corticosteroid-replacing drugs for dose reduction of the more classical immunosuppressants in graft rejection seems realistic in the years to come.

Role of calcitriol or its analogs in keratinocyte differentiation and hyperproliferative skin disease

The skin is responsible for producing precholecalciferol from 7-dehydrocholesterol during exposure to the UV light from the sun, and is thereafter thermally converted into cholecalciferol. Keratinocytes, the most important cells of the skin, also possess both the 25- and 1α-hydroxylase enzymes and thus can produce small amounts of calcitriol. Under normal circumstances keratinocyte production of calcitriol does not appear to contribute to circulating levels (74) but can serve in an autocrine or paracrine function, acting together with calcium, growth factors and other hormones to regulate keratinocyte proliferation and differentiation. Hence keratinocytes express VDRs, express a specific calbindin and are responsive to the presence of calcitriol by both genomic and non-genomic mechanisms, with marked effects on proliferation (decreased) and differentiation (increased). Furthermore, the receptors for calcitriol and the production of calcitriol vary with the differentiation stage in a manner that suggests feedback regulation, because both are reduced in the later stages of differentiation (74). This further argues for a paracrine role of keratinocyte-derived calcitriol in epidermal differentiation.

Normal process of epidermal differentiation

The epidermis is composed of four layers of cells at different stages of differentiation: the basal cells, the spinous cells, the cells of the granular layer and the cells of the stratum corneum (see Ref. 75 for review). The basal cells are the dividing cells that compose the
germinative layer of the epidermis. They make keratins or precursors of intermediate filaments that comprise a significant portion of the cytoskeleton. Basal layer cells make specific keratins: keratin 5 and keratin 14 (keratin 16 is associated with hyperproliferation). As the cells move up, they become spinous cells. In a normal epidermis one observes 4–8 layers of spinous cells; they are postmitotic but still metabolically active. The spinous cells synthesize two new keratins, keratin 1 and keratin 10, which make microfilbrils. Spinous cells also synthesize involucrin, a precursor of the cornified envelope, which will become a component of the stratum corneum. Expression of involucrin, keratin 1 and keratin 10 is associated with early events of differentiation. As spinous cells reach the granular layer, they stop generating keratins and envelope proteins and start the synthesis of profilaggrin and loricin, which are keratin-organizing proteins. The cells now also express transglutaminase K protein. Finally, the stratum granulosum cells become permeable to calcium and this calcium influx activates transglutaminase, which cross-links the envelope proteins into a cage. The stratum corneum is formed, composed of terminally differentiated keratinocytes (skelets filled with keratin filaments); which are also called cornified envelopes. The time needed for cells to migrate from the basal layer to the stratum corneum is 28 days. The process of epidermal differentiation is tightly regulated by a number of hormones, growth factors and ions. This review will limit itself to the role of calcitriol and calcium in epidermal differentiation.

Calcium is the best-studied prodifferentiating agent. In vivo, a calcium gradient exists in the epidermis, which may provide the driving force for differentiation in intact epidermis. In the basal and spinous layers calcium is found primarily intracellularly, whereas in the upper granular layers calcium accumulates in large amounts in the extracellular matrix (74). Most information about the regulation of epidermal differentiation by hormones, growth factors and ions is learned from in vitro studies with cultured keratinocytes. Keratinocytes in culture can go at least partly through this differentiation process, which is further optimized when the keratinocytes are grown on a floating raft of collagen and fibroblasts at a air/liquid interface.

In vitro, the differentiation process of human keratinocytes in culture is highly dependent on the calcium concentration of the medium, known as the calcium switch. When cells are switched to higher calcium they stop proliferating and start to differentiate, making involucrin, loricin, transglutaminase, keratin 1 and 10, fillagrin and cornified envelopes, which represents genomic actions as the mRNAs for the different proteins are increased.

Calcitriol has been studied in vitro on keratinocytes in culture where it has been demonstrated to inhibit keratinocyte proliferation and to stimulate keratinocyte differentiation. The antiproliferative effect of calcitriol is enhanced by calcium (74) and epidermal growth factor (EGF) (76), while, at least in vitro, insulin appears to counteract the synergistic effect of EGF (76). Several molecular factors could be involved in the antiproliferative effect of calcitriol. The antiproliferative effects are accompanied by a decrease in the number of high-affinity EGF receptors (77) (total number of receptors remains the same) and at least in breast cancer cells decreased EGF receptor mRNA expression (78). Other effects of calcitriol on gene expression, possibly contributing to its antiproliferative effect, include decreased expression of the proto-oncogene c-myc (77), which is closely associated with cell proliferation. A recent study demonstrates that the growth inhibition effect of calcitriol is linked to the dephosphorylation of the tumor suppressor retinoblastoma gene product (its phosphorylation is necessary for progression through the cell cycle) (79). Moreover, calcitriol increases the synthesis of TGF-β1 (80); TGF-β1 is known to inhibit keratinocyte proliferation alone or in combination with calcitriol and, like calcitriol, is capable of decreasing the phosphorylation of the retinoblastoma gene product and of reducing the c-myc concentration.

