The pleiotropic actions of vitamin D

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Summary

General knowledge of the role of vitamin D3 in human physiology has been shaped by its discovery as a preventive agent of nutritional rickets, a defect in bone development due to inadequate uptake of dietary calcium.

Studies on the function of the biologically active vitamin D3, 1,25-dihydroxyvitamin D3, have been greatly accelerated by the molecular cloning and structural analysis of the vitamin D3 receptor, which is a ligand-activated regulator of gene transcription. Molecular genetic techniques including genomics have helped to reveal that 1,25-dihydroxyvitamin D3 can control more than calcium homeostasis. It has effects on cellular differentiation and proliferation, and can modulate immune responsiveness, and central nervous system function. Moreover, accumulating epidemiological and molecular evidence suggests that 1,25-dihydroxyvitamin D3 acts as a chemopreventive agent against several malignancies including cancers of breast, prostate and colon.

KEY WORDS: vitamin D, pleiotropic actions, bone.

Introduction

Vitamin D can be produced in adequate amounts by moderate exposure of skin to solar ultraviolet X rays. Exposure to sunlight remains an important source of vitamin D, as many people in northern countries become deficient in circulating 25(OH)D3 during winter, and therefore deficient in 1,25(OH)2D3 synthesized in peripheral tissues (1). Vitamin D has been widely known for decades for its primary physiological role in regulating calcium homeostasis. However, accumulating evidence from epidemiological, animal, cellular, biochemical and, most recently, molecular genetic studies has revealed new actions of vitamin D.

Vitamin D can regulate the proliferation and differentiation of a wide variety of cell types, which has led to the analysis of the potential therapeutic uses of its synthetic analogues as anticancer agents, and as modulators of immune and nervous system function. These lines of investigation have been accelerated by two recent developments: the determination of the crystal structure of the vitamin D receptor and the use of large-scale gene expression profiling with microarrays to identify the molecular genetic events underlying vitamin D action. Here we will focus on the impacts of recent experimental and technological advances on the potential uses of its analogues in cancer therapy and prevention, in the treatment of autoimmune disorders, and as neuroprotective agents.

Moreover, we also discuss the vitamin D status in inflammatory bowel disease (IBD), infectious diseases, asthma, diabetes mellitus type I and II.

Vitamin D receptor

By 1975, the presence of the vitamin D receptor (VDR) was confirmed in the nuclei of cells incubated with radiolabelled hormonal 1,25-dihydroxyvitamin D3 [1,25(OH)2D3] (2, 3). The cDNA encoding the human vitamin D receptor (VDR) was cloned in 1988 (4), and confirmed that, similar to other steroid receptors, it is a member of the nuclear receptor family. Nuclear receptors are ligand-activated regulators of gene transcription (5) with a conserved domain structure. The highly conserved DNA-binding domain (DBD) contains two zinc fingers that form a single structural domain containing an α-helical reading head that controls specific DNA sequence recognition. The VDR ligand-binding domain (LBD) not only binds ligand but also contains a ligand-regulated C-terminal AF-2 domain (activating function-2) that is essential for its capacity to activate transcription. Similar to several nuclear receptors, the VDR functions as a heterodimer with members of the retinoid X receptor (RXR) family of receptors. Strong interactions between VDR and RXR LBDs are essential for ligand-dependent dimerization and high-affinity DNA binding.

Nuclear receptors regulate target gene transcription by ligand-controlled recruitment of several accessory proteins known collectively as coregulators. Coregulators are essential for the histone modifications, chromatin remodeling and recruitment of RNA polymerase and ancillary factors necessary for initiation of transcription. Nuclear receptors regulate transcription in part by binding specific DNA sequences called hormone response elements (6).

