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1,25-Dihydroxyvitamin D_3 – a hormone with immunomodulatory properties

1,25-Dihydroxyvitamin D_3 – ein Hormon mit immunmodulierenden Eigenschaften

Summary The active vitamin D metabolite, 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$], exerts immunosuppressive activity. At a cellular and molecular level, the hormone preferentially targets helper T cell activity (Th1) by inhibiting the secretion of both IL-2 and IFN-γ by Th1 and by suppressing the secretion pro-Th1 cytokine IL-12 by antigen-presenting cells. The active metabolite further inhibits class II antigen expression and enhances suppressor cell activity.

In animal models of autoimmunity, 1,25-$(OH)_2D_3$ prevents the development of experimental autoimmune encephalomyelitis, reduces the incidence of diabetes, and attenuates murine lupus. The hormone also prolongs graft survival in animal models of transplantation.

In humans, non-classical use of 1,25-$(OH)_2D_3$ has led to an anti-proliferative effect in psoriasis, antineoplastic effect in prostate cancer, and immunomodulatory effect in scleroderma. The development of less hypercalcemic analogs might open a new therapeutic area for vitamin D_3.

Key words 1,25-Dihydroxyvitamin D_3 – immunosuppression – cytokines – Th1 autoimmunity – transplantation – scleroderma

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Introduction

Over 15 years ago, the discovery of specific 1,25-dihydroxyvitamin D3 [1,25-$(OH)_2D_3$] receptors on monocytes/macrophages and activated lymphocytes (1, 2) led to a novel property of the hormone, until then the domain of calcium homeostasis. From the beginning, it was clear that the hormone exerted antiproliferative, prodifferentiating, and immunosuppressive activities. The following years further defined the cellular and molecular effects of the metabolite in these respective areas. But the demonstration that these activities were also seen in animal models has led to an intense investigation leading to the development of analogs with reduced calcemic effects, allowing one to potentiate the activity of 1,25-$(OH)_2D_3$ in vivo. Recently, these properties of 1,25-$(OH)_2D_3$ have been studied in humans: antiproliferation (psoriasis), antineoplastic (prostate cancer), and immunosuppressive (scleroderma).

We will review the immunomodulatory role of the hormone at a cellular and molecular level, reassess the role and effectiveness of the hormone and analogs in animal models of autoimmunity, and summarize the application of 1,25-$(OH)_2D_3$ in human studies.

Immunomodulatory role of 1,25-$(OH)_2D_3$ at a cellular and molecular level

The effect of 1,25-$(OH)_2D_3$ on immune cells with relevance to autoimmunity is best understood by its predilection for helper T cells type 1 (Th1). The precursor helper T cell type 0 (Th0) upon IL-12 secretion by monocytes/macrophages develops into Th1, characterized by the secretion of both IL-2 and IFN-γ. This lymphocyte subset might play a role in various autoimmune diseases: multiple sclerosis (3), thyroiditis (4), rheumatoid arthritis (5), type 1 diabetes (6).

The vitamin D metabolite has a direct anti-proliferative effect on Th1 cells and directly inhibits the secretion of IL-2 and IFN-γ by committed human T cell clones of the Th1 phenotype (7). No effect of 1,25-$(OH)_2D_3$ on IL-4 secretion was seen in Th2 cells, a subset promoting IgE production and involved in allergic disorders (7). In addition, the hormone inhibits in a dose-dependent fashion the production and secretion of the pro-Th1 cytokine, IL-12, by monocytes (7, 8). In a similar fashion to that for the lymphokines IL-2 and IFN-γ, 1,25-$(OH)_2D_3$ inhibits IL-12 p35 and p40 subunits at a transcriptional level (9).

Besides its effects on lymphocytes, 1,25-$(OH)_2D_3$ inhibits accessory cell function of monocytes, reduces class II antigen expression, and inhibits monokine production (10, 11). While there is an anti-proliferative effect on B cells, the inhibition of immunoglobulin production by 1,25-$(OH)_2D_3$ is mediated through a partial effect on B cells. In the *in vitro* model of allograft response, the mixed lymphocyte reaction (MLR) 1,25-$(OH)_2D_3$ enhances suppressor cell activity, reduces cytotoxic cell generation, and again reduces the expression of class II antigen expression (12). The hormone also inhibits the development of new cytotoxic natural killer (NK) cells and the NK activity of non-adherent cells (13–15). In general, 1,25-$(OH)_2D_3$ analogs exert a similar action to that of the natural hormone but certain analogs appear more potent than others (7).

