VITAMIN D : ACTIONS FOR THE NEW MILLENNIUM

Harbans Lal , Rajesh Pandey and S.K. Aggarwal

Department of Biochemistry, Pt. B.D. Sharma PostGraduate Institute of Medical Sciences, Rohtak (Haryana)

ABSTRACT

The past two decades have witnessed new facets in vitamin D metabolism. Free vitamin D₃ till now considered to be limited to animal sources, has been shown to occur in plants. Further, the extrarenal synthesis of active vitamin D₃ or calcitriol has been documented in physiological as well as in certain pathological conditions. At the cellular level, calcitriol acts through a dual mechanism. The genomic pathway involves the vitamin D receptor (VDR) which is of ubiquitous distribution and linked with various diseases. Besides, a nongenomic VDR - independent pathway also exists. The biological effects of calcitriol can no longer be dismissed by merely referring to calcium/bone homeostasis. It enhances the perinatal growth and suppresses the abnormal growth, as well. The effects on the endocrine, immune, cardiovascular, reproductive and nervous systems have also been characterized. It is compelling to believe that calcitriol and/or its analogues will find extensive applications at least in the treatment of osteoporosis, perinatal growth retardation, neoplasia, psoriasis, insulin resistance and transplantation.

KEY WORDS: Vitamin D, calcitriol, vitamin D receptor, diltanoids, growth, nonclassical effects.

Vitamin D has long been considered to be exclusive amongst the various micronutrients, the foremost reason being that it is derived by the photochemical conversion of the precursor sterols. Fortified foods and foods of animal origin, though are considered to be the only dietary sources, recently vitamin D (as free D₃) has also been shown to be present in the leaves of the tomato plant. These leaves which are usually discarded thus have a potential use in human and/or veterinary medicine (1).

Activation

Ultraviolet irradiation of the skin produces a family of secosteroids called vitamin D. Sunlight is not only the major provider but also the most

important regulator of its cutaneous synthesis. This may be a reason that chronic exposure to sunlight does not result in vitamin D intoxication (2). Whatever be the source of vitamin D, it circulates in the blood bound to α - globulin called vitamin D - binding protein (DBP). In the liver, vitamin D is converted to 25 - hydroxy vitamin D [25 - (OH)D] or calcidiol by the enzyme 25 -hydroxylase. Subsequently, by the action of 1, α - hydroxylase, 25 - (OH)D is converted to 1 α , 25 - dihydroxy vitamin D [1 α , 25 - (OH)₂ D] or calcitriol (active vitamin D), in the kidney. This critical step is under autoregulation by circulating calcitriol. Parathyroid hormone (PTH), hypocalcemia as well as hypophosphatemia are the other important positive modulators (3).

The synthesis of calcitriol is no longer considered to be restricted to the kidneys. Physiologically, the placenta is a major site of extrarenal calcitriol production. Further, an ectopic source is also likely, at least in certain pathological conditions such as peritonitis, inflammatory arthritis, tuberculosis, leprosy, fungal infections, silicon -

Author for correspondence

Dr. Harbans Lal
15/8 FM, Medical Enclave
Rohtak 124 001
E-mail: harbansh@ndb.vsnl.net.in

induced granulomas and lymphomas. The extrarenal $1, \alpha$ - hydroxylase is independent of the classical regulators of its renal counterpart (3,4).

Mode of action

At the cellular level, calcitriol regulates gene expression by acting through its specific receptors called vitamin D receptor (VDR). This is the well - characterized genomic pathway. However, a distinct non - genomic pathway has also been described to explain such varied effects as the rapid increase in cytosolic calcium (transcaltachia), phosphoinositide turnover as well as the induction of protein kinase C, phospholipase A_2 , guanylate cyclase and alkaline phosphatase (5-7). The two pathways however, may not be functionally independent and may involve a 'cross - talk' (8).

Deltanoids

The 'deltanoids' is a term coined for the various analogues of vitamin D and/or calcitriol, which may have dual mode of action, i.e. genomic as well as the nongenomic pathways as described above(9). They however, differ in their effectiveness in relative stimulation of the two pathways (5). The magnitude of biological response obtained from the various deltanoids has been determined by the following factors - (i) binding affinity for DBP ; (ii) role of the cell membrane or other site of activity ; (iii) relative ability to penetrate into the target cells ; (iv) binding affinity for the VDR ; (v) ability to convert into a competent transactivator; (vi) rate of metabolism ; and (vii) the experimental conditions (10). Considering the above effects of deltanoids a variety of them have now emerged and their structures have been manipulated to give desirable clinical effects (11).

