

Review

Promise of vitamin D analogues in the treatment of hyperproliferative conditions

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Abstract

1 α ,25-Dihydroxyvitamin D₃ [1 α ,25-(OH)₂D₃; calcitriol] is best known as a hormone involved in calcium homeostasis but is also a potent antiproliferative agent in many cell types, particularly epithelial cells. 1 α ,25(OH)₂D₃ mediates its actions through a classic steroid hormone-like transcriptional mechanism by influencing the expression of hundreds of genes. Effects of 1 α ,25(OH)₂D₃ have been observed on expression of cell cycle regulators, growth factors and their receptors, apoptotic machinery, metastatic potential, and angiogenesis; all of which have some effect on hyperproliferative conditions. This minireview focuses on the anticancer potential of 1 α ,25(OH)₂D₃ and its analogues by summarizing the promising data from animal and human trials of 1 α ,25(OH)₂D₃ and some of the more interesting synthetic vitamin D analogues in the treatment of a variety of different animal cancer models and in human patients with advanced cancer. Optimal administration of vitamin D analogues is only just being achieved with high-dose intermittent administration overcoming bioavailability and hypercalcemia problems and combination therapy with cytotoxic agents (taxols and cisplatin), antiresorptive agents (bisphosphonates), or cytochrome P450 inhibitors being attempted. Although the potential of vitamin D as an antiproliferative drug has been realized in the treatment of psoriasis and in parathyroid cell hyperplasia associated with secondary hyperparathyroidism, the search for an anticancer treatment incorporating a vitamin D analogue remains elusive. [Mol Cancer Ther 2006;5(4):797–808]

Introduction

The nutritional term vitamin D was coined almost a century ago to denote an antirachitic substance that can be derived from dietary sources, but in mammals vitamin D is also made in the skin on exposure to sunshine containing UVB light. Today, the term vitamin D is more commonly but erroneously used as an abbreviation for its active form, 1 α ,25-dihydroxyvitamin D₃ [1 α ,25-(OH)₂D₃; also known as calcitriol], which is synthesized from vitamin D by a two-step enzymatic process. This metabolite, 1 α ,25-(OH)₂D₃, interacts with its ubiquitous nuclear vitamin D receptor (VDR) to regulate the transcription of a wide spectrum of genes involved in calcium and phosphate homeostasis as well as in cell division and differentiation. It is these latter actions that have attracted tumor biologists to explore the effects of vitamin D and to use it as an antiproliferative agent in cancer cells *in vitro* and *in vivo*. This minireview is designed to update the expert and nonexpert on (a) current views of the mechanism of action of 1 α ,25-(OH)₂D₃, (b) promising new anticancer vitamin D analogues, and (c) potential of vitamin D-related anticancer therapies.

Biochemistry of Vitamin D

Vitamin D₃ can be synthesized in the skin from 7-dehydrocholesterol (1) or be derived in the form of vitamin D₃ or vitamin D₂ (collectively known as vitamin D) from dietary sources. Vitamin D and its metabolites are mostly hydrophobic and thus require a protein (vitamin D-binding protein) for their transport in the aqueous environment of the bloodstream to sites of storage (adipose tissue) or sites of activation (liver and kidney) of sites of action. Vitamin D undergoes its first step of activation (i.e., 25-hydroxylation in the liver), a step carried out by both liver mitochondrial (CYP27A1) and microsomal cytochrome P450 isoforms CYP2R1, CYP3A4, and CYP2J3 (2–4). The product of the 25-hydroxylation step, 25-OH-D₃, is the major circulating form of vitamin D₃ and in humans is present in plasma at concentrations in the range 10 to 80 ng/mL (25–200 nmol/L; ref. 5). The main reason for the extended plasma half-life of 25-OH-D₃ is its strong affinity for vitamin D-binding protein, and the vitamin D-binding protein-null mouse shows accelerated rates of vitamin D clearance and low 25-OH-D₃ levels (6). Serum levels of 25-OH-D₃ represent a measure of the vitamin D status of the animal *in vivo* and have proven particularly valuable in

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epidemiologic studies because they inversely correlate with the incidence of various types of cancers (breast, prostate, and colon) as well as multiple sclerosis and diabetes (7). Recently, 25-OH-D levels have been reclassified into the following categories: (a) <25 nmol/L (<10 ng/mL) are considered to be indicative of vitamin D deficiency; (b) between 25 and 80 nmol/L (10-32 ng/mL) indicative of vitamin D insufficiency; (c) between 80 and 200 nmol/L (32-80 ng/mL) indicative of vitamin D sufficiency; and (d) >200 nmol/L (>80 ng/mL) indicative of vitamin D toxicity. The public health strategy has now become the use of health intervention approaches (sunlight exposure, D fortification, and D supplementation) to raise 25-OH-D levels in the whole population toward vitamin D sufficiency (8), a strategy that is predicted to reduce cancer incidence.

The circulating metabolite, 25-hydroxyvitamin D₃, is activated to the hormonal form, 1 α ,25-(OH)₂D₃, primarily in the kidney (2) and the synthesis of circulating 1 α ,25-(OH)₂D₃ in the normal mammal [human reference range, 37.5-150 pmol/L (15-60 pg/mL); ref. 5] is the exclusive domain of that organ. Patients with chronic renal failure exhibit frank rickets or osteomalacia due to a deficiency of circulating 1 α ,25-(OH)₂D₃ caused by lack of renal 1 α -hydroxylase, a situation that is reversed by 1 α ,25-(OH)₂D₃ hormone replacement therapy. The main component of the 1 α -hydroxylase enzyme is the cytochrome P450, CYP27B1 (9), which is defective in a rare human-dependent rickets (VDDR type I; ref. 10). *Cyp27b1* gene ablation in the mouse results in rickets (11). CYP27B1 is strongly down-regulated by its product, 1 α ,25-(OH)₂D₃, and up-regulated by parathyroid hormone (PTH) as part of the calcium homeostatic loop (12) and up-regulated by fibroblast-like growth factor 23 as part of the phosphate homeostatic loop (13).