Concerning its prodifferentiating effect, calcitriol has been demonstrated to stimulate differentiation morphologically and to modulate structural (differentiation associated) proteins both at the protein and mRNA level. At the morphological level, in vitro studies with keratinocytes in culture have shown that calcitriol increases the number of squamous cells (or differentiated cells) and increases the number of cornified envelopes (which is the most distinctive marker of terminal differentiation). Calcitriol also increases biochemical markers of differentiation as it increases the transglutaminase activity and cytosolic involucrin protein content, which appear at early stages of differentiation (74). A recent study has demonstrated that these increases are accompanied by similar changes in mRNA and thus represent genomic effects. At this level of gene expression there is also interaction with calcium, with a synergistic effect at intermediate calcium concentrations but inhibition at higher calcium concentrations (74). In contrast to the in vivo observations using immunofluorescence methods on biopsies of psoriasis plaques treated with calcitriol or its analogs, no direct effects of calcitriol on keratin gene expression has yet been observed in in vitro cultures of keratinocytes (81).

Whether calcitriol effects on keratinocytes are mainly due to its genomic or non-genomic effects is presently not clear. The genomic pathway involves the binding of calcitriol to the intranuclear receptor VDR, which binds to specific vitamin D response elements thereby directly regulating the transcription of genes. Although the VDR may in certain circumstances act as a homodimer or as a heterodimer with retinoic acid receptor (RAR),
most of its action is probably only possible after heterodimerization with RXR (82). No VDR, RXR or RAR response elements have been found in the promoter region of the transglutaminase gene (but putative response elements for c-myc and AP1 sites were identified) (83). Similarly, no VDR response elements could be detected in the promoter region of several keratins, whereas RAR and TSH-R response elements were clearly present.

The non-genomic mechanism involves the rapid increase of intracellular calcium, which can decrease proliferation and is closely correlated with the ability of epidermal cells to form cornified envelopes. A rise in intracellular calcium has indeed been observed within minutes after exposure to calcitriol.

Clinical applications of calcitriol

Psoriasis is a chronic erythemat-squamous skin disorder found in approximately 2% of the Caucasian population. Psoriasis presents itself in different clinical forms. The most characteristic and frequent form is the chronic plaque-type psoriasis, characterized by well-demarcated infiltrated erythematous patches covered with silvery scales. The typical histological picture is characterized by acanthosis or regular thickening of the stratum spinosum, a loss of stratum granulosum and by hyperkeratosis or regular thickening of the stratum corneum. Typical also is the parakeratose, indicating abnormal keratinization or the presence of nuclear remains in the cell. At the level of cell kinetics, psoriasis is characterized by an increase in growth fraction or active proliferating cells, an eightfold shortening of the cell cycle and a marked decrease in epidermal cell turnover time. There is incomplete differentiation, as suggested histologically by the loss of the granular layer and incomplete formation of cornified envelopes or parakeratosis. This incomplete differentiation is reflected further at the biochemical level by a modified keratin profile or decreased expression of differentiation-associated keratins (keratin 1 and keratin 2) and the appearance of keratin 16 and keratin 6, markers of hyperproliferation. Furthermore, involucrin and transglutaminase appear prematurely in the epidermis (84).

The rationale behind using calcitriol in psoriasis is its ability to reverse keratinocyte proliferation and to increase biochemically and morphologically the differentiation of keratinocytes. Calcitriol is therapeutically active in psoriasis, because treatment with topical calcitriol results in reappearance of the granular layer, regression of acanthosis and parakeratosis and resumption of the orthokeratotic horny layer. Immunohistopathology demonstrates an early decrease in the recruitment of cycling epidermal cells (Ki-positive cells), a rapid reduction of infiltration of mononuclear cells later (4 weeks of therapy) by reduction of T-cell infiltration, a normalization of filaggrin staining and a decrease in involucrin staining of the dermal papillae (84). These changes are not specific for calcitriol and are also observed after treatment with local steroids, PUVA and cignoline.