Chemopreventive action of 1,25(OH)2D3

a) Molecular evidences

Microarray studies have provided insights into the molecular events underlying the chemopreventive effects of 1,25(OH)2D3. Gene expression profiling revealed that EB1089 treatment induced the growth-arrest and DNA damage gene (GADD45a) in head and neck squamous cell carcinoma (HNSCC) cells in culture (7, 8), and in tumour xenografts of a mouse model of HN-SCC (8). Induction of GADD45a expression was recently observed in studies of the antineoplastic effects of 1,25(OH)2D3 in insulinoma cells (9). Ablation of the GADD45a gene in mice disrupts normal DNA repair and maintenance of global genomic stability (10). This suggests that treatment with 1,25(OH)2D3...
or its analogues has genoprotective effects; i.e. they protect the genome against accumulation of mutations that underlie cellular transformation and cancer progression. This notion is supported by observations that EB1089 induces expression of several genes controlling redox balance in HNSCC, including glucose-6-phosphate dehydrogenase, which lies at the head of the pentose phosphate shunt, a source of reducing equivalents, glutathione peroxidase and thioredoxin reductase (11). The enzymatic activities encoded by these genes are also induced in treated cells (our unpublished results). Induction of thioredoxin reductase activity has also been observed in 1,25(OH)2-treated prostate and breast carcinoma cells (12, 13). These results are consistent with the observation that treatment of leukemic cells reduces intracellular levels of reactive oxygen species (ROS) (14). The protective effects of 1,25(OH)2D3 against oxidative DNA damage may represent a physiological feedback loop to the photochemical synthesis of vitamin D in skin by ultraviolet light, which is a DNA damaging agent and an inducer of ROS (15). Indeed, direct photoprotective effects of 1,25(OH)2D3 were observed in UV-irradiated keratinocytes in vitro and in mouse skin, and were linked to increased expression of free radical scavenging metallothionein (16).

1,25(OH)2D3 also stimulated expression of the gene encoding the NRF2 transcription factor (17). NRF2 is induced by a number of chemopreventive agents, and in turn stimulates expression of several phase II detoxifying enzymes. Ablation of the NRF2 gene in mice rendered them more sensitive to carcinogenesis and eliminated the beneficial effects of chemopreventive agents (17). An enhancement of xenobiotic metabolism by 1,25(OH)2D3 is also consistent with its direct induction of several genes encoding members of the cytochrome P450 family of oxidative enzymes (18-20).

b) Evidences in animal studies

Animal studies have provided evidence of chemopreventive actions of 1,25(OH)2D3 analogues in models of colon, hamster cheek pouch, hepatocellular, gastrointestinal and skin carcinogenesis (21-26). Chemoprevention likely arises in part from the capacity of 1,25(OH)2D3 to regulate cellular differentiation and proliferation (27). The potent growth inhibitory effects of 1,25(OH)2D3 analogues on cells in culture (28-31, 7) and in xenograft models of cancer (8, 32-35) coupled with their low subsequent risk of colorectal cancer or adenoma, the cancer precursor, have found a lower risk associated with higher 25(OH)D3 concentrations (47-52), with one exception (53). In studies that have examined circulating 25(OH)D3 levels and subsequent risk of colorectal cancer or adenoma, the cancer precursor, have found a lower risk associated with higher 25(OH)D3 concentrations (47-52), with one exception (53). In the Washington County, Maryland cohort, an inverse relation between circulating 25(OH)D3 was observed in the first eight years after the blood sample collection (47), but no associ-
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...was found to be inversely associated with colorectal cancer risk (59), but not with prostate cancer risk (84). This finding suggests the effect of dietary vitamin D may differ between prostate and colorectal cancer.