Physiologic or pathologic situations in which extrarenal 25-OH-D₃-1 α -hydroxylase activity becomes prominent include pregnancy where placental 1 α -hydroxylase provides 1 α ,25-(OH)₂D₃ for the developing fetus or, in the granulomatous condition, sarcoidosis where sarcoid tissue expresses an unregulated 1 α -hydroxylase that results in elevated plasma 1 α ,25-(OH)₂D₃ levels causing hypercalciuria and hypercalcemia (14). Extrarenal 1 α -hydroxylase in sarcoid macrophages is induced by IFN- γ and other cytokines (15). There is also evidence that a few solid tumors express extrarenal 1 α -hydroxylase that in turn causes hypercalcemia, although the vast majority of hypercalcemia of malignancy is due to excessive production of PTH-related peptide (16). Recently, CYP27B1 mRNA and immunodetectable protein have been detected in many normal extrarenal tissues (e.g., skin, prostate, colon, and breast; ref. 17), where pathologic problems do not exist. The recognition of the wider distribution of extrarenal CYP27B1 has generated a new hypothesis that the enzyme exists to augment circulating 1 α ,25-(OH)₂D₃ with local production of 1 α ,25-(OH)₂D₃ (18, 19). Locally high concentrations of 1 α ,25-(OH)₂D₃ in certain sites, such as skin, prostate, and breast, are

believed to result in altered patterns of gene expression, which in turn limit cell division and lead to tissue-specific differentiation of particular cell types. This emerging physiologic role for extrarenal 1 α -hydroxylase emphasizes the importance of the circulating 25-OH-D pool that provides substrate for "target cell" enzyme as well as for the renal enzyme responsible for circulating 1 α ,25-(OH)₂D₃. Thus, the prevailing opinion is that the level of plasma 25-OH-D is more useful than the level of plasma 1 α ,25-(OH)₂D₃ for predicting the full spectrum of vitamin D actions around the body (20).

Irrespective of where 1 α ,25-(OH)₂D₃ is synthesized, it represents the sole active molecule responsible for the biological response inside vitamin D target cells. The functions of vitamin D have been shown to occur through a steroid hormone-like mechanism involving a nuclear VDR that specifically regulates the transcription of vitamin D-dependent genes coding for proteins that in turn regulate cellular events, such as intestinal calcium transport and cell division (ref. 21; Fig. 1). In the classic steroid hormone model, 1 α ,25-(OH)₂D₃ enters the cell by traversing the plasma membrane in a free form and binds tightly to the VDR inside the nucleus ($K_d = 2 \times 10^{-10}$ mol/L). The liganded or occupied VDR specifically targets only vitamin D-dependent genes by interacting with a specific sequence found upstream of the vitamin D-dependent gene known as a vitamin D-responsive element that is a tandem repeating oligonucleotide of 6 bp with a 3-nucleotide spacer, normally situated upstream of the 5' end of the vitamin D-responsive gene. A consensus vitamin D-responsive element (AGGT-CAnnnAGGTCA) is found in several positively regulated genes (e.g., osteocalcin), whereas more complex elements are found in the negatively regulated collagen type I and pre-pro-PTH genes. Vitamin D-responsive element-containing genes now number around several hundred, suggesting that 1 α ,25-(OH)₂D₃ through a VDR-mediated process regulates many physiologic processes besides intestinal calcium and phosphate homeostasis (22). Transactivation of genes requires a heterodimeric partnership with the retinoid X receptor and recruitment of a plethora of other transactivating proteins, termed the DRIP complex (23). The current model suggests that occupation of the ligand-binding domain of the VDR triggers a protein conformational change in the AF-2 domain of the COOH terminus of the VDR (24), which allows recruitment of positive transcription factors and/or shedding of transcriptional inhibitory factors that lead to increased formation of a transcription initiation complex and an increased rate of gene transcription.

Reports of the broad distribution of the VDR protein and other components of the vitamin D machinery in different tissues besides the classic targets of bone, intestine, and kidney have encouraged researchers to look for vitamin D involvement in other physiologic processes. This was further reinforced by reports of diverse effects of exogenous vitamin D analogues on biological systems *in vitro* and nonclassic tissues *in vivo*. Although it was unclear if

