D-Hormone analog alfacalcidol: an update on its role in post-menopausal osteoporosis and rheumatoid arthritis management

Florent Richy[1,3], Rita Deroisy[2], Marie-Paule Lecart[1,2,3], Linda Hanssens[1,3], Audrey Mawet[1,3], and Jean-Yves Reginster[1,2,3]

[1]Public Health, Epidemiology and Health Economics Unit, Faculty of Medicine, University of Liège, Sart-Tilman, [2]Bone and Cartilage Metabolism Research Unit, University Hospital, Liège, [3]WHO Collaborating Center for the Public Health Aspects of Osteoarticular Disorders, Liège, Belgium

ABSTRACT. Alfacalcidol (1-alpha-hydroxyvitamin D_3) is a non-endogenous analog of vitamin D which can bypass the renal and intestinal regulatory mechanisms that control the production of calcitriol (1,25-hydroxyvitamin D_3, the active form of vitamin D, D-Hormone). Alfacalcidol may be metabolized into calcitriol with a limited risk of hypercalcemia. Alfacalcidol and calcitriol have been evaluated in animal and human studies assessing their effects on bone mineral density and fracture rates. More recently, they have been shown to produce beneficial effects in muscle, immune system, and autoimmune diseases, including rheumatoid arthritis. This paper discusses the therapeutic efficacy of alfacalcidol in reports in which it has been proposed as an interesting alternative to vitamin D or calcitriol. Some recent findings about general metabolism and regulation of vitamin D and its analogs are discussed. The biological and clinical effects of alfacalcidol in post-menopausal osteoporosis are reviewed, followed by critical appraisal of its efficacy in preventing bone loss and falls in the elderly. The last two sections discuss the role of D-analogs in regulating the immune system, with particular regard to rheumatoid arthritis. The main results of this review show that alfacalcidol may have a wider range of therapeutic applicability, beyond simply restricting it to patients in hemodialysis or peritoneal dialysis with high serum levels of intact PTH.

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INTRODUCTION

Until 1980, no-one imagined that vitamin D and its metabolites 25(OH)D, $24,25(OH)_2D$ and $1,25(OH)_2D$, well-known for their central roles in calcium and bone metabolism, might play an important role in regulating the immune system. Recent advances in our understanding of their mechanism of action opened up new research fields, due not only to their unique interaction with bone cells but also to their modulation of the immune system. Although alfacalcidol, $1\text{-}\alpha(OH)D_3$, a synthetic D-Hormone analog, has been studied for several decades, its extensive mechanism of action is still not fully elucidated. The aim of this paper was to review the available evidence of the biological and clinical effects of alfacalcidol in post-menopausal osteoporosis (PMOP) and rheumatoid arthritis (RA).

GENERAL METABOLISM OF VITAMIN D AND ITS ANALOGS (FIGS. 1-2)

Vitamins D_2 (ergocalciferol) and D_3 (cholecalciferol) are produced by the skin or absorbed from the gut. They are metabolized into their active form, calcitriol, by two successive steps: 25-hydroxylation in the liver to 25(OH)D, followed by 1α-hydroxylation in the renal proximal tubules to $1,25\text{-}(OH)_2D$, yielding the biologically active form of vitamin D, calcitriol (1). Some other cells exhibit 1α-hydroxylase activity, including osteoblasts, placental cells, keratinocytes, macrophages and some tumor cells. The role of the extrarenal production of $1,25(OH)_2D$ is still debated but, in normal conditions, it does not significantly contribute to the circulating levels of the hormone (2, 3). 24-hydroxylation, producing 24,25-dihydroxyvitamin D [$24,25(OH)_2D$] or 1,24,25-trihydroxyvitamin D, occurs in a wide range of normal tissues and is believed to be important both to catabolise vitamin D metabolites and to regulate the active forms of vitamin D (4). $24,25(OH)_2D$ is not in fact inactive. Recent animal studies have shown that this

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Correspondence: F. Richy, PhD, University of Liège, CHU B23, B4000 Sart-Tilman, Belgium.
E-mail: florent.richy@ulg.ac.be