Immuno-modulatory effects of 1,25(OH)2D3

The VDR is expressed in most cells of the immune system, including T lymphocytes, and antigen-presenting cells (APC) such as macrophages and dendritic cells (87-91). Growing evidence indicates that 1,25(OH)2D3 is a modulator of immune system function, consistent with its capacity to control cellular differentiation. Helped by Th (cells are central to all antigen-specific immune responses. The microenvironment in which naive Th cells develop determines which of 2 subtypes predominates (Th1 or Th2). Th1 and Th2 cells are direct targets of 1,25(OH)2D3. Quiescent CD4+ T cells expressed VDRs but only at low concentrations (52). 1,25(OH)2D3 increased the proliferation of purified Th cells and decreased the production of IFN-γ, IL-2, and IL-5 (92). In Th2 cells, 1,25(OH)2D3 increased the production of IL-4 (92). The effectiveness of 1,25(OH)2D3 for suppression of autoimmune diseases in vivo has been shown to depend on IL-2 (93) and IL-4 (94) secretion. CD4+ T cells from VDR knockout (KO) mice (which do not respond to vitamin D) produced more IFN-γ and less IL-2, IL-4, and IL-5 than did CD4+ T cells from wild-type (WT) mice (95). Consistent with this finding, in vivo antigen stimulation of VDR KO mice resulted in increased antigen-specific IFN-γ response (95). Furthermore the mixed lymphocyte reaction with CD4+ T cells from VDR KO mice was twice that with CD4+ T cells from WT mice (95). The data suggest that T cells from VDR KO mice secrete more IFN-γ and less of the Th2 cytokines IL-4 and IL-5. Furthermore, 1,25(OH)2D3 reduced Th1 cell-associated cytokine production and increased Th2 cell IL-4 secretion. In the absence of vitamin D signaling, the T cell compartment has a potentially stronger Th1 phenotype.

One study of mice in which the VDR gene had been ablated concluded that altered immune responses were an indirect consequence of VDR disruption because they could be restored by normalization of calcium homeostasis (96). However, another study revealed abnormal development of pro-inflammatory T helper 1 (Th1) cell development in VDR knockout mice (96). Moreover, mice rendered 1,25(OH)2D3 deficient by knockout of the gene encoding 25-hydroxyvitamin D3 1α-hydroxylase were deficient in peripheral T lymphocytes (98). These findings help to provide a molecular basis for the therapeutic potential of 1,25(OH)2D3 analogues in treatment Th1-stimulated autoimmune diseases. Indeed, in mice, 1,25(OH)2D3 can prevent systemic lupus erythematosus, experimental autoimmune encephalomyelitis (EAE), collagen-induced arthritis, inflammatory bowel disease and autoimmune diabetes (96). For example, treatment of mice with myelin basic protein induces EAE, a multiple sclerosis-like disease whose progression is driven by activated T cells. Dietary 1,25(OH)2D3 prevented the onset of EAE and the progression of established disease (99, 100). The most firmly established clinical use of 1,25(OH)2D3 analogues is in the treatment of the Th1-driven chronic inflammatory skin disease psoriasis, which affects 2% of the population. 1,25(OH)2D3 analogues account for 50% of all drugs used to treat mild to moderate disease. Analogues are used topically, and one of the most thoroughly tested is the secosteroidal compound calcipotriol (101-103), which is effective either alone or when administered in combination with anti-inflammatory steroids (104).
Inflammatory bowel diseases (IBD) and vitamin D status

In IBD, the immune system-mediated attack is against the gastrointestinal tract (105, 106). T cells that preferentially produced the Th1 cytokines (IL-2, IFN-gamma, and tumor necrosis factor alpha) were shown to transfer Crohn’s disease-like symptoms to naive mice (107, 108), and the production of Th1 cytokines is associated with IBD among humans subjects (109). In conventional animal facilities, IL-10 KO mice develop enterocolitis within 9-12 wk of life (110). Approximately 30% of IL-10 KO mice die after the development of severe anemia and weight loss (110).

Vitamin D deficiency accelerated the development of IBD symptoms among IL-10 KO mice (111). In the clinical practice, vitamin D deficiency is common among patients with Crohn’s disease, even when the disease is in remission (112, 113). It is unclear why vitamin D deficiency occurs more frequently in IBD; it is probably attributable to the combined effects of low vitamin D intake, malabsorption of many nutrients including vitamin D, and decreased outdoor activities in climates that are not optimal for vitamin D synthesis in the skin. The standard treatments for patients with IBD include short-term high-dose and long-term low-dose prednisone therapy (113, 105, 106). Prednisone and other corticosteroid therapies result in decreased bone mineral density, which increases the risks for vertebral fractures. Vitamin D deficiency has been linked to bone loss among patients with IBD, and bone loss is a problem for up to 50% of patients with IBD (112, 113). A placebo-controlled study showed that calcium and vitamin D supplementation were effective for preventing bone loss among patients with Crohn’s disease (112, 113, 114). The hormonally active form of vitamin D, 1,25-dihydroxyvitamin D3 (1,25(OH)2D3), is known to increase bone mineralization when administered to experimental animals (115) and human subjects (116). Therefore, vitamin D and/or 1,25(OH)2D3 supplementation is warranted for patients with IBD, to maintain bone mineral density and to normalize circulating vitamin D concentrations.