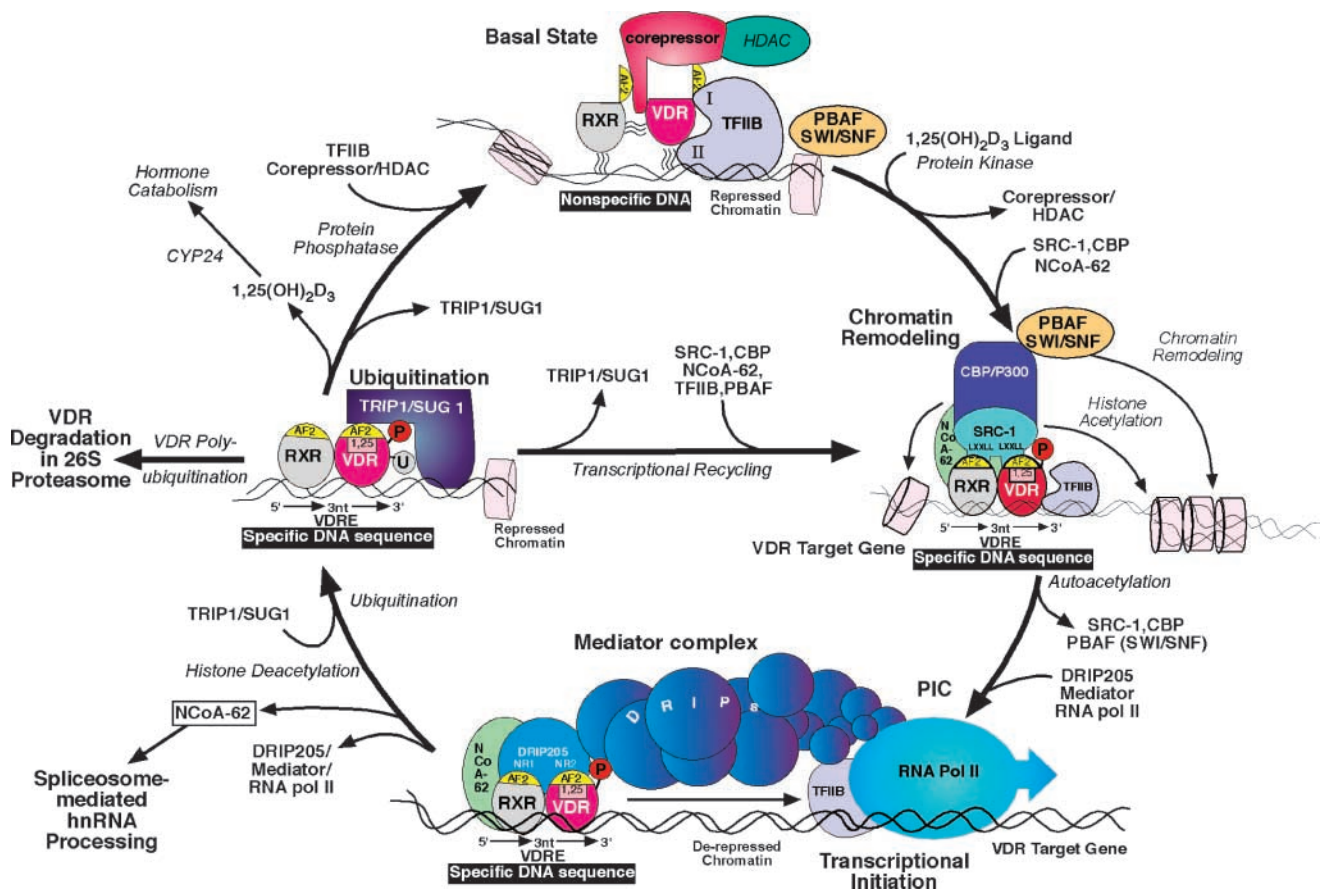


Figure 1. Model of the transcriptional mechanism of action of $1\alpha,25\text{-(OH)}_2\text{D}_3$ enters the picture at 1 o'clock interacting with the unliganded VDR-retinoid X receptor basal complex and locating the vitamin D-responsive element-containing vitamin D-dependent gene. The initial steps in activation involving conformational changes in the VDR and recruitment of coactivators are shown in the 3 o'clock complex. At this point, a SWI/SNF chromatin remodeling complex affects changes in chromatin allowing recruitment of the transcription initiation complex, including attraction of DRIPs shown at 6 o'clock. Active transcription of the vitamin D-dependent gene occurs. The complex at 9 o'clock is involved in turning over the VDR and recycling the rest of the transcriptional machinery. Note that the ligand $1\alpha,25\text{-(OH)}_2\text{D}_3$ is also subject to degradation by CYP24A1, an inducible cytochrome P450 found in the target cell. Reproduced from ref. 20; Figure 9 in Chapter 13 in "Vitamin D 2nd Edition" published by Elsevier, New York. With kind permission from G. Kerr Whitfield and Mark R. Haussler and Elsevier.

the effects were physiologic or pharmacologic, these observations have certainly broadened our view of vitamin D as more than just a calcemic hormone. The consensus view stemming from various mouse models and gene microarray experiments is that $1\alpha,25\text{-(OH)}_2\text{D}_3$ influences many physiologic processes (2), which are usually divided into:

- classic roles, which include the regulation of blood calcium and phosphate concentrations by actions on intestine, bone, kidney, and parathyroid gland, and
- nonclassic roles, which include cell differentiation and antiproliferative actions on various cell types, including bone marrow (myeloid and lymphoid), skin, muscle, and intestine.

Although most of the classic actions of vitamin D have been known for decades since dietary vitamin D deficiency was first shown, the nonclassic roles have only emerged from more subtle studies involving experiments

probing the mechanism of action of vitamin D at the molecular level, from studies of children with mutations of the VDR (VDDR type II) and from studies of the VDR knockout mouse (25).

24-Hydroxylation of both circulating precursor 25-OH-D₃ and hormonal form $1\alpha,25\text{-(OH)}_2\text{D}_3$ has been shown to occur *in vivo* to give short-lived 24-hydroxylated products with reduced biological activity (26). The virtually ubiquitous 25-OH-D₃-24-hydroxylase, now called CYP24A1, catalyzes a five-step, vitamin D-inducible, C-24 oxidation pathway that changes the vitamin D molecule into water-soluble truncated products, such as calcitric acid (27, 28). 24-Hydroxylation is an important step destroying excess precursor 25-OH-D₃ and inactivating $1\alpha,25\text{-(OH)}_2\text{D}_3$ inside target cells, suggesting that C-24 oxidation primarily exists as a target cell attenuation or desensitization process that constitutes a molecular switch to turn off vitamin D responses inside target cells (29). Consistent with this hypothesis is the finding that 50% of *Cyp24a1* null mice die prematurely of

hypercalcemia and nephrocalcinosis, whereas their surviving homozygous littermates show a greatly reduced ability to clear a bolus dose of [1β - ^3H] $1\alpha,25$ - $(\text{OH})_2\text{D}_3$ from their circulation compared with normal wild-type mice (30). The importance of target cell degradation has reinforced the utility of designing vitamin D analogues with modifications around C-24 to resist the catabolic effects of CYP24A1 and the screening of CYP24A1 inhibitors. Furthermore, the chromosomal region 20q13.2, which contains the *CYP24A1* gene, is amplified in certain human cancers justifying the development of CYP24A1 inhibitors (31). Such CYP24A1 inhibitors based onazole chemistry (Novartis, Vienna, Austria; ref. 32) and substrate-like competitors (Cytochroma, Markham, Ontario, Canada; ref. 33) have been developed and look promising, although there are also claims that the phytoestrogen, genistein, is an effective CYP24A1 inhibitor (34).

Rationale for Using Vitamin D Analogues in Cancer Therapy

There has been considerable interest shown by the pharmaceutical industry in the design and synthesis of

compounds, which mimic some or all of the biological actions of $1\alpha,25$ - $(\text{OH})_2\text{D}_3$ and acting through VDR-mediated mechanisms (2). This has been particularly true of the class of molecules known as low-calcemic vitamin D analogues that purportedly have reduced calcemic actions and enhanced antiproliferative activity. Such anticancer vitamin D molecules are the central focus of this minireview (examples provided in Fig. 2). Note that vitamin D analogues are now widely used drugs in the treatment of psoriasis, a hyperproliferative skin condition (35) and in suppression of secondary hyperparathyroidism and parathyroid hyperplasia resulting from chronic kidney disease (36). Typically applied vitamin D analogues used in psoriasis (e.g., calcipotriol) were designed to be susceptible to target and liver cell breakdown, making them less likely to cause calcemic side effects should they gain access to the general circulation. Systemically administered vitamin D analogues [e.g., 19-nor- $1\alpha,25$ - $(\text{OH})_2\text{D}_2$ and 1α - $\text{OH}-\text{D}_2$], used to suppress PTH synthesis in the parathyroid gland in the individual with chronic kidney disease, were designed to circulate for longer periods but are often operating in patients with