The vitamin D endocrine system

The classical target organs of calcitriol are the intestine, the bone, the kidneys and the parathyroid glands, whose complex interplay serves to maintain normocalcemia as well as bone homeostasis. As shown in Fig. 1, hypocalcemia stimulates the parathyroid gland to secrete PTH as

well as chromogranin A (CgA), a regulatory protein that inhibits the stimulated secretion of PTH (12). The PTH in turn enhances the renal synthesis of calcitriol by stimulating $1, \alpha$ - hydroxylase. The calcitriol thereby increases the intestinal and renal calcium absorption, besides bone resorption, to restore normocalcemia. Excess calcitriol however, checks the synthesis of PTH as well as thyrocalcitonin and the scheme of events are reversed during hypercalcemia (3,13).

Fig. 1. Role of calcitriol in the physiological response to hypocalcemia.

Normal bone density is maintained when the rate of bone formation equals the rate of resorption and the net effect of calcitriol on bone

depends on serum calcium levels. Various genes are regulated by calcitriol in bone cells and their protein products modulate bone mineralization as well as resorption (8). Further, calcitriol has been shown to regulate the effect of growth hormone on osteoblasts (14). Besides the hormonal regulation, the physico-chemical exchange of calcium between bone fluid and extracellular fluid is also critical in determining the set point, i.e. mean serum calcium at steady state (15). Recently, the contribution of VDR gene polymorphisms to postmenopausal osteoporosis, hereditary vitamin D - resistant rickets as well as sporadic primary hyperparathyroidism has been recognized and is being further explored (16-18).

Non-classical effects

Various other functions have been assigned to calcitriol which are unrelated to calcium metabolism. These non - classical effects have been substantiated by the fact that the distribution of VDR is virtually ubiquitous and includes bone (cortex / marrow), intestine, kidney, parathyroid gland, pancreas, breast, gonads, placenta, thyroid gland, immune cells, brain, muscle, heart, skin as well as various cancer cells (5).

Growth

Enough evidence has accumulated to suggest the effect of vitamin D / calcitriol on nonskeletal growth by regulating cell proliferation and differentiation - (i) calcitriol regulates polyamine synthesis, cAMP / cGMP levels, prostaglandin synthesis, creatine kinase activity, membrane protein synthesis and the levels of calmodulin (4,19) ; (ii) the receptors for various growth factors may be modulated by calcitriol in its target cells (14) ; (iii) by upregulating the protein connexin 43, calcitriol may affect gap junctional intercellular communication (20) ; (iv) modulation of proto-oncogene expression and antitumor activity (19) ; (v) various systemic effects (to be discussed later) unexplained by the skeletal actions; and (vi) direct demonstration of enhancement of cellular growth. For example, calcitriol modulates the synthesis of various muscle proteins and plays a crucial role in adipocyte differentiation, as well (4,21).

Several experimental studies conducted in our laboratory have also supported the role of vitamin D on nonskeletal growth. It has been shown that vitamin D supplementation in albino rats results in not only a gain in body weight but also in increased liver weight, hyperplasia of the liver cells and decreased liver cell size (22). Further, the pups of pregnant female rats on vitamin D supplementation weighed more at birth, the effect however, was more conspicuous from day 10 onwards. An increase in the mean weight of the liver, muscle and brain also occurred. However, compared to the liver, optimum growth in the brain/muscle required a lower dose of vitamin D supplementation. This variation could be attributed to the differences in the intrinsic growth profile of various organs as well as their sensitivity to vitamin D metabolites (23). Preliminary observations in human studies also showed that pregnant women receiving vitamin D supplementation gave birth to babies with greater weight and improvement in other anthropometric measurements (24).

In lactating rats also, vitamin D supplementation led to enhanced skeletal as well as soft tissue growth supporting the view that the mammary gland is one of the target tissues for calcitriol. It also indicated that improved milk yield, as observed in the study, might be chiefly responsible for growth acceleration in the pups because direct vitamin D supplementation to the pups failed to improve their growth (25). The true relationship between vitamin D and the perinatal growth was established in a subsequent study which confirmed the onset of anabolic action of vitamin D during gestation. It also showed that the various organs differ in their time course of perinatal growth in a given species as well as in different species (26).

Calcitriol is therefore an important growth - link in the maternal - placental- fetal unit (Fig. 2). In the mother, calcitriol stimulates the pituitary gland as well as the pancreas to secrete prolactin and insulin respectively, which, along with calcitriol exert mammatrophic effects. Further, insulin favours protein anabolism. Compounded by the effect of

Fig. 2. Calcitriol (CT) - a growth link in the maternal-placental-fetal unit.

calcitriol on calcium metabolism, the lactational performance is improved quantitatively as well as qualitatively, supporting the neonatal growth. On the other hand, calcitriol promotes the growth of the placenta which itself is also a source of calcitriol since placenta has now been well documented as an extrarenal source of calcitriol (4). A direct anabolic effect of calcitriol on the intrauterine fetal growth also occurs. Both of these actions encourage the fetal growth, extending into the neonatal period (27,28).