Vitamin D and infectious diseases

On the basis of the ability of 1,25(OH)2D3 to suppress the development of various autoimmune and lymphoproliferative diseases and to prolong allograft survival, 1,25(OH)2D3 has been recognized as an immunosuppressive hormone (117, 118). However, 1,25(OH)2D3 has been shown to have no effect on the susceptibility of mice to infections with Herpes simplex virus or Candida albicans (115). The doses of 1,25(OH)2D3 chosen were the same doses that had been shown previously to prolong allograft survival (115, 118). Surprisingly, little is known about the effect of vitamin D status on the ability of the host to fight infections. There is an interesting but mechanically unsubstantiated link between vitamin D deficiency and cases of tuberculosis (119). Experimentally, vitamin D deficiency and host resistance to infectious diseases have not been studied extensively. One experiment in VDR KO mice showed that VDR KO mice exhibited increased granulomatous inflammation (slightly more severe infection) during Schistosoma mansoni infection, compared with WT mice (95). Little is known about the role of vitamin D and 1,25(OH)2D3 in regulating immune responses to infectious diseases. What is known is somewhat paradoxical, on the basis of the ability of this nutrient/hormone to suppress autoimmune diseases and prolong transplant survival.

Vitamin D and experimental asthma

Experimental allergic asthma was induced in VDR KO, WT, and 1,25(OH)2D3-treated WT mice. WT mice developed asthma, which was characterized by many inflammatory cells infiltrating the lungs. Lung histopathologic scores reflected the amount of epithelial hyperplasia and inflammation on a scale of 0 to 4 (maximum). The Th2 cell-driven disease experimental asthma failed to develop in VDR KO mice (120). 1,25(OH)2D3 treatment of WT control mice had no effect on asthma severity. VDR KO mice did develop antigen-specific Th2 cell responses in the periphery but failed to develop lung inflammation or airway hyperresponsiveness (120). The absence of vitamin D signaling through VDRs protected these mice from developing experimental asthma. The Th2 cell response develops in the absence of VDRs; however, Th2 cells may not traffic to the lung and cause disease. It is also possible that epithelial cells in the lungs of VDR KO mice are unable to respond to an inflammatory challenge.

Vitamin D and diabetes

Several studies in rats and humans (121, 122) have demonstrated that vitamin D deficiency causes reduced insulin secretion, and that 1,25(OH)2D3 improves insulin secretion and consequently in glucose tolerance (123). In vitamin D-deficient rats, glucose tolerance and insulin secretion were improved with 1,25(OH)2D3 treatment (124). In gestational diabetes mellitus, Rudnicki and Moldt-Petersen (125) reported that the glucose level decreased after intravenous treatment with 1,25(OH)2D3. Vitamin D also corrects glucose intolerance and normalizes insulin sensitivity in uremic patients (126, 127).

