The pharmacology of cell differentiation
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VITAMIN D_3 ANALOGS AND HEMATOPOIESIS

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INTRODUCTION

Vitamin D_3 is classically recognized as a hormone which regulates bone and mineral metabolism (1). The seco-steroid 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$] is the major biologically active metabolite of vitamin D_3. Recent studies have suggested a wider biological role of 1α,25$(OH)_2D_3$ in various tissues not primarily related to mineral metabolism. In this chapter, we focus on the affects that vitamin D_3 and its analogs have on hematopoietic cells.

Vitamin D_3 Hormone

Production and metabolism: Vitamin D_3 is synthesized in the skin from 7-dehydrocholesterol in a reaction catalyzed by ultraviolet light. Further processing of this molecule occurs in the liver where hydroxylation of vitamin D_3 occurs at carbon 25 to produce 25$(OH)D_3$. Physiologically active 1α,25$(OH)_2D_3$ is synthesized with the addition of 1α hydroxy group by the enzyme 25$(OH)D_3$-1α-hydroxylase in the kidney.

The conversion of 25$(OH)D_3$ to the active hormone 1α,25$(OH)_2D_3$ is under stringent control, so that the renal output of the hormone is related to a person's calcium needs (2). Parathyroid hormone and 1α,25$(OH)_2D_3$ principally regulate synthesis of the hormone. Parathyroid hormone stimulates directly the 1α-hydroxylase in the kidneys, whereas 1α,25$(OH)_2D_3$ inhibits this enzyme. Other factors which modulate the production of the hydroxylase include [$PO^{=}_4$] and several hormones including estrogen, calcitonin, growth hormone, prolactin and glucocorticoid (2,3).

Extrarenal 1α,25$(OH)_2D_3$ production can occur, especially under certain circumstances. For example, elevated 1α,25$(OH)_2D_3$ serum levels have been found to be elevated in patients with hypercalcemia who have either sarcoidosis or tuberculosis and have no kidneys (4). Extrarenal presence of 1α-hydroxylase and 1α,25$(OH)_2D_3$ production from 25$(OH)D_3$ has been shown in vitro. Homogenates of either granuloma or cultured pulmonary alveolar macrophages from patients with pulmonary sarcoidosis can produce 1α,25$(OH)_2D_3$ (5). In addition, alveolar and bone marrow macrophages from normal subjects stimulated by gamma interferon (γ-IFN) or lipopolysaccharide, as well as cultured keratinocytes from neonatal human foreskin can also synthesize the hormone (6-8).

Studies have shown that 1α,25$(OH)_2D_3$ inhibits the synthesis of DNA of T-lymphocytes as well as inhibiting their production of lymphokines including γ-IFN, interleukin-2 (IL-2) and granulocyte-macrophage colony-stimulating factor (GM-CSF) (9-11). We previously suggested that 1α,25$(OH)_2D_3$ might play a paracrine role in the communication between T-lymphocytes and macrophages (7). Antigens might activate both lymphocytes and macrophages. The γ-IFN producted by the T-lymphocytes could further stimulate macrophages to produce IL-1 and other T-cell stimulators. Possibly as

a feedback inhibitor, macrophages synthesize $1\alpha,25(OH)_2D_3$ which decreases cell division and lymphokine production by the activated T-lymphocytes.

Mode of actions of $1\alpha,25(OH)_2D_3$: Substantial evidence has accumulated that the mechanism of action of $1\alpha,25(OH)_2D_3$ is similar to that of other steroid hormones. The hormone enters the cell after release from binding to the vitamin D binding protein (DBP) in serum. Intracellularly, it noncovalently but steroid-specifically, binds with an intracellular receptor protein. This steroid-receptor complex binds to specific DNA [vitamin D response element (VDRE)] in the nucleus of target cells to modulate either transcriptional activation or repression of vitamin D_3 responsive genes.

The $1\alpha,25(OH)_2D_3$ receptors (VDR) are about 60,000 dalton proteins that bind both DNA and $1\alpha,25(OH)_2D_3$ with high affinity (dissociation constant = 1 to 50 x 10^{-11}M). The recent cloning of VDR from several species including human, revealed that the protein belongs to the steroid, thyroid, retinoic acid receptor gene family (12-15). It is composed of a distinct N-terminal DNA recognition domain containing two zinc finger structures and a large C-terminal binding domain for $1\alpha,25(OH)_2D_3$. The receptor protein is expressed in almost all tissues including various types of tumors and cancer cell lines (16).

The VDR which is bound to ligand [$1\alpha,25(OH)_2D_3$], recognizes and binds directly with VDRE, located within the vicinity of vitamin D_3 responsive genes (17). Over 60 genes has been shown to be transcriptionally modulated by $1\alpha,25(OH)_2D_3$ (18), but precise VDRE sequences and definitive evidence for $1\alpha,25(OH)_2D_3$/VDR-VDRE interaction has been shown in only several genes including the human and rat osteocalcin, and murine osteopontin genes (17,19,20). The DNA consensus binding sequences of vitamin D, thyroid hormone and retinoic acid are the same, a hexanucleotide half-site (AGGTCA) separated by either three, four or five "spacing" nucleotides, respectively (21,22).