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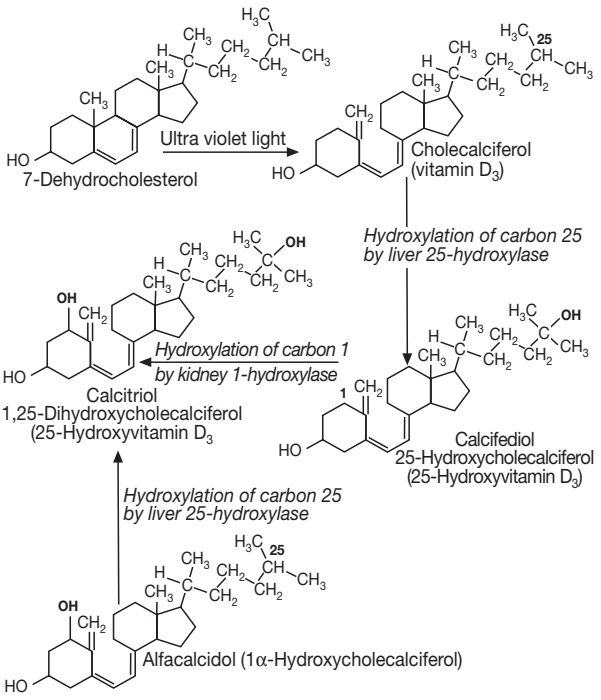

Figure 1 - *General vitamin D pathways.*

metabolite can stimulate chondrocyte maturation (5), increase bone mineral density in vitamin D-replete rats, rabbits and dogs (6), and play a beneficial role in fracture repair in chicks (7). $24,25(OH)_2D$ has also been shown to be a potent inhibitor of PTH secretion in humans (8). The interactions among calcitriol, alfacalcidol and $24,25(OH)_2D$ are still under study. Recent findings in animal models suggest that both metabolites must be present for optimal changes in bone metabolism (Fig. 2). The major enzymes involved in vitamin D hydroxylation are mitochondrial mixed-function oxidases containing cytochrome P450 with ferredoxin and heme-binding domains (9). Until today, four cytochrome P450 molecular species (CYP27A1, CYP2C11, CYP2D25, CYP3A4) have been identified as vitamin D_3 25-hydroxylases (10, 11). Alfacalcidol ($1\text{-}\alpha(OH)D_3$) is a synthetic derivate of vitamin D. Important for its metabolization is the fact that hepatic 25-hydroxylation is unregulated and exclusively substrate-dependent. Conversely, the renal 1α-hydroxylase enzyme is stringently regulated by: the PTH cAMP-mediated pathway, calcitonin in a different region of the proximal tubule, and by $1,25(OH)_2D$ itself, through negative feedback regulation via its receptor (VDR) (2, 4). Thus, in situations in which the general vitamin D pathways are disturbed, exogenous alfacalcidol may bypass these regulatory systems to produce bioavailable calcitriol. Circulating vitamin D metabolites measured in clinical practice are 25(OH)D and $1,25(OH)_2D$ (calcitriol). As 25(OH)D synthesis is substrate-, i.e., vitamin D-dependent, serum levels of this metabolite are taken as a measure of vitamin D status. Besides its classical actions in calcium metabolism, it is now suggested that the hormonal form of vitamin D has many "uncommon" functions, which have only been revealed as a result of the identification and characterization of its receptor. The VDR complex was discovered in 1975 (12). Many tissues express VDR, including osteoblasts, intestinal, muscle, distal renal, liver, parathyroid, and T-cells and monocytes (13, 14). Calcitriol functions as a steroid hormone which binds to a cytosolic VDR, resulting in selective demasking of the genome of the nucleus (Fig. 3).