Immunomodulatory function of 1,25-$(OH)_2D_3$ in vivo

A variety of animal models have been studied for the immunomodulatory properties of 1,25-$(OH)_2D_3$ and related analogs (16). The key properties of the vitamin D metabolites can be best assessed through the models of experimental autoimmune encephalomyelitis (EAE), murine lupus, and murine diabetes of NOD mice.

In EAE, administration of 1,25-$(OH)_2D_3$ given at 5 μg/kg every other day from day 3 prior and up to day 5 following immunization with neuroantigen can effectively prevent the onset of disease, usually occurring within 10 to 15 days post immunization (17). Similar treatment can interfere with the onset of relapses characteristically associated with this disease (18). A blunting of the IgG2a response, driven by Th1 cells has been observed. While the "ideal" analog producing similar or enhanced immunosuppressive properties but with less hypercalcemic effects remains to be found, there is some evidence that when used in combination with existing immunosuppressive agents such as cyclosporine or rapamycine, the dosage of 1,25-$(OH)_2D_3$ could be reduced to produce effective suppression of disease while preventing the development of hypercalcemia (19).

In murine lupus of MRL/lpr mice, a potent autoimmune process leads to multiorgan disease and various serologic abnormalities. In these inbred mice, the disease is spontaneous, and treatment initiated at an early age can dramatically prevent the skin lesions associated with the disease process (20). With the same dosages used to completely prevent skin lesions, dosage similar to prevent EAE, a reduction of proteinuria and attenuation of the intense interstitial inflammation was also observed (unpublished data).

In murine diabetes of NOD mice, a model for type 1 diabetes, 1,25-$(OH)_2D_3$ reduces the incidence of the histopathological lesion, insulitis, and prevents the clinical onset of diabetes (21). Vitamin D3 analogs with reduced hypercalcemic activity are also effective in the prevention of disease (22). Further investigation into pathogenic aspects of the disease has recently revealed that treatment with 1,25-$(OH)_2D_3$ in NOD mice might be independent from the presence of suppressor cells and might involve increased apoptosis of autoimmune effector cells of the Th1 subset (23).

In transplantation, the rejection process remains the impediment for long-term allograft survival. The advent of powerful immunosuppressive agents has dramatically reduced the onset of acute cellular rejection at the expense of significant side effects, such as, infection, nephrotoxicity, and risks of lymphoproliferative disorders. The natural hormone, 1,25-$(OH)_2D_3$, exerts minimal immunosuppressive activity when administered as a sole agent in many experimental models of transplantation (24–26). Some analogs have shown enhanced activity compared to the natural metabolite but long-term administration led to some degree of hypercalcemia (24). At this time, no single analog used as a single agent, can be comparable to cyclosporine or other established anti-rejection therapy, with acceptable serum calcium levels.

Non-classical use of 1,25-$(OH)_2D_3$ in human studies

There is evidence that levels of 1,25-$(OH)_2D_3$ in some autoimmune diseases such as systemic lupus erythematosus are low (27) but the relevance of these findings on pathogenesis of the disease remains to be elucidated. The first disease for which a non-calcium role of vitamin D3 showed application was psoriasis. Topical administration of an analog calcipotriol has now gained widespread acceptance in dermatology (28). A systemic application of calcitriol has also been found to be effective in this disease and the results are summarized in Table 1. A similar dosage of calcitriol has also recently found application for prostate cancer (29). An open prospective trial of calcitriol for the treatment of scleroderma had also shown some benefits (30). The results of both of these studies are also presented in Table 1.