$1,25(OH)_2D_3$ and Hematopoiesis

The recognition that $1\alpha,25(OH)_2D_3$ may interact with hematopoietic cells was suggested by a variety of evidence including: 1) A number of cultured hematopoietic cells including those of leukemic origin as well as activated lymphocytes possess high affinity receptors for $1\alpha,25(OH)_2D_3$ (23). 2) Many murine and human myeloid leukemic cell lines and normal myeloid progenitor cells can be induced to differentiate towards macrophage-like cells when exposed to $1\alpha,25(OH)_2D_3$ (24-26). 3) Activated macrophages are capable of synthesizing $1\alpha,25(OH)_2D_3$ (4,5). 4) The $1\alpha,25(OH)_2D_3$ can have profound effects on proliferation and cytokine production of T-lymphocytes and action of this seco-steroid is dependent on these cells possessing receptors for $1\alpha,25(OH)_2D_3$ (9,10).

In vitro studies suggest that $1\alpha,25(OH)_2D_3$ plays a role in the generation of functional macrophages. The addition of $1\alpha,25(OH)_2D_3$, at concentrations between 10^{-7} and 10^{-9}M, to mononuclear bone marrow cell in soft agar culture in the presence of GM-CSF had minimal effect on the number of colonies obtained from committed myeloid progenitor cells (27). However, the composition was altered by the hormone; 85% and 54% of colonies produced in plates containing $1\alpha,25(OH)_2D_3$ at 10^{-7} and 10^{-9}M, respectively were composed of solely macrophages, compared with only 25% macrophage colonies in the absence of $1\alpha,25(OH)_2D_3$.

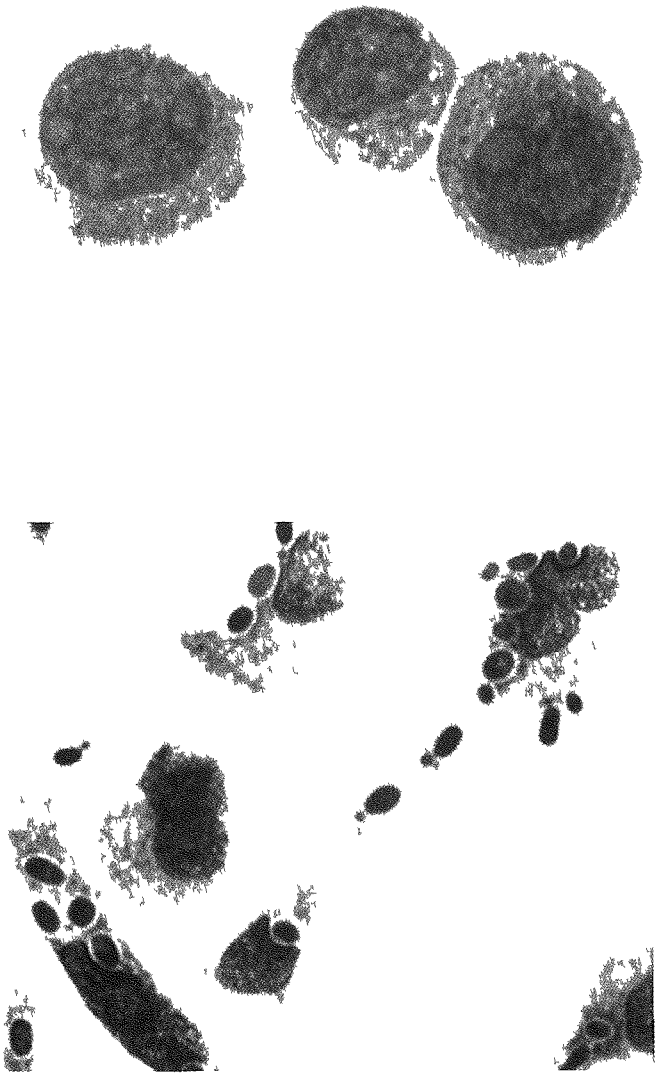

Fig 1. (A) HL-60 promyelocytes. (B) HL-60 cells induced to macrophage-like cells after exposure to 10^{-7}M $1\alpha,25(OH)_2D_3$ for seven days. Cells are adherent and have phagocytosed Candida Albicans.

Liquid cultures of normal human marrow cells in the presence of $1\alpha,25(OH)_2D_3$ ($5x10^{-9}M$) for 5 days contained 68% monocytes and macrophage, compared with 12% in control cultures (28).