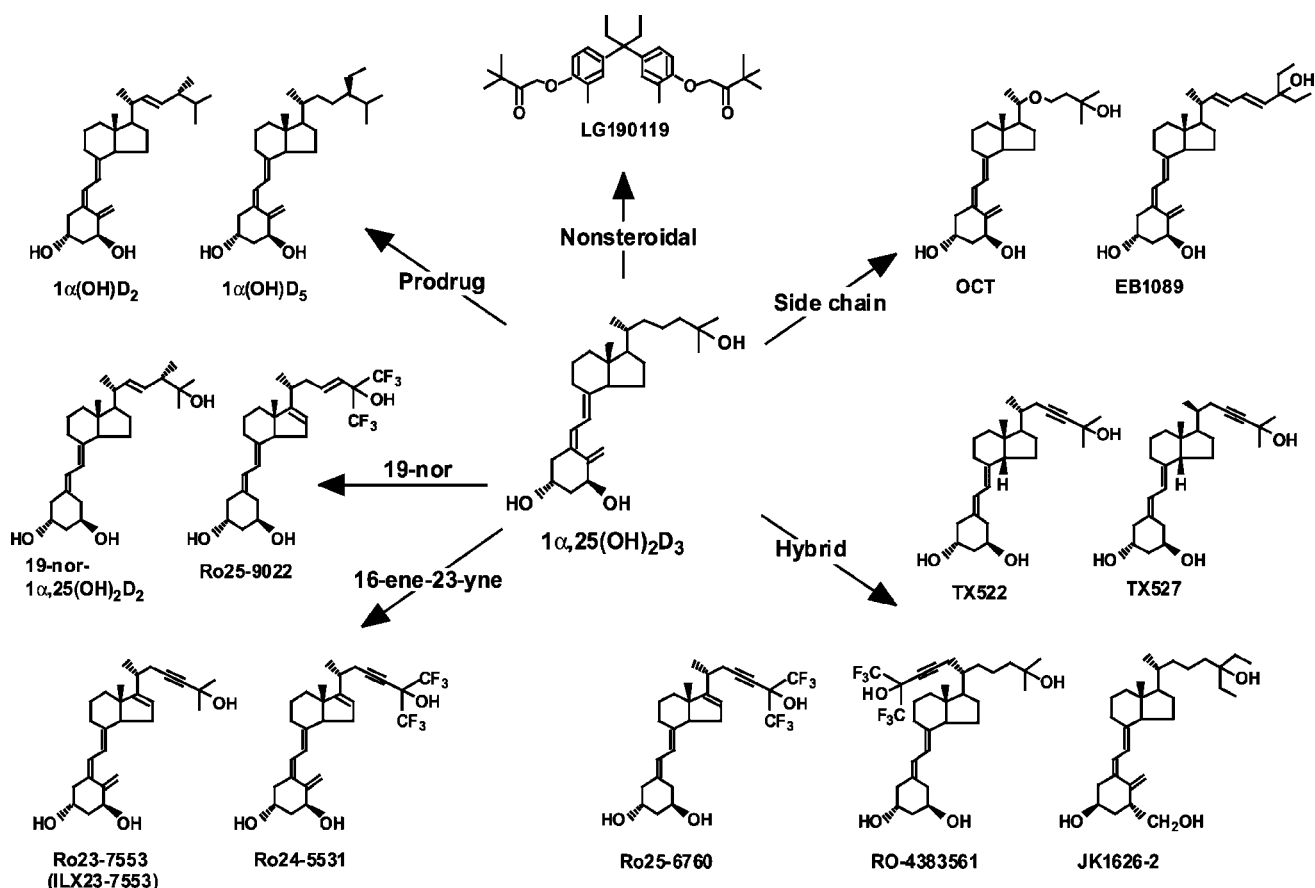


Figure 2. Chemical structures of potential anticancer vitamin D analogues referred to in the text are provided. Note that several different strategies have been used by the organic chemist to modify the properties of the vitamin D molecule. The most basic molecule is the prodrug version that is inactive as given but activated by CYPs *in vivo*. The most popular analogues have modified (hybrid) side chains and these include double side chain versions known as Gemini analogues. Many analogues possess side chains with double or triple bonds in the C-24 position that make them resistant to the actions of CYP24A1. The nonsteroidal vitamin D analogues were designed to occupy the VDR-ligand-binding pocket.

hypocalcemia where there may be a broader therapeutic window. Even so, low-calcemic vitamin D analogues can still cause hypercalcemic episodes and such serious side effects can reduce the clinical effectiveness of these drugs. This is particularly true in cancer therapy where the analogue must be given systemically at relatively high concentrations for a long period of time. The consequence of this growing clinical experience with topical and systemic vitamin D analogues in dermatology and nephrology is a renewed effort to try novel vitamin D compounds and therapeutic strategies in various types of cancer. The succeeding sections discuss the mechanisms involved in the anticancer effects of vitamin D analogues and results to date of *in vitro* and *in vivo* experimentation with vitamin D analogues in cancer therapy.

Mechanisms Involved in the Anticancer Effects of Vitamin D

The earliest findings by Abe et al. (37) in 1981 that $1\alpha,25(\text{OH})_2\text{D}_3$ inhibited proliferation of a variety of human leukemic cell lines and stimulated the differentiation of normal and leukemic myeloid precursors toward more mature, less aggressive phenotypes rendered $1\alpha,25(\text{OH})_2\text{D}_3$ potentially useful in the treatment of myeloid leukemias and other types of cancer. Over the past two decades, evidence has been accumulated that $1\alpha,25(\text{OH})_2\text{D}_3$ and its analogues have anticancer properties in several tissues. Although the exact mechanism underlying the growth inhibitory actions of vitamin D and its analogues in cancer cells is not fully understood, the volume of data support multipronged effects involving growth arrest at the G_1 phase of the cell cycle, apoptosis, tumor cell differentiation, disruption of growth factor-mediated cell survival signals, and inhibition of angiogenesis and cell adhesion. Most observations indicate that VDR-mediated pathways constitute potential therapeutic targets for cancer prevention and treatment.

Cell Cycle Regulators

The $1\alpha,25(\text{OH})_2\text{D}_3$ -VDR system arrests the cancerous cell cycle at the G_0 - G_1 transition through multiple mechanisms. This G_1 arrest is achieved by induction of the cyclin-dependent kinase inhibitors, $p21^{\text{WAF1/CIP1}}$ and $p27^{\text{KIP1}}$, which causes a decrease of cyclin-dependent kinase 2 activity leading to dephosphorylation of retinoblastoma protein and repression of E2F transcriptional activity in several cell types (38, 39). In this cascade, $1\alpha,25(\text{OH})_2\text{D}_3$ and analogues induce gene transcription of the $p21^{\text{Waf1/Cip1}}$ and induce the synthesis and/or stabilization of the $p27^{\text{KIP1}}$ protein (40–42). In breast cancer cells, $1\alpha,25(\text{OH})_2\text{D}_3$ enhances the expression of HOXA10, a homeobox protein that causes G_1 arrest (43). In a VDR-serine/threonine protein phosphatase PP1c/PP2A-p70S6 kinase complex, $1\alpha,25(\text{OH})_2\text{D}_3$ seems to induce the VDR-associated phosphatase activity that in turn inactivates their substrate p70S6 kinase (44). Because p70S6 kinase is essential for G_1 -S-phase transition, these results provided further evidence of a $1\alpha,25(\text{OH})_2\text{D}_3$ -mediated G_1 block in colon cancer cells.