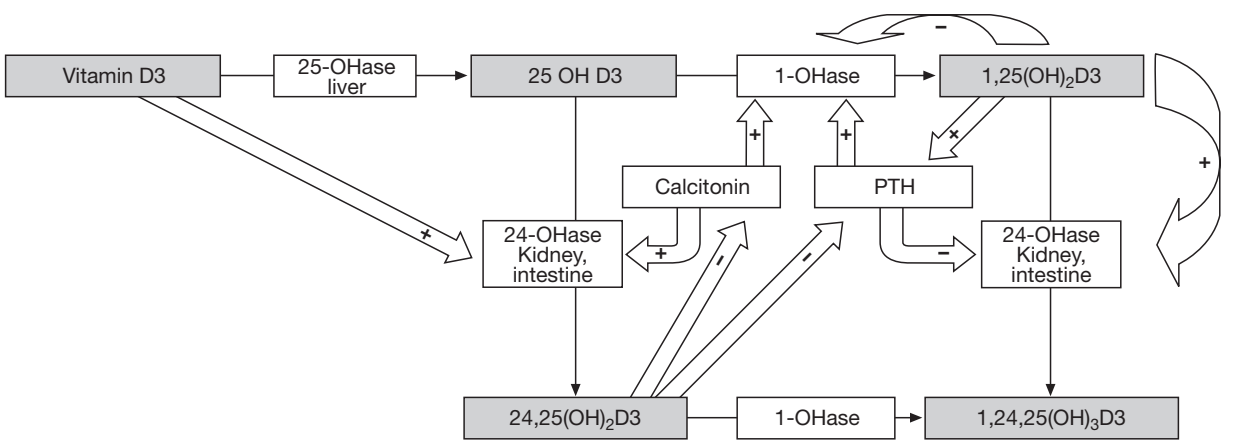

Figure 2 - *Regulation of vitamin D metabolism.*
OHase: OH-hydroxylase.

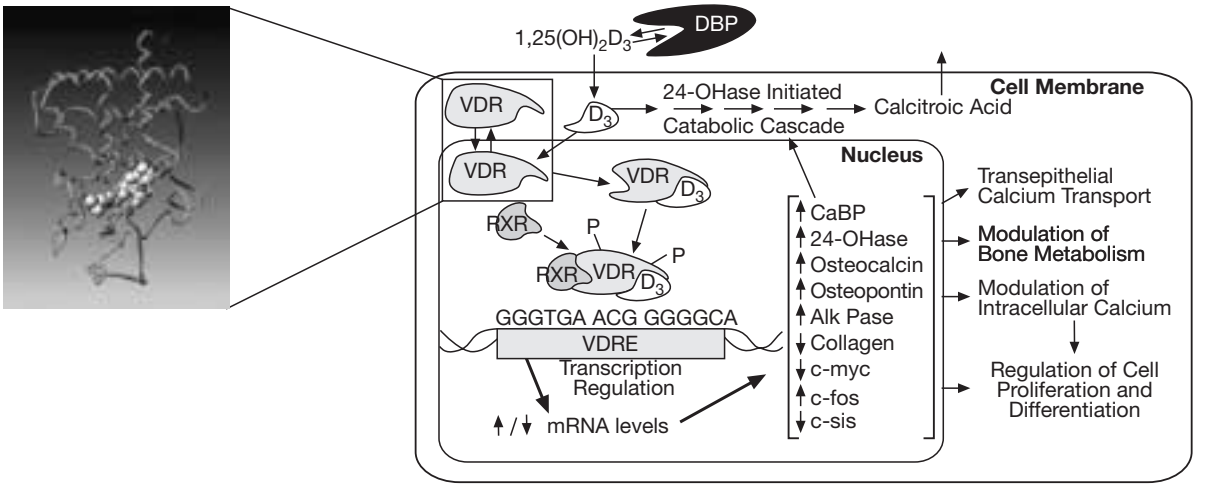

Figure 3 - *Vitamin D Receptor: structure and function.*
DBP: vitamin D binding protein; VDRE: vitamin D responsive elements (specific DNA sequences); CaBP: calcium-binding protein; 24-OHase: 24-OH-hydroxylase; Alk Pase: Alkaline phosphatase; c-myc, c-fos, c-sis: protooncogen regulating cell proliferation and differentiation; RXR: Retinoid X Receptor

Two subtypes of VDR have recently been discovered using knock-out mice models. 1α,25(OH)$_2$D(3)-dependent regulation of DNA synthesis in chondrocytes requires the presence of the 1,25-nVDR, although other physiological responses to the vitamin D metabolite, such as proteoglycan sulfating, involve regulation *via* the 1,25-mVDR(5). Because of the wide variety of tissues in which VDR has been identified, the exploitation of vitamin D compounds in treating various diseases has been expanded.