a) Type 1 diabetes mellitus

Some studies (128, 129) suggested that vitamin and its metabolites act in the regulation of the endocrine and exocrine pancreas not only via the plasma calcium levels but also directly on the β-cells. 1,25(OH)2D3 may influence both endocrine and exocrine pancreatic function (130). The effects of 1,25(OH)2D3, a biologically active metabolite of vitamin D, and its analogues have been examined regarding binding to nuclear VDR (nVDR) and membrane VDR (mVDR), through which they might induce genomic and nongenomic responses respectively. In humans, Baumgartl et al. (131) reported that serum 25OHD3 levels measured at matched time points throughout the year are lower in patients newly diagnosed with type 1 diabetes than in healthy controls. In 1999, the EURODIAB Substudy 2 Study Group (132) studied the correlation between vitamin D supplements during the first year of life with the development of type 1 diabetes. They reported that vitamin D supplement during the first year of life is associated with a decreased risk of type 1 diabetes. In another study, Stene et al. (133) investigated whether cod liver oil or vitamin D supplements taken either by the mother during pregnancy or by the child in the first year of life is associated with lower risk of type 1 diabetes in children. They found a lower risk of diabetes in children when mothers took cod liver oil during pregnancy. It was noted that newborn children of mothers who had taken cod liver oil during pregnancy had higher concentrations of 25(OH)D3 in the cord blood than children of mothers who had taken other vitamin D supplements during pregnancy (133). However, there was no significant protection from type 1 diabetes risks when infants were fed either cod liver oil or vitamin D supplements. They suggested that exposure in utero could be relevant for the development of type 1 diabetes. In addition, Hypponen et al. (134) assessed the risk of type 1 diabetes and vitamin intake during infancy of 10.821 children in Oulu and Lappland of
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northern Finland. They reported that dietary supplementtion is also associated with reduced risk of type 1 diabetes.

b) Type 2 diabetes mellitus

Vitamin D deficiency was linked to IGT (impaired glucose tolerance) and type 2 diabetes in humans many years ago (135, 136). These observations were confirmed in animal models, which demonstrated that pancreatic insulin secretion is inhibited by vitamin D deficiency (137). Several reports have assigned an active role to vitamin D in the functional regulation of the endocrine pancreas, particularly the beta cells. Not only receptors for 1,25(OH)2D3 are found in beta cells (138), but the effector part of the vitamin D pathway is also present in the form of vitamin D-dependent calcium-binding protein, also known as calbindin-D28k (139). The expression of calbindin-D28k has been shown to protect beta cells from cytokine-mediated cell death (140). Several studies have demonstrated a link between VDR gene polymorphisms and type 2 diabetes, although the findings differ from one population to another. A study in Bangladeshi Asians demonstrated that the ApaI RFLP (Restriction Fragment Length Polymorphism) influences insulin secretion in response to glucose (141), while associations between the VDR ApaI RFLP (restriction fragment length polymorphism) and higher fasting plasma glucose levels and glucose intolerance were observed in a community-based study of older adults without known diabetes (142). More recently, genotyping for TaqI, ApaI, BsmI and FokI RFLPs revealed that the BsmI RFLP is associated with high fasting glucose levels in older adults without known diabetes (143).

Effects of 1,25(OH)2D3 in the central nervous system

While 1,25(OH)2D3 can protect against progression of neurodegenerative disorders such as EAE through its effects on the immune system, recent evidence suggests that it can act directly on the central nervous system (CNS) itself. The VDR is widely expressed throughout the CNS (144), and is a strong inducer of nerve growth factor expression (145). Several studies have suggested that 1,25(OH)2D3 has neuroprotective effects. In vivo experiments in rodents have shown that 1,25(OH)2D3 retards age-related decreases in hippocampal neuronal density (146), and protects against neuronal cell death in a rodent model of stroke (147). Moreover, 1,25(OH)2D3 can act directly on primary cultures of rat hippocampal neurons to inhibit expression of markers associated with neuronal aging (148). Part of the neuroprotective effects of 1,25(OH)2D3 in the CNS may also lie in its capacity to protect cells from ROS. Studies in cultured rat neurons showed that 1,25(OH)2D3 protected against the neurotoxic effects of agents that caused oxidative damage by increasing intracellular levels of glutathione (149), consistent with its effects on redox balance observed in cancer cells.

Conclusions

Vitamin D action is not only involved in bone metabolism. The broad expression pattern of the VDR, and the widespread effects of its hormone on cellular differentiation and proliferation have opened up a number of new fields of investigation for basic researchers interested in 1,25(OH)2D3 function as well as those more concerned with the therapeutic potential of its synthetic analogues as chemopreventive agents against cancer and neuronal aging, as well as their potential in combating a number of autoimmune disorders.

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