Growth Factors and Growth Factor Receptors

The growth inhibition of cancer cells by $1\alpha,25(\text{OH})_2\text{D}_3$ is also associated with growth factor signaling. Transforming growth factor- β (TGF- β) is a potent inhibitor of the proliferation of many cell types and is involved in cell cycle control and apoptosis. Vitamin D analogues induce autocrine TGF- β activity through increasing expression of TGF- β isoforms and/or TGF- β receptors in nonmalignant and malignant breast cells (45). In addition, Smad3, a TGF- β signaling mediator, coactivates VDR to further enhance TGF- β signaling and vitamin D signaling, suggesting the synergistic action of TGF- β and vitamin D analogues. $1\alpha,25(\text{OH})_2\text{D}_3$ analogues also blocked the mitogenic activity of insulin and insulin-like growth factor (IGF)-I on breast cancer cells most probably by inducing the expression of IGF-binding protein 3 and 5 (46, 47). Interestingly, in the human osteosarcoma cell line MG-63, $1\alpha,25(\text{OH})_2\text{D}_3$ and TGF- β act synergistically to increase IGF-binding protein 3 secretion (48). Furthermore, induction of IGF-binding protein 3 has been found to be obligatory for the $1\alpha,25(\text{OH})_2\text{D}_3$ -mediated growth inhibitory effect on the prostate cancer cell line LNCaP, and IGF-binding protein 3 neutralizing antibodies block $1\alpha,25(\text{OH})_2\text{D}_3$ -mediated induction of p21 expression (49). Thus, IGF-binding protein 3 induction by $1\alpha,25(\text{OH})_2\text{D}_3$ may, at least in part, explain the antiproliferative and proapoptotic actions of $1\alpha,25(\text{OH})_2\text{D}_3$ in primary cancer cells.

Apoptosis

$1\alpha,25(\text{OH})_2\text{D}_3$ -induced apoptosis is an important contributor to the growth-suppressing properties of the sterol in cancer. In cancer cells, $1\alpha,25(\text{OH})_2\text{D}_3$ analogues induce apoptosis through reciprocal modulation in the antiapoptotic protein Bcl-2 and the proapoptotic protein Bax content (50). $1\alpha,25(\text{OH})_2\text{D}_3$ analogues also increase intracellular calcium, which activates the calcium-dependent proapoptotic protease, μ -calpain (51), and increase the antitumoral and proapoptotic properties of ionizing radiation in MCF-7 breast tumor xenografts in nude mice and tumor necrosis factor- α -induced apoptosis in MCF-7 cells through caspase-dependent and caspase-independent mechanisms (52, 53). Other suggested mechanisms for the apoptotic effects of vitamin D include down-regulation of the antiapoptotic IGF receptor, up-regulation of the proapoptotic signaling molecule mitogen-activated protein kinase/extracellular signal-regulated kinase kinase-1, activation of the sphingomyelin-ceramide-ganglioside GD3 signaling pathway, and reduced expression of Akt, a kinase that regulates cell survival signals (54).

Differentiation

In addition to proliferation and apoptosis, the other major cellular process is differentiation. The coupling between proliferation and differentiation has been most widely studied in cells of hematopoietic system and in keratinocytes. In general, $1\alpha,25(\text{OH})_2\text{D}_3$ inhibits cell proliferation and induces myeloid differentiation along the monocyte-macrophage lineage. In the monocytic cell line, HL60 (55), $1\alpha,25(\text{OH})_2\text{D}_3$ induces CCAAT/enhancer-binding protein β expression, a protein recently identified as a potent

suppressor of the oncogenic cyclin D1 signature in human epithelial tumors (56). $1\alpha,25(\text{OH})_2\text{D}_3$ induction of CCAAT/enhancer-binding protein β in HL60 cells promotes the differentiation of these immature myeloid precursors into normal macrophages through interaction with retinoblastoma protein (55), which can directly interact with the promoter for CD14, a gene characteristically expressed in monocytes. Similarly, $1\alpha,25(\text{OH})_2\text{D}_3$ analogues induce differentiation of leukemic cells with intact potential for monocytic differentiation by changing the intracellular balance of transcription factor activity and allow the cells to bypass the granulocytic differentiation block by diverting the cells to monocytic lineage (57). Effects on differentiation have also been reported for other cell types. Recent microarray studies of gene expression profiles in cancer cells have highlighted the capacity of $1\alpha,25(\text{OH})_2\text{D}_3$ analogues to drive malignant cells to a more differentiated state. EB1089 treatment of head and neck squamous cell carcinoma repressed expression of several markers of cancer progression (e.g., N-cadherin, squamous cell carcinoma antigen, tenascin-C, and tumor antigen L6) and induced expression of several genes associated with epithelial cell differentiation (cystatin M, protease M, type XIII collagen, and desmoglein 3; ref. 22).

Invasion and Metastasis

For tumor suppressive activity, besides growth inhibition, vitamin D and its analogues decrease the invasiveness of several cell types *in vitro*, and they inhibit angiogenesis and metastasis in xenograft and transgenic mouse models *in vivo* (58, 59). In cultured malignant cells, $1\alpha,25(\text{OH})_2\text{D}_3$ and its analogues down-regulate cell invasion-associated proteases include matrix metalloproteinases 2 and 9 (60, 61) and serine proteinases (62). $1\alpha,25(\text{OH})_2\text{D}_3$ also inhibits prostate cancer cell invasion by decreasing in expression of α_6 and β_4 integrins, both of which are receptors for laminin (63). In prostate and colon cancer cells, $1\alpha,25(\text{OH})_2\text{D}_3$ and its analogues increase expression of E-cadherin, a tumor suppressor associated with the metastatic potential of cells, and inhibit the oncogenic β -catenin signaling (64). Furthermore, $1\alpha,25(\text{OH})_2\text{D}_3$ inhibits tenascin-C, an extracellular matrix protein, which promotes growth, invasion, and angiogenesis and is up-regulated in many cell types during tumorigenesis (65). Moreover, $1\alpha,25(\text{OH})_2\text{D}_3$ and its analogues inhibit the proliferation of some tumor-derived endothelial cells (66) and inhibit the expression of vascular endothelial growth factor that induces angiogenesis in tumors (67).

Factors Involved in Vitamin D Resistance

Although the presence of a functional VDR is also a prerequisite for a growth regulatory response and a relationship between VDR levels and growth inhibition has been suggested for several cancer cells, the presence of the VDR is not always predictive of a growth inhibitory effect of exogenous $1\alpha,25(\text{OH})_2\text{D}_3$. Human colorectal cancer cells highly express the transcription factor, Snail, which is recruited to the native VDR promoter, thereby limiting VDR responses and the efficacy of $1\alpha,25(\text{OH})_2\text{D}_3$ therapy

(68). In prostate cancer, altered levels of expression of the steroid receptor corepressor, SMRT, or defective nuclear VDR localization, but not reduced VDR levels, are responsible for the resistance to the antiproliferative properties of $1\alpha,25(\text{OH})_2\text{D}_3$ (69).

More recent studies have shown that the histone deacetylation inhibitor trichostatin A can reverse the resistance to $1\alpha,25(\text{OH})_2\text{D}_3$ therapy, which develops in the breast cancer cell line MDA-MB-231 resulting from decreased VDR and increased corepressor NCoR1 content (70).