VITAMIN D DEFICIENCY: A HETEROGENEOUS CONDITION

"Vitamin D deficiency" collectively describes a number of pathological conditions which include primary vitamin D deficiency, calcitriol deficiency, and resistance to calcitriol. Primary vitamin D deficiency is due to inadequate dietary intake or exposure to sunlight, and is not simply a biochemical abnormality. It is regularly associated with secondary hyperparathyroidism, increased bone turnover, bone loss, osteoporosis, and increased risk of fracture (15). Vitamin D deficiency impairs the intestinal absorption of calcium. PTH maintains normal serum calcium levels for a time, despite decreased calcium absorption, by increasing bone resorption; however, vitamin D deficiency progressively causes resistance to the osteoclastic effects of PTH on bone and a decrease in calcium renal tubular reabsorption (16). PTH increases serum calcium but also stimulates phosphaturia, resulting in hypophosphatemia (16). These combined effects lead to a reduced bone mineralization. Unlike primary vitamin D deficiency, primary calcitriol deficiency is not due to the limitation of precursors but to a defect in the synthesis of calcitriol, reducing intestinal Ca absorption and increasing PTH and bone resorption. The pathogenesis of primary calcitriol deficiency is related to impaired ability of the kidney to synthesize adequate amounts of calcitriol. It is common in patients with renal insufficiency or failure. Resistance to calcitriol is related to aging-associated decline in the functions of various tissues and organs, leading to reduced calcitriol biological action despite its normal serum levels (17). The potential cause of this resistance may be due to age-related or unknown defects in regulation in the number or a decrease in the affinity of VDR, which mediate genomic actions of vitamin D (18, 19).

BIOLOGICAL EFFECTS OF ALFACALCIDOL IN POST-MENOPAUSAL OSTEOPOROSIS

Alfacalcidol is a synthetic precursor of calcitriol, and is converted into 1,25(OH)$_2$D$_3$, predominantly in the liver, by 25-hydroxylation. 25-hydroxylation has been reported to be catalyzed by both mitochondrial CYP27A and a microsomal CYP2D25 vitamin D$_3$ 25-hydroxylase in the liver (20, 21). Northern blotting and reverse transcription-polymerase chain reaction experiments have revealed that porcine CYP2D25 mRNA (showing 77% identity with that of humans) is expressed at the highest level in the liver and in small amounts in other tissues, including muscle (20) and bone. As noted above, alfacalcidol bypasses endogenous regulation by renal 1-α hydroxylase and its pharmacokinetic profile is therefore very different from that of calcitriol: after oral ingestion of calcitriol, peak

serum $1,25(OH)_2D_3$ is reached within 2 hours, whereas oral ingestion of alfacalcidol causes a slow rise in serum calcitriol with peak values after 8-18 hours. Calcitriol, upon absorption, acts immediately and directly on the VDR in the intestinal mucosal cells to promote Ca absorption, leading to a rapid increase in serum calcium. In contrast, alfacalcidol has very limited intestinal action, since 25-hydroxylase required for its metabolization into calcitriol acts predominantly in the liver. Compared with calcitriol, alfacalcidol allows for more progressive and longer production of calcitriol together with a lower risk of hypercalcemia. This allows the lack of calcium absorption due to VDR deficiency to be counterbalanced.