The biological effects of calcitriol are not restricted to the stimulation of normal skeletal and soft tissue growth, but the micronutrient has been shown to have various other effects, as well (Table 1).

Protection from abnormal growth

Extensive research over the past two decades has revealed that calcitriol causes a dose - dependent inhibition of cell proliferation. Further, it switches the cellular activity from proliferation to differentiation. The antiproliferative and pro - differentiating effects of calcitriol have been

correlated with cellular VDR levels, the relationship becoming stronger with a higher receptor content (29).

Table 1. New aspects of vitamin D metabolism

- Identification of plant sources - Extrarenal synthesis - Genomic and nongenomic modes of cellular action - Ubiquitous distribution of VDR - Diseases linked to VDR alleles - Evolution of diltanoids - Increased perinatal growth - Decreased abnormal growth – use in neoplasia and psoriasis - Increased glucose tolerance – potential use in DM - Immunomodulation – use in autoimmune disorders and transplantation - Hemodynamic, reproductive and neuronal functions

Vitamin D status is an important consideration which can influence the colonic malignant transformation process, a condition known to be multifactorial. Studies have suggested a protective role of vitamin D against colon cancer as well as small adenomas (30,31). Further, suitable diltanoids may guard against the development of colorectal cancer in patients suffering from ulcerative colitis, thus providing a therapeutic option in disease - control in patients who relapse despite conventional therapy (32). It is also emphasized that the evaluation of vitamin D/ diltanoids in colon cancer has helped in unravelling crucial details regarding the dual mechanism of action of this micronutrient (33).

Calcitriol has also been shown to inhibit the growth of renal cell carcinoma (34). An interesting speculation is that calcitriol possibly exploits the anti - cancer activity of the immune system to back up its role against renal cancer. This is based on the fact that calcitriol enhances the activity of the VDR - positive immune cells. Further, it increases the sensitivity of target cells to various cytokines secreted by immune cells. The corollary is that the calcitriol - cytokine synergism may provide a rationale for using diltanoids in immune anti - cancer therapy (35,36).

Evaluation of the role of calcitriol in breast cancer has produced far - reaching results - (i) majority of the breast tumors possess VDR (37) ; (ii) VDR - positivity is linked with a longer disease - free survival following treatment, however, it is also linked with the ability of tumor cells to grow as bony metastases (38,39) ; (iii) calcitriol may decrease the sensitivity of cancer cells to autocrine growth factors (40) ; (iv) both the extracellular as well as the intracellular calcium concentration may play a pivotal role in mediating the effect of calcitriol on breast cancer cells (41); and (v) co-operativity is likely between the actions of calcitriol and sex-steroids on malignant breast cells (39). This is supported by the finding that the combined use of diltanoids and antiestrogens may be useful in estrogen receptor - positive breast tumors, whereas diltanoids alone might suffice in receptor - negative tumors (42).

Carcinoma of the prostate is another hormone - dependent tumor in which some beneficial role for calcitriol has emerged. Certain endocrine factors produced by prostate cancer cells have been shown to disturb the regulation of serum calcitriol (43). The cancer cells possess VDR and calcitriol exerts antiproliferative and differentiating effects on prostatic epithelial cells as well as on stromal cells. The effect on the latter is however, limited which is consistent with their lower receptor content (44). It has been suggested that determining the VDR status of the patient's tumor may be useful prior to therapy with diltanoids because only the receptor - positive tumors would show a favourable response (45).

The reports are also available about the role of calcitriol in various other neoplasms such as leukemia, retinoblastoma, lung cancer, endometrial cancer, head and neck cancer as well as melanoma (46-49).

The observation that calcitriol is a potent antiproliferative agent for keratinocytes and a stimulator of epidermal cell differentiation however, has encouraged its use in certain non - neoplastic cutaneous disorders involving abnormal proliferation / differentiation such as psoriasis (50). It has been reported that disturbances in vitamin D metabolism or in the responsiveness of epidermal cells to calcitriol may be involved in the pathogenesis of psoriasis. This has been confirmed by the effectiveness of oral as well as topical calcitriol / diltanoids on psoriatic lesions (51,52). However, since the disease requires long term treatment, it is essential to select those analogues which have minimal side effects.

Nonclassical systemic effects

Endocrine system : Although the parathyroid glands are the main endocrine target tissues for calcitriol, the pancreas, the pituitary, the gonads, the placenta and the thyroid gland have been shown to be the other endocrine target glands.