Promising Vitamin D Analogues under Development for Types of Cancer

The search for new vitamin D analogues with potential clinical usefulness has focused on candidates with good systemic bioavailability, potent anticancer/chemopreventive activity, and low-calcemic activity, the structures of which are shown in Fig. 2. In the sections below, we have summarized progress in trials of vitamin D analogues in the common epithelial cancers in animal models (Table 1) and human trials (Table 2).

Breast Cancer and Vitamin D Analogues

$1\alpha,25(\text{OH})_2\text{D}_3$ and its analogues have been shown to inhibit the proliferation of breast cancer cells *in vitro* and tumor progression *in vivo*. One of the most interesting aspects of the action of vitamin D analogues is their efficacy in both estrogen receptor-positive and estrogen receptor-negative breast cancer cells. In addition to direct growth inhibitory effects, some analogues also inhibit angiogenesis and decrease the metastatic potential of breast cancer cell *in vitro* and *in vivo*.

The beneficial effects of EB1089 (Fig. 2; Table 1) on breast cancer in tumor-bearing animals result in a marked increase in survival time (71) and an inhibition of the development of bone metastases without causing hypercalcemia (72). 22-Oxa- $1\alpha,25(\text{OH})_2\text{D}_3$ suppressed tumor growth in athymic mice implanted with human breast carcinoma with or without estrogen receptor (73). Moreover, the beneficial effects of 22-oxa- $1\alpha,25(\text{OH})_2\text{D}_3$ on breast cancer can be augmented by combination with the antiestrogen, tamoxifen, or an aromatase inhibitor (73, 74). Suppression of tumor growth by 22-oxa- $1\alpha,25(\text{OH})_2\text{D}_3$ was accompanied by inhibiting neovascularization (58, 67). An additional benefit of EB1089 and 22-oxa- $1\alpha,25(\text{OH})_2\text{D}_3$ is that they inhibit PTH-related peptide production (75, 76); thus, both analogues may also have utility in treating malignancy-associated hypercalcemia. Following up these analogues, TX522 [19-nor-14-epi-23-yne- $1\alpha,25(\text{OH})_2\text{D}_3$] and TX527 [19-nor-14,20-bisepi-23-yne- $1\alpha,25(\text{OH})_2\text{D}_3$] have been shown to retard tumor progression in an *in vivo* model of MCF-7 breast cancer cells established in nude mice (77). Other potential analogues, Ro24-5531 [16-ene-23-yne-26,27-hexafluoro- $1\alpha,25(\text{OH})_2\text{D}_3$] and $1\alpha(\text{OH})\text{D}_5$ extended the latency of tumor incidence as well as multiplicity of mammary carcinomas induced by *N*-methyl-*N'*-nitrosourea in rats when the analogue was fed for 2 to 7 months (78, 79), which indicated the possibility for

Table 1. *In vivo* effects of vitamin D analogues in animal models of cancer

Tumor, model	Analogue	Dose	Effect	Hypercalcemia	Ref.
Breast					
<i>N</i> -methyl- <i>N'</i> -nitrosourea induced	EB1089	1 µg/kg p.o.	Tumor suppression	Marked	(71)
	Ro24-5531	1.25 and 2.5 nmol/kg diet	Reduced tumor incidence	None	(78)
	1α(OH)D ₅	58.4 and 116.8 nmol/kg diet*	Reduced tumor incidence	None	(79)
MCF-7 xenografts	22-Oxa-1α,25(OH) ₂ D ₃	1.0 µg/kg p.o.	Tumor suppression	None	(73)
	TX522	80 µg/kg/2 d i.p.	Tumor suppression	None	(77)
	TX527	25 µg/kg/2 d i.p.	Tumor suppression	Marked	(77)
MDA-MB-231 xenografts	EB1089	14 pmol/L/24 h, infusion	Inhibited skeletal metastasis	None	(72)
Prostate					
LNCaP xenografts	EB1089	0.5 µg/kg i.p.	Tumor suppression	None	(81)
	EB1089	1 µg/kg p.o.	Tumor suppression	Mild	(86)
	LG190119	3 and 10 mg/kg p.o.	Tumor suppression	None	(86)
	LG190119	10 mg/kg p.o.	Reduced tumor incidence	None	(86)
PC-3 xenografts	Ro23-7553	1.6 µg/animal i.p.	Tumor suppression	None	(84)
MAT LyLu tumors in rats	EB1089	0.5 and 1.0 µg/kg i.p.	Inhibited lung metastasis	Marked	(82)
MDA-PCa 2b xenografts	JK-1626-2	4 µg/kg s.c.	Inhibited metastatic bone lesions	None	(83)
Colon					
1,2-Dimethylhydrazine induced	22-Oxa-1α,25(OH) ₂ D ₃	30 µg/kg i.p. †	Reduced aberrant crypt foci	None	(91)
	Ro25-5317	3.5 nmol/kg diet	Reduced tumor incidence	None	(88)
	Ro25-9022	3.0 and 3.5 nmol/kg diet	Reduced tumor incidence	None	(88)
Azoxymethane induced	Ro24-5531	2.5 nmol/kg diet	Reduced tumor incidence	None	(89)
	1α(OH)D ₅	58.4 nmol/kg diet	Reduced aberrant crypt foci	None	(79)
LoVo xenografts	EB1089	0.1 and 0.5 µg/kg p.o.	Tumor suppression	None	(87)
HT-29 xenografts	Ro25-6760	0.1 and 0.2 µg/animal i.p.	Tumor suppression	None	(90)
MC-26 xenografts	RO-4383561	0.02 µg E i.p.	Reduced tumor growth	None	(92)

*1α(OH)D₅ 58.4 and 116.8 nmol/kg diet = 25 and 50 µg/kg diet.

†22-Oxa-1α,25(OH)₂D₃ 30 µg/kg i.p. = 72.5 nmol/kg i.p.

prevention of breast cancer in the rat. Of note, the combination of a vitamin D analogue with paclitaxel and cisplatin suppressed tumor growth more than either drug alone (80), indicating that treatment of breast cancer could be expanded to include combination therapies of vitamin D analogues with anticancer agents.

Prostate Cancer and Vitamin D Analogues

1α,25(OH)₂D₃ has been shown to inhibit the proliferation of both androgen-dependent and androgen-independent prostate cancer cells. The beneficial effects of EB1089 on prostate cancer in tumor-bearing animals result in a marked decrease in tumor volumes (81) and an inhibition of the development of lung metastases without severe weight loss (82). Prostate cancer-mediated osteoblastic bone lesions were inhibited by the low-calcemic hybrid analogue JK-1626-2 (1α-hydroxymethyl-16-ene-26,27-bishomo-25-hydroxyvitamin D₃; ref. 83). In an *in vivo* animal model carrying with human prostate cancer cells, Ro23-7553 [16-ene-23-yne-1α,25(OH)₂D₃] has been shown to inhibit tumor growth without increasing the serum calcium concentration (84). However, dietary supplemented Ro24-5531 (1.25 or 2.5 nmol/kg diet) was only moderately effective at inhibiting the development of androgen-induced carcinoma of the prostate and seminal vesicles (85). Several nonsteroidal analogues have interesting biological properties resembling those of 1α,25(OH)₂D₃ but with extremely low systemic calcemic effects. One of these compounds, LG190119, effectively

inhibited LNCaP prostate cancer xenograft tumor growth without causing hypercalcemia (86). Nonsteroidal analogues may represent promising new therapeutics in the cancer armamentarium.