It has been suggested (22) that pharmacological or suprapharmacological doses of $1,25(OH)_2D(3)$ stimulate bone resorption by inducing RANKL, the ligand from pre-osteoblastic cells binding to RANK on pre-osteoclastic cells to promote the differentiation of osteoclasts (23). Conversely, a certain range of physiological doses of vitamin D inhibit PTH-induced bone resorption, the latter mechanism appearing to be mediated, at least partly, by suppression of PTH/PTHrP receptor-mediated signaling. This may highlight the central role played by $1,25(OH)_2D_3$ in bone formation and resorption coupling. Indeed, the most important endocrine regulator of PTH is calcitriol (24), which regulates PTH through its receptor by suppressing both the expression of the preproparathyroid gene and parathyroid cell proliferation (25). Alfacalcidol, by enhancing D-analog levels, indirectly suppresses secondary hyperparathyroidism, which is common in osteoporotic and elderly patients. The mechanism includes inhibition of the proliferation of parathyroid cells by reducing their apoptosis (26, 27) as well as PTH synthesis and release (28), and of the effects of PTH on bone (29, 30). Reduction of alkaline phosphatase activity has also been shown (31). Vitamin D metabolites calcitriol and $24,25(OH)_2D$ modulate the response of bone and cartilage cells to 17 beta-estradiol and dihydrotestosterone in both cell cultures and in vivo rat models (32). They both reduce, by one order of magnitude, the amount of sex steroids needed to stimulate cultured osteoblast-like cells or rat embryo epiphyseal cartilage cells, and synergistically increase the maximal response of these cells (32). Notwithstanding this, it has been shown that interactions among D-analogs, VDR and oestrogen receptor (ER) are largely dependent on gender groups, suggesting complex ER-VDR-sex, ER-age-sex and VDR-age-sex interactions may exist (33). The current data do not support the strong role of $24,25(OH)_2D$ alone in the regulation of osteoblast action and mineralization (34). Conversely, alfacalcidol induces an increase in calcitonin secretion (35) and normalization of uncoupled bone turnover through an increase in transforming growth factor beta (TGF-beta), which stimulates osteoblastic maturation, and osteoprotegerin, which inhibits osteoclastic maturation by inhibiting the RANKL-RANK system (36). Calcitriol, by inhibiting bone-resorbing cytokines, specifically TNF-alpha or osteoblastic apoptosis, induces a modification in osteoclastic apoptosis, and an impact on the remodeling process has been observed (37). Correction of the helper/suppressor ratio in patients with high bone loss due to an increase in CD8 also appears to be involved (38).

CLINICAL EFFECTS OF ALFACALCIDOL IN POST-MENOPAUSAL OSTEOPOROSIS

Several studies have investigated the clinical effects of D-analogs (mainly calcitriol, alfacalcidol and $24,25(OH)_2D$) on bone mineral density, fractures and bone metabolism markers in PMOP. $24,25(OH)_2D$ is still a poorly studied compound, which has not shown any beneficial effects on BMD and calcium metabolism in clinical studies (34).

We previously performed two meta-analyses reviewing the clinical effects of D-analogs calcitriol and alfacalcidol (39), and compared their efficacies against that of native vitamin D (40). Our first systematic review included all randomized controled trials on alfacalcidol or calcitriol versus calcium or placebo. Eight studies of alfacalcidol (41-48) and a similar number of studies of calcitriol (49-56) specifically focused on their respective clinical efficacies on bone loss and/or fracture. Among trials of alfacalcidol, four (41-44) investigated post-menopausal osteoporosis in women aged 60 and more. Meta-analysis of these studies revealed the highly significant effect of alfacalcidol on global and particularly on spinal BMD, at a median duration of 18 months. Regarding non-spinal BMD, the data were too sparse for a proper meta-analysis. Regarding fracture prevention, we found a highly significant reduction (-47%) in the relative risk of lumbar spine fractures in the alfacalcidol arms, compared with placebo or calcium alone, at a median follow-up of 12 months. We were not able to find relevant data on hip fracture prevention by alphacalcidol in PMOP. However, we did find a trend toward increased efficacy of alfacalcidol, compared with calcitriol, in preventing decrease with BMD, and more specifically spinal BMD. In addition, studies on alfacalcidol, pooled together, provided remarkably homogenous results ($p_{heterogeneity}=0.66$); studies on calcitriol did not ($p_{heterogeneity}=0.01$). These results suggested not only that alfacalcidol and calcitriol have similar efficacies, but also that alfacalcidol may exert its BMD-preserving capabilities in a wider range of clinical patterns.