Psoriasis is not only characterized by an imbalance in epidermal growth but also by inflammatory and immunological abnormalities. There is an influx of mononuclear cells, which is extremely pronounced in the pustular form of psoriasis. At the immunological level, active psoriasis plaques are characterized by an intraepidermal influx of activated (HLA-DR+) CD4+ (helper) T lymphocytes, while resolution of psoriasis is characterized by an intra-epidermal influx of activated CD8+ (suppressor) T lymphocytes and a reduction in the number of CD4+ cells (85). The presence of elevated levels of cytokines with immunological and pro-inflammatory properties is further evidence for the pathogenic role of immunological and pro-inflammatory processes in psoriasis. Hence, there is an increased production of chemotactic and pro-inflammatory cytokines (TNF-α, INF-γ, IL-6, IL-8), growth factors (TGF-α) and adhesion molecules (ICAM-1) (85–88). Some of the abnormalities of the skin immune system can be modified by calcitriol treatment. In keratinocytes the production of IL-6 and IL-8 is inhibited by calcitriol (90), and the EGF receptor, (the receptor for TGF-α) is down-regulated (77). Moreover, calcitriol induces TGF-β, which inhibits keratinocyte and lymphocyte proliferation and suppresses their cytokine secretion (IL-2 and INF-γ) (90).

Clinical applications of calcitriol analogs

The limitation of calcitriol in the treatment of hyper-proliferative dermatoses like psoriasis, skin cancer and dyskeratoses is its ability to cause hypercalcuria, hypercalcemia, nephrocalcinosis, nephrolithiasis and soft-tissue calcification. Even when the use of calcitriol is restricted to local application, the window between effective and safe dose is low. The use of calcitriol analogs with potent cell-regulating properties but a lower risk to induce these calcium-related side effects could increase their clinical applicability.

Calcipotriol or MC903, one of the non-calcemic calcitriol analogs, is widely approved for use in the topical treatment of psoriasis. Calcipotriol is a synthetic 1,24-dihydroxycholecalciferol analog, containing a double bond and a cyclopropane ring in the side chain. Hence, MC903 is 200 times less potent than calcitriol in producing hypercalcemia and hypercalcicuria after oral administration in rats, but is as effective as calcitriol on keratinocyte growth (84). During treatment there is also a decrease in the recruitment of cycling epidermal cells (Ki-positive cells), an early reduction in the number of neutrophiles and later on a decrease in CD4+ and CD8+ cells. Improvement of the psoriatic lesions is further accompanied by a reduction in the amount of keratins 5, 16 and 18 (markers of
basal and hyperproliferating keratinocytes) and an increase in keratins 1, 2 and 10 (markers of differentiating keratinocytes) (57, 91, 93). Furthermore, MC903 decreases the ornithine decarboxylase activity necessary for the polyamine and thus for DNA synthesis (94). Clinical studies indicate further that the effect of local MC903 on psoriatic skin is at least equivalent to the beneficial effects of topical application of the potent steroid 17-betamethasone valerate (84).

Tacalcitol (TV02) or 1.24-dihydroxycholecalciferol is slightly less active than calcitriol in the induction of hypercalcemia but it entirely inhibits epidermal cell proliferation and stimulates their differentiation in vitro (95, 96). Tacalcitol also inhibits IL-1-induced IL-8 production by peripheral blood mononuclear cells (97). In vivo, TV-02 inhibits ornithine decarboxylase and transglutaminase activity and also decreases the proliferation of mouse skin. Like calcipotriol, the clinical efficacy of topical Tacalcitol is comparable to that of the potent steroid betamethasone valerate (98). The 22-oxa analog of calcitriol (OCT) is also a likely candidate for the treatment of psoriasis (57).

General conclusions

Vitamin D is an essential substrate that can either be obtained from dietary intake or by photosynthesis in the skin from 7-dihydrocholesterol. After sequential biotransformation in the liver and the kidney this secosteroid becomes an important calcitropic hormone. It probably also plays a role as a paracrine factor in the immune system and the skin, where it regulates cell proliferation and differentiation. Analogs of the natural hormone can therefore be used to modulate immune or hyperproliferative disorders.

Modifications in the side chain (homologation, 20-epimerization, replacement of a carbon by oxygen, unsaturation, fluorination or a combination of these modifications) have been particularly successful in increasing the intrinsic activity and/or decreasing the calcemic potency of the parent vitamin D hormone. Several analogs therefore have a 10–100-fold dissociation of their biological activity profile (cell differentiation or immune activity versus calcemic effect).

Indeed, several autoimmune animal models respond well to non-hypercalcemic vitamin D analogs and such compounds might find a role as immunomodulators for autoimmune disorders or in the prevention of graft rejection. Moreover, non-hypercalcemic calcitriol analogs are at least equipotent to topical glucocorticoid for therapy of psoriasis. Their possible use in malignant hyperproliferative diseases requires further study.

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