Colon Cancer and Vitamin D Analogues

The vitamin D analogues reduce the proliferation of colon cancer cells *in vitro*, but they also reduce tumorigenesis in xenograft and chemically induced tumors *in vivo* (87, 88). Several epidemiologic studies show that vitamin D deficiency may accelerate colon cancer growth. Conversely, dietary supplementation of Ro24-5531 or 1α(OH)D₅ prevented azoxymethane-induced crypt cell hyperproliferation, aberrant crypt foci development, and tumor incidence (79, 89). Using dietary supplementation with the low-calcemic analogue, Ro 25-9022 [16,23E-diene-26,27-F6-19-nor-1,25(OH)₂D₃], studies showed a reduced tumor incidence and inhibition of spontaneous metastasis in a 1,2-dimethylhydrazine-induced colon carcinogenesis model (90). Otoshi et al. (91) showed that the i.p. injection of 22-oxa-1α,25(OH)₂D₃ also suppressed the development of aberrant crypt foci in rats. Using the novel double side chain 19-nor-Gemini compound, injection of RO-4383561 reduced tumor growth in a MC-26 xenograft model (92). Notably, neither analogue significantly influenced animal growth rates or serum calcium levels. The results from these studies indicate that vitamin D analogues may be an effective new approach for colon cancer prevention and treatment.

Clinical Development of Vitamin D Analogues for Cancer Treatment

Clinical trials with $1\alpha,25(\text{OH})_2\text{D}_3$ in cancer patients set the stage for the development of vitamin D analogues for cancer treatment (93–95). Table 2 summarizes cancer clinical trials done with $1\alpha,25(\text{OH})_2\text{D}_3$ and vitamin D analogues to date. Earlier studies showed the therapeutic efficacy of systemically applied $1\alpha,25(\text{OH})_2\text{D}_3$ for treating cancer, but subsequently it has not fulfilled its early promise (93). A main drawback of systemically applied vitamin D analogues has been side effects, primarily hypercalcemia, at the doses needed to effect clinical improvement. One approach to limiting hypercalcemia was i.v. or s.c. administration of $1\alpha,25(\text{OH})_2\text{D}_3$ at doses of 1.5 to 4 μg thrice weekly (93) that have been used in individuals with renal disease as i.v. pulse therapy (96), but renal stones developed in some cancer patients with this regimen. Alternatively, controversial hepatic arterial infusion of $1\alpha,25(\text{OH})_2\text{D}_3$ (0.2–10 $\mu\text{g}/\text{d}$) allowed a high dosage to be administered without inducing hypercalcemia in liver cancers (97). In recent years, much progress has been made in cancer treatment with very high dose intermittent $1\alpha,25(\text{OH})_2\text{D}_3$ therapy based on laboratory data, suggesting that intermittent exposure to high levels of $1\alpha,25(\text{OH})_2\text{D}_3$ may be sufficient to produce its antiproliferative effects. Weekly high-dose pulse oral $1\alpha,25(\text{OH})_2\text{D}_3$

(0.5 $\mu\text{g}/\text{kg}$) was better tolerated and led to an increase in median prostate-specific antigen doubling time without the development of hypercalcemia or hypercalciuria in prostate cancer (94, 98). Weekly high-dose administration of $1\alpha,25(\text{OH})_2\text{D}_3$ in combination with chemotherapy (docetaxel or carboplatin) produced a minimal 50% reduction in prostate-specific antigen values in 30 of 37 patients with metastatic cancer and suggested that this was better than the response in other trials of the chemotherapy alone (99–101). Although no patient experienced severe side effects from the treatment, pharmacokinetic studies showed that a dose of 0.48 $\mu\text{g}/\text{kg}$ produced mean peak $1\alpha,25(\text{OH})_2\text{D}_3$ levels of 1,625 pg/mL that is 30-fold higher than normal levels. This suggests that hypercalcemia or hypercalciuria is likely to occur at early times within the first 24 to 48 hours after administration and that prolonged high-dose pulse $1\alpha,25(\text{OH})_2\text{D}_3$ administration would be expected to enhance the potential for stone formation. Accordingly, the reduction of dietary calcium intakes to <500 mg may be important to minimize the development of hypercalcemia in these patients (94).

Vitamin D analogues may therefore be an effective therapy for treatment of early as well as advanced cancer. Several analogues, dosed daily, have entered clinical trials. Seocalcitol (EB1089) was evaluated in a phase I clinical trial of advanced breast and colorectal cancer; although less

Table 2. Clinical development of $1\alpha,25(\text{OH})_2\text{D}_3$ and its analogues for cancer treatment

Study	Treatment	Patients	Protocol	Ref.
Phase II	$1\alpha,25(\text{OH})_2\text{D}_3$ + dexamethasone	Prostate cancer before prostatectomy	Twice weekly p.o.	Ongoing study*
Phase II	$1\alpha,25(\text{OH})_2\text{D}_3$ + mitoxantrone + prednisone	Prostate cancer	High-dose pulse p.o.	Ongoing study*
Phase II	$1\alpha,25(\text{OH})_2\text{D}_3$ + docetaxel	Pancreatic cancer	Weekly p.o.	Ongoing study*
Phase II	$1\alpha,25(\text{OH})_2\text{D}_3$	Prostate cancer before prostatectomy	Weekly p.o.	(98)
Phase II	$1\alpha,25(\text{OH})_2\text{D}_3$ + docetaxel	Prostate cancer	Weekly p.o.	(99)
Phase II	$1\alpha,25(\text{OH})_2\text{D}_3$ + carboplatin	Prostate cancer	Weekly p.o.	(100)
Phase II	$1\alpha(\text{OH})\text{D}_2$	Prostate cancer before prostatectomy	Daily p.o.	Ongoing study*
Phase II	$1\alpha(\text{OH})\text{D}_2$	Prostate cancer	Daily p.o.	(105)
Phase II	EB1089	Liver cancer	Daily p.o.	(103)
Phase II	EB1089	Pancreatic cancer	Daily p.o.	(104)
Phase I	$1\alpha,25(\text{OH})_2\text{D}_3$ + ketoconazole + dexamethasone	Advanced solid tumors	Days 1-3/wk p.o.	Ongoing study*
Phase I	$1\alpha,25(\text{OH})_2\text{D}_3$ + gefitinib + dexamethasone	Advanced solid tumors	Weekly i.v.	Ongoing study*
Phase I/II	$1\alpha,25(\text{OH})_2\text{D}_3$ + docetaxel + estramustine	Prostate cancer	High-dose pulse p.o.	(101)
Phase I	$1\alpha,25(\text{OH})_2\text{D}_3$ + paclitaxel	Advanced solid tumors	Days 1-3/wk p.o.	(95)
Phase I	$1\alpha,25(\text{OH})_2\text{D}_3$	Liver cancer	Daily hepatic infusion	(97)
Phase I	$1\alpha,25(\text{OH})_2\text{D}_3$	Advanced malignancy	Weekly p.o.	(94)
Phase I	$1\alpha,25(\text{OH})_2\text{D}_3$	Advanced malignancy	Every other day s.c.	(93)
Phase I	19-Nor- $1\alpha,25(\text{OH})_2\text{D}_2$ + gemcitabine	Advanced malignancy	Weekly i.v.	Ongoing study*
Phase I	16-Ene-23-yne- $1\alpha,25(\text{OH})_2\text{D}_3$	Advanced malignancy	Daily p.o.	(108)
Phase I	$1\alpha(\text{OH})\text{D}_2$	Prostate cancer	Daily p.o.	(106)
Phase I	EB1089	Breast and colorectal cancer	Daily p.o.	(102)
No phase specified	19-Nor- $1\alpha,25(\text{OH})_2\text{D}_2$ + zoledronate	Multiple myeloma or other plasma cell disorder	Weekly i.v.	Ongoing study*