Our second quantitative review (40) assessed the relative efficacies of D-analogs alfacalcidol and calcitriol against their parent compound, vitamin D. Regarding BMD, D-analogs exerted a significantly higher efficacy on BMD at any site compared with native vitamin D, at a median duration of 24 months. When restricted to the lum-

bar spine, this intertreatment difference remained significant whereas there were no significant differences regarding their efficacies on other measurement sites, including hip. When comparing the adjusted global relative risks for spinal and non-spinal fracture, alfacalcidol and calcitriol appeared to be significantly more effective approaches compared with vitamin D. Analysis of the difference between spinal and non-spinal fracture rates confirmed the benefits of D-analogs, with significantly lower spinal and non-spinal fracture rates for D-analogs, on the basis of 30 to 36 months follow-up. Despite the lack of head-to-head trials in this field and the need for more careful follow-up of calcemia, alfacalcidol may be considered as an interesting alternative to native vitamin D in preventing bone loss and fractures in PMOP.

ALFACALCIDOL AND MUSCULAR FUNCTION

Muscle function, together with bone mineral density, is an important determinant of fracture risk, especially in the elderly (57). It was assumed for a century that vitamin D deficiency was linked to disturbed muscle metabolism (58). Vitamin D deficiency can impair intracellular Ca metabolism in muscle cells. The Ca-depleted content of mitochondria isolated from vitamin D-depleted chicks has been shown to be low (59), and Ca uptake into the sarcoplasmic reticulum is reduced during vitamin D deficiency (60, 61). Animal studies have shown that the actinomyosin content of myofibrils is reduced during experimental rickets (62).

Several studies in humans have demonstrated the relationship between D-hormone analogs and muscle function (63-65). Patients with osteomalacia suffer from muscle weakness and have low serum levels of muscle enzymes (66). A recent study on chick embryonic muscle cells provided direct evidence for the participation of the VDR in non-genomic $1,25(OH)_2D_3$ signal transduction. Activation of tyrosine phosphorylation cascades through this mechanism may contribute to hormone regulation of muscle growth (67). The results of a prospective, population-based study showed that lower 25-OHD and higher PTH levels increase the risk of sarcopenia in older men and women (68). A recent study has shown that congestive heart failure is associated with low vitamin D status (69).

Several trials have provided evidence for the involvement of D-analogs in preserving muscle function. Supplementation with 357 or 1250 µg vitamin D or 50 µg 25(OH)D for 1 or 2 months normalized muscle strength in patients with myopathy (66, 70). Leg extension power was positively correlated with serum 25(OH)D levels in elderly males and with serum $1,25(OH)_2D_3$ in the whole group of males and females (64). Grady et al. (71) and Lips et al. (72) did not show statistical improvement in muscle function using calcitriol or vitamin D. Glerup et al. (73), Pfeifer et al. (74), and Bischof et al. (75) showed a significant decrease in body sway and number of falls, corresponding to an improvement in muscle function using native vitamin D.

More recently, research has focused on alfacalcidol. Dukas et al. (76) demonstrated that, in calcium-replete patients, alfacalcidol treatment significantly and safely reduced the number of fallers in an elderly community-dwelling population (OR 0.45, 95% CI 0.21-0.97, p=0.042). Sato et al. (44) reported a highly significant differential efficacy of alfacalcidol on BMD depending on body side, in hemiplegic patients. BMD on the intact side increased by 3.5%, but decreased by 2.4% on the hemiplegic side over 6 months. Some local factors, such as paralysis and immobilization, may diminish the effect of alfacalcidol on bone formation on the hemiplegic side. This is an argument favoring the involvement of muscle 25-hydroxylase, which converts alfacalcidol into active calcitriol on a local basis. Janssen et al. (77) assessed the effects of alfacalcidol in vitamin D-deficient elderly people. Muscle strength improved, as well as walking distance and functional ability, which resulted in a reduction in the number of falls and fractures. Additional research is needed to clarify further to what extent alfacalcidol supplementation can preserve muscle strength and prevent falls and fractures in elderly people.