Experimental studies have demonstrated low circulating calcitriol levels in diabetes mellitus

(DM) (53). Ozono and associates (54) have reported that in the fed state, calcitriol increases the preproinsulin mRNA in the pancreas. This effect may or may not be calcium - mediated. Calcitriol also modulates the biosynthesis of several β -cell proteins implicated in the different steps of the insulin excitation-secretion coupling (55). An inhibitory effect of calcitriol on glucagon secretion has also been proposed (56). Based on the fact that calcitriol results in a significant improvement in the oral glucose tolerance of human diabetics, the use of calcitriol as an adjuvant to the conventional therapy in DM has been suggested (57). Further, the ability of calcitriol to improve insulin resistance is being exploited in patients with chronic renal failure, though its role in acute renal failure awaits evaluation (58,59).

It has been found that calcitriol-(i) enhances the prolactin gene transcription; (ii) inhibits thyrotrophin (TSH) stimulated iodide uptake by the thyroid gland; (iii) inhibits triiodothyronine - induced growth hormone secretion; and (iv) inhibits the release of norepinephrine from the adrenal gland (19,60). The physiological relevance of these findings however, is not yet clear.

Hemopoietic system: Studies have shown that calcitriol regulates the differentiation of hemopoietic stem cells into monocyte-macrophages and osteoclasts, whereas the bone marrow derived stromal cells may be directed towards the osteoblastic phenotype (61).

Vitamin D deficiency is associated with an impaired immune response and an increased susceptibility to infections. It has been shown that calcitriol promotes the early immune response by increasing the activity of the macrophages as well as the T-cells. In this regard, calcitriol behaves like a cytokine (62). Further, as a part of the feed back control of the immune response, it inhibits the activated T - cells and B - cells (63,64). By modulating the activity of natural killer (NK) cells, it may also regulate the immune response to viral infections and neoplasia (62).

It has been reported that DBP itself can be converted into a protein called macrophage activating factor (MAF) by sialidase (from T - cells) and β -galactosidase (from B - cells). The MAF contains N - acetylgalactosamine as the remaining sugar moiety. However, cancer cells in some patients secrete an enzyme called α -N-acetylgalactosaminidase that causes DBP-deglycosylation. The DBP then loses its MAF-precursor activity and the cancer patients become immunosuppressed. The α -N-acetylgalactosaminidase as well as the MAF-precursor activity of DBP in blood may serve as a diagnostic and prognostic indicator in cancer patients (65). The other important functions of DBP are its ability to bind G-actin, thereby preventing its polymerization into F-actin after cellular trauma as well as the enhancement of complement-mediated chemotaxis (66).

The efficacy of calcitriol has also been shown in autoimmune diseases such as psoriatic arthritis, as well as in controlling graft rejection (67,68).

Cardiovascular system: Studies concerned with the effect of calcitriol on blood pressure though are inconclusive, calcitriol has been implicated in the increased peripheral resistance of gestational hypertension in the third trimester of pregnancy (69,70). Marya and associates have stated that in pregnant women receiving vitamin D supplementation, the blood pressure is lowered although the incidence of toxemia may be unaffected (71). Hopefully, when the role of calcitriol on hemodynamics is clearly defined, suitable diltanoids may find their application in cardiovascular disorders.

Other systems: Evidence shows that vitamin D may be essential for normal reproductive functioning (72). Retardation of spermatogenesis in vitamin D deficiency has been found to be corrected by an optimal vitamin D supplementation (73).

Since the cutaneous synthesis of vitamin D depends on sunlight, it has also been called as "soltriol". Its effects on the nervous system have led researchers to propose a sun-skin-soltriol regulated

neural "timing circuit". Thus, the organizational as well as activational effects of calcitriol on the developing and mature brain regions, respectively, are likely to regulate neuronal function including biorhythms (74). Further, the discovery that calcitriol induces the synthesis of nerve growth factor, may open new vistas for the management of neurodegenerative diseases (50).

Future perspectives

Keeping in view the diverse effects of calcitriol, it is expected that besides osteoporosis, calcitriol and/or the deltanoids may find extensive applications in various other pathological conditions, as well. Its property to enhance perinatal growth

may be exploited in cases of perinatal growth retardation. Though its use in psoriasis has already been practised, the ability to suppress abnormal growth may also be utilised in various neoplastic conditions. The efficacy to enhance glucose tolerance as well as the modulation of immune response holds good therapeutic promise in cases of diabetes mellitus and organ-transplantations, respectively. Finally, as the details of the role of calcitriol in the cardiovascular and nervous system are unravelled, one may even expect its use in hypertension and neurodegenerative diseases. Thus, the focus on vitamin D research in the millennium will be in the aforesaid conditions besides its possible use in certain other pathological states.

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