*Cancer clinical trial currently under way in the United States (<http://www.cancer.gov>).

calcemic than $1\alpha,25(\text{OH})_2\text{D}_3$, hypercalcemia limited the tolerable dose of EB1089 to $7 \mu\text{g}/\text{m}^2/\text{d}$ (102). In phase II trials, daily oral administration of EB1089 (10–15 $\mu\text{g}/\text{d}$) was well tolerated in hepatocellular carcinoma (103) and pancreatic cancer (104), but no objective antitumor activity in advanced disease was observed. The failures of the clinical trials of EB1089 in breast cancer and hepatic cancer have led to its withdrawal.

Several different strategies have been tried to optimize the application of vitamin D analogues for the treatment of various malignancies. One of these strategies was to use a low-calcemic analogue [e.g., the prodrug-type analogue, $1\alpha(\text{OH})\text{D}_2$], with its relatively mild side effects, already proven to be superior to $1\alpha,25(\text{OH})_2\text{D}_3$ in chronic kidney disease patients. Other strategies have been to use vitamin D analogues in combination with chemotherapeutic cytotoxic agents (such as taxols and cisplatin) where the benefits arise from the synergism of vitamin D and the chemotherapy taking advantage of different mechanisms of action, including effects on angiogenesis and metastasis. Additional strategies include attempts to limit the hypercalcemic side effects of the vitamin D analogue by coadministration of prednisone or bisphosphonates to limit the bone resorptive effects and maximize effective dose of the vitamin D analogue, especially in metastatic prostate cancer.

Clinical trials with $1\alpha,25(\text{OH})_2\text{D}_3$ in prostate cancer patients have highlighted the potential of vitamin D analogues either alone or in combination with chemotherapy (104–108). The analogue doxercalciferol [$1\alpha(\text{OH})\text{D}_2$] evaluated in a phase II study in patients with androgen-independent prostate cancer (105). Following the phase I study (106), the recommended phase II dose was $12.5 \mu\text{g}/\text{d}$ on a daily p.o. medication. During the trial, plasma levels of $1\alpha,25(\text{OH})_2\text{D}_2$, an active form of $1\alpha(\text{OH})\text{D}_2$, was confirmed to reach a plateau by week 4 despite continuous drug dosing, and disease stability for >6 months resulted in 30% of the patients. Another clinical study using paricalcitol [$19\text{-nor-}1\alpha,25(\text{OH})_2\text{D}_2$] i.v. thrice weekly on an escalating dose of 5 to 25 μg in patients with androgen-independent prostate cancer resulted in a significant reduction of elevated serum levels of PTH, often observed as a common feature of advanced prostate cancer (107). Further clinical investigation of $19\text{-nor-}1\alpha,25(\text{OH})_2\text{D}_2$ in combination with chemotherapy (gemcitabine) or bisphosphonate (zoledronate) is currently ongoing in patients with advanced malignancy.

Perspectives

The anticancer potential of vitamin D analogues has been discussed for over a decade, but clinically approved anticancer vitamin D drugs remain elusive, although the antiproliferative effects of vitamin D analogues have been realized in other hyperproliferative conditions: psoriasis and the parathyroid hyperplasia in chronic kidney disease. Vitamin D analogues are potent antiproliferative regulators of many cancer cell lines *in vitro* (35). Preclinical studies have revealed that vitamin D analogues are also effective growth regulators of tumors in animals *in vivo*, although

occasionally daily administration of certain vitamin D analogues causes mild hypercalcemia, which is still less severe than with $1\alpha,25(\text{OH})_2\text{D}_3$. According to researchers involved in clinical trials using $1\alpha,25(\text{OH})_2\text{D}_3$ and vitamin D analogues, part of the reason for the failure has been the inability of such trials to achieve adequate drug levels in the blood/tumor of patients as well as occasional hypercalcemia (109, 110). Recent protocols have attempted to overcome poor bioavailability by advocating use of very high dose intermittent therapy to try to transiently raise $1\alpha,25(\text{OH})_2\text{D}_3$ concentrations to supraphysiologic levels, well in excess of 1 nmol/L (109), but such approaches have had limited success. Whether the limited effectiveness is also due to reduced affinity of vitamin D analogues for transport proteins, such as vitamin D-binding protein, leading to rapid hepatic clearance or whether it is due to increased “drug resistance” caused by up-regulated CYP24A1 and accelerated destruction is unknown.

Another possibility for an effective cancer therapy is to use a low-calcemic vitamin D analogue in combination with another therapy to achieve a synergistic effect on the tumor. Combination therapy with cytotoxic agents (taxols or cisplatin) or cytochrome P450 inhibitors (e.g., general CYP or CYP24A1 inhibitors) might prove to be more effective ways of using these valuable antiproliferative agents. Combination therapy with glucocorticoids (e.g., dexamethasone or prednisone) might potentiate the anti-tumor effects while reducing the hypercalcemia induced by $1\alpha,25(\text{OH})_2\text{D}_3$. In addition, other strategies to limit the calcium-mobilizing bone resorptive effects (bisphosphonates) or intestinal calcium absorptive side effects (low calcium diets or nighttime administration) are other techniques that might be used to refine the eventual combination therapy that proves effective. Furthermore, understanding how analogues exert their tissue-specific selective actions may allow for the design of more effective and safer vitamin D compounds for the treatment of hyperproliferative disorders, including cancer.

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