IMMUNOLOGICAL ROLE OF ALFACALCIDOL (FIG. 4)

In recent years, there has been an effort to understand the possible non-calcemic roles played by vitamin D, including its role in the immune system and, in particular, on T-cell-mediated immunity. Vitamin D receptor is in fact found in significant concentrations in T-lymphocytes and macrophage populations (78, 79), but its highest concentration is found in immature immune cells of the thymus and mature CD-8 T lymphocytes (80). Calcitriol has recently aroused great interest as an immune modulator with immunosuppressive activity, because of its

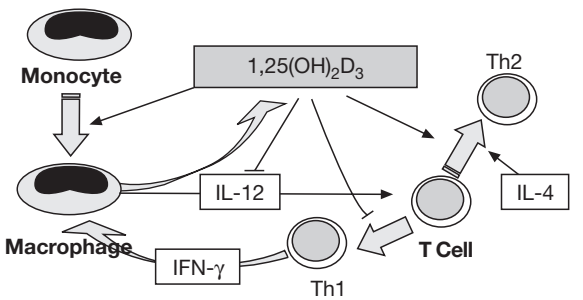

Figure 4 - *Interactions between calcitriol and the immune system. Pietschmann et al. Bedeutung von Vitamin D im Immunsystem. Journal Für Mineralstoffwechsel 2003; 10: 13-15. Reproduced by kind permission of Krause & Pachernegg GMBH.*

ability to shift T-cell responses from Th1 to Th2. The hormone inhibits the production of lymphokines (IL-2, IFN-gamma) and monocyte-derived cytokine (IL-12), leading to inhibition of helper T-cell subset type 1 (Th1) (81, 82). The significant role of vitamin D compounds as selective immunosuppressants is illustrated by their ability to prevent or even suppress animal models of autoimmune disease. Several studies on animal models have shown that 1,25-dihydroxyvitamin has a significant impact on the development of encephalomyelitis (83), rheumatoid arthritis (84), systemic lupus erythematosus (85), type I diabetes (86), and inflammatory bowel disease (87). Possible mechanisms of suppression of these autoimmune disorders by calcitriol have been presented. Notably, calcitriol stimulates transforming growth factor TGFß-1 (88) and interleukin production which, in turn, may suppress inflammatory T-cell activity. In support of this, calcitriol was unable to suppress a murine model of human multiple sclerosis in IL-4-deficient mice (80). It was shown to suppress proliferation of promyelocytes and promote their differentiation into monocytes (89). Peripheral lymphocytes contain variable amounts of VDR. CD-8 lymphocytes have the highest concentrations, whereas CD-4 and macrophages have lower ones (90).

The intrinsic effects of alfacalcidol, i.e., not those from its metabolite calcitriol, on various immunological parameters including lymphocyte subsets are still not clearly demonstrated. However, interestingly, Yamauchi et al. (91) showed that, in patients with rheumatoid arthritis, the CD-4/CD-8 ratio remains stable in patients whose initial value was normal, whereas it decreases after alfacalcidol treatment in patients whose initial values were abnormally high. It has been demonstrated that the delayed hypersensitivity response to dinitrobenzene is impaired in vitamin D deficient mice (92), which tends to confirm the modulator control of 1,25-$(OH)_2D_3$ in T cell-mediated immunity. The T-cell immune response depending on D hormone levels displays a characteristic inverted "U" curve (93). While this field requires more research investment, this particular activity of D-hormone analogs is the rationale for studies and trials on alfacalcidol and calcitriol for treating autoimmune disorders, including RA.