Treatment with calcitriol has shown beneficial effects in all instances. The efficacy of the hormone is clearly limited by its hypercalcemic/hypercalciuric effects. Despite a reduced calcium diet, all patients developed hypercalciuria as defined by a urinary calcium excretion of >350 mg/24 hours, and dosage of 1,25-$(OH)_2D_3$ had to be reduced. While some patients received up to 2.5 µg/day of the drug, on an empty stomach at bed time, the dosage that seemed to be most tolerated was 1.5 to 1.75 µg per day. Interestingly, not hypercalcemia but hypercalciuria was a limiting factor in drug administration with potential risk for nephrocalcinosis.

A 10 year old girl with systemic sclerosis presented with Raynaud's phenomena, rapid skin changes, and joint limitation with weakness. Penicillamine therapy was ineffective while prednisone initially provided symptomatic relief to the myositis. After steroid tapering, a therapeutic trial of 1,25-$(OH)_2D_3$ (calcitriol) was initiated. Figure 1 illustrates the course of the therapy.

Administration of daily calcitriol led an improvement in skin lesions as assessed by the Rodman score. However, the development of hypercalciuria prompted discontinuation of the drug, rapidly followed by a deterioration in the skin changes. Reinstitution of therapy with calcitriol improved the clinical manifestations of the disease. After one year of treatment, calcitriol was discontinued and the patient's disease remained in remission. A safety and tolerability trial for the administration of calcitriol to patients with scleroderma is actually underway.

In summary, the properties of 1,25-$(OH)_2D_3$ observed *in vitro* have found application in animal models *in vivo* and in humans in pathologic states from neoplasia to autoimmune diseases. Diseases in which Th1 cells might play a pathogenic role appear to be the main potential of the drug. These would include a variety of autoimmune diseases such as scleroderma, lupus, multiple sclerosis, rheumatoid arthritis, and diabetes. The intrinsic property of 1,25-$(OH)_2D_3$ of mobilizing calcium remains the main obstacle for the widespread application of the hormone for non-calcium related diseases. However, the promise of analogs with reduced calcemic effects and/or the synergistic combination of 1,25-$(OH)_2D_3$ with existing immunosuppressive agents might open a new area of therapeutic intervention.

Table 1 1,25-Dihydroxyvitamin D_3 therapy of non-calcium metabolism states

Indication Autor	Psoriasis Perez (31)	Prostate cancer Gross (29)	Scleroderma Humbert (30)
# Patients	85	7	11
Duration of study	36 mo	15 mo	6–36 mo
Diet	800 mg CA	800 mg CA	–
Dosage of 1,25-$(OH)_2D_3$ (µg/day)	2.4±0.6	1.5 (Max: 2.5)	1.75 (Max: 2.5)
Serum CA (mg/dl)	9.9±0.5	9.6±0.1	10.5
Urine CA (mg/24 hrs)	273.9±37.7	309±15	<305
Effect	↓Erythema (41%) ↓Scaling (71%) ↓Plaque thickness (57%)	↓PSA * rise in 6/7 pts	↓Oral aperture (28 → 32 mm ↑Palmar flexion index (28.7 → 12 mm)

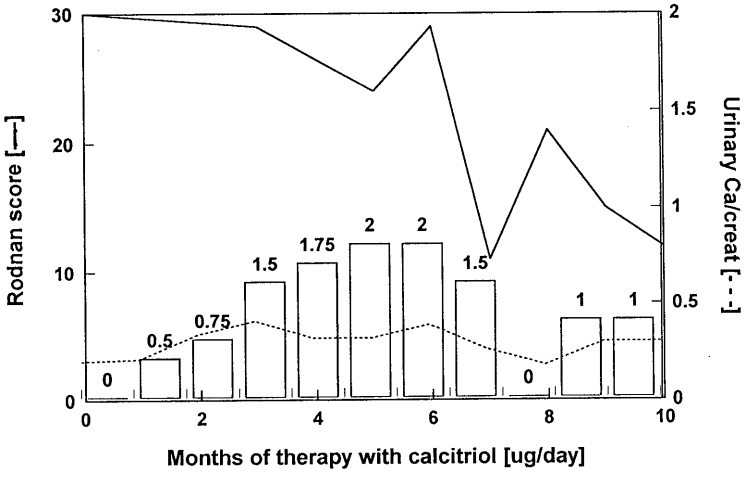

Fig. 1 A 10 year old with systemic sclerosis

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