ALFACALCIDOL IN RHEUMATOID ARTHRITIS

Patients with RA are at high risk of developing both generalized and periarticular osteoporosis (94) and are thus at even higher risk of fractures (95). Local and systemic osteoporosis are linked to increased production of inflammatory cytokines (TNF alpha, IL-1 beta, IL-6), resulting in increased formation and activation of osteoclasts (96-100). TNF-alpha may also interfere with bone formation by promoting apoptosis of osteoblasts (101). Bone loss appears very early and is correlated directly with disease activity (102). Later in the process, it is associated with the negative effects of limited mobility, which may be related to decreased muscle function in the elderly. Goertz et al. (103) and Lee et al. (104) showed that VDR polymorphisms do not play a major role in RA predisposition, but Gough et al. (105) did obtain inverse results in female patients with early RA. In parallel, predisposition towards osteoporosis has been shown in certain VDR genotypes (106). High disease activity in patients with RA has been associated with alterations in vitamin D metabolism and increased bone resorption (84). The decrease in 1,25$(OH)_2D_3$ levels in these patients may lead to a negative calcium balance and inhibition of bone formation. Furthermore, low levels of 1,25$(OH)_2D_3$ may raise levels of activated T-cells and proliferation of lymphokine-activated killer cells, thus accelerating the arthritic process (107).

Whether glucocorticoids work positively or negatively on generalized/periarticular osteoporosis in RA is still controversial (108-110). The pathogenesis of corticosteroid-induced osteoporosis is complex. As a pathogenetic co-factor, corticosteroids reduce intestinal calcium absorption and increase renal calcium excretion, resulting in compensatory increased PTH release and increased sensitivity of bone to PTH. In addition, corticosteroids inhibit osteoblastic function (111) as well as the favorable effects of growth factors and sex hormones on bone (112, 113). Thus, bone loss in RA is centered around primary inhibition of osteoblastic activity, compounded by the effects of secondary hyperparathyroidism (114). It has recently been suggested that the expression of D-hormone receptors (VDR) may be decreased by corticosteroids, and that they probably reduce the number of functional VDR (115, 116). This may be an explanation for the efficacy of D-analogs in treating PMOP. Corticosteroids inhibit IL-12 production in human monocytes and enhance their capacity to induce IL-4 synthesis in CD4+ lymphocytes (117).

D-analogs have been shown to inhibit cytokines IL-1, IL-6, TNF-alpha and particularly IL-12 (118). At the cellular level, D-hormone may directly or indirectly reduce the expression of Th1 helper cells by inhibition of IL-12 from monocytes (119). Therapy with alfacalcidol or calcitriol results in increased production of Th2 helper cells, which produce bone-protective cytokines like IL-4 and IL-10 (120). D-analogs have been shown to have a protective effect on osteoblasts against TNF-alpha-induced cell death (101).

Five intervention trials aimed at quantifying the effect of vitamin D and its metabolites on the clinical expression of RA have been published. Andjelkovic et al. (121) (alfacalcidol 2 µg/day/3 months), Brohult et al. (122) (vitamin D 2500 µg/day/1-2 years) and Dottori et al. (123) (25(OH)D 50 µg/day/1 month) have shown reductions in disease activity or pain symptoms, whereas Yamauchi et al. (124) (alfacalcidol 2 µg/day/4 months)

and Hein et al. (125) (alfacalcidol 1 µg/day/2 months) have not. With respect to these contradictory findings, the exact role of alfacalcidol in the management of RA remains unclear.

Various clinical studies have investigated the efficacy of alfacalcidol in corticosteroid-induced bone loss, regardless of the underlying disease. The main results of its use in RA have been to preserve bone mass, not to increase it (126). Gukasian et al. (127) reported an analysis of the anti-osteoporotic efficacy of alfacalcidol in 50 patients with RA. 30 RA patients received alfacalcidol (0.75-1.0 µg/day) for 12 months and 20 control RA patients received a placebo. Alfacalcidol stabilized bone mineral density at the femoral neck and lumbar spine. A significant BMD increase was observed in those areas of the proximal femur where cortical bone tissue prevails. In a double-blind, placebo-controlled comparative trial of 16 weeks by Yamauchi et al. (91), 1.0-2.0 µg/day alfacalcidol revealed 10% more patients with improvement compared with the placebo group, but the difference between them did not reach significance. The OKT-4/OKT-8 ratio was found not to change in patients whose initial value was normal, whereas it decreased after alfacalcidol treatment in patients whose initial values were higher. Bone mineral density was conserved in the alfacalcidol group. Among all trials, taking efficacy on bone loss into consideration, a dose of 1.0 µg/day was judged to be suitable for safe, long-term treatment with regard to the limited risk of hypercalcemia.

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