Differentiation induction of leukemic cell lines in vitro

About a decade ago, the M1 murine myeloid leukemic cell line was found to differentiate in the presence of $1\alpha,25(OH)_2D_3$ (24). Since then, other murine and human leukemic cell lines including the HL-60 human myeloblastic line have been found to differentiate and to cease to proliferate in the presence of $1\alpha,25(OH)_2D_3$ (25-28). Vitamin D_3 and analogs induce these cells to acquire morphological and functional characteristics of macrophage (25-28). For example, HL-60 cells become adherent to charged surfaces, develop long-filamentous pseudopodia, stain positively for non-specific esterase, reduce nitroblue tetrazolium (NBT), acquire the ability to phagocytose yeast and increase their expression of type II Fc receptors after incubation with $1\alpha,25(OH)_2D_3$ (10^{-10} to 10^{-7} M) for 5 to 7 days (29,30) (Fig. 1). The HL-60 cells also acquire the ability to degrade bone matrix in vitro (31). The effective dose (ED_{50}) that induces approximately 50% of the HL-60 cells to differentiate is about 6×10^{9}M (Table 1). In addition to the induction of differentiation, $1\alpha,25(OH)_2D_3$ inhibits their clonal growth in vitro. For example, HL-60 colony formation in soft agar is reduced by 50% in the presence of 8×10^{-10}M $1\alpha,25(OH)_2D_3$.

TABLE 1
Functional and Morphological Changes in HL-60 Cells Induced by Various Concentrations of $1,25(OH)_2D_3$

$1,25(OH)_2D_3$ (M)	NBT Reduction (%)	Phagocytic Cells (%)	Blasts (%)	Nonspecific Acid Esterase-Positive (%)
0	2	2	90	2
10^{-11}	10	2	95	3
10^{-10}	18	13	82	10
10^{-9}	37	20	66	25
10^{-8}	64	26	45	54
10^{-7}	82	44	32	82
10^{6}	86	60	27	98

HL-60 cells were cultured in the presence of various concentrations of $1,25(OH)_2D_3$; after 7 days, cells were assessed for various differentiation parameters. All data are expressed as the percentage of total cells. NBT, nitro-blue tetrazolium assay measures the ability of cells to produce superoxide, a function of mature cells.

Other myeloid leukemic cell lines, particularly those with relatively mature

phenotypes, have been shown to differentiate and to experience growth inhibition in response to vitamin D compounds. Clonal growth by U-937 and THP-1 monoblasts, and HEL bipotential erythro-monoblasts were inhibited by 50% in the presence of $1\alpha,25(OH)_2D_3$ at
4×10^{-9} to 4×10^{-8} M (32). These concentrations are comparable to those required for differentiation induction of those cell lines in liquid cultures (Table 2)(29,33,34). In contrast, HL-60 blast (an early myeloblast clone of HL-60), KG-1 and KG-1a early myeloblasts, and K562 bipotent cells were resistant to the effects of $1\alpha,25(OH)_2D_3$ (Table 2). The differential responsiveness to the hormone may therefore reflect the stage of myeloid differentiation of the cell lines.

TABLE 2
Effects of $1\alpha,25(OH)_2D_3$ on Clonal Growth of Cells from Myeloid Leukemia Lines

		50% Inhibitory Concentration
Cell line	Description	$1\alpha,25(OH)_2D_3$ (M)
HL-60	Human promyelocytes	8×10^{-10}
U937	Human monoblasts	4×10^{9}
HEL	Bipotent[a]	2×10^{8}
THP-1	Human monoblasts	3×10^{8}
M1	Murine myeloid leukemia	1×10^{-8}
HL-60 blast	Human early myeloblasts	No inhibition
KG-1A	Human early myeloblasts	No inhibition
KG-1	Human myeloblasts	No inhibition[b]
K562	Bipotient[a]	No inhibition

[a]Monoblast and erythroblast characteristics. [b]Stimulation at suboptimal concentrations of CSF.

Scatchard analysis of saturation kinetics revealed that HL-60 cells have approximately 4000 VDRs with a dissociation constant (Kd) of 5.4×10^{-9}M (29). The difference in numbers of VDR between the myeloid cell lines can not explain their differential responses to vitamin D_3. The HL-60 blast and KG-1 possess almost the same number of VDRs as HL-60 cells, but the former are resistant to the differentiative effects of $1,25(OH)_2D_3$ (23).

Mechanism by which $1\alpha,25(OH)_2D_3$ exerts effects on myeloid cells presently is unclear. Involvement of VDR has been suggested by several lines of evidence: 1.) The efficacy of the various vitamin D_3 analogs parallels their ability to bind to the cellular VDRs (32). 2.) Hematopoietic cell lines derived from patients with vitamin D_3 resistant rickets type II do not have functional $1\alpha,25(OH)_2D_3$ receptors and do not respond to $1\alpha,25(OH)_2D_3$ (35).

What genes in myeloid cells are targets for $1\alpha,25(OH)_2D_3$/VDR is unknown. Alterations of phenotype and proliferative status by $1\alpha,25(OH)_2D_3$ are precede by modulations in the expression of several growth-related proto-oncogene; for example, a rapid decline in the levels of mRNA for c-myc (36,37), the transient expression c-fos (37), and the sustained expression of c-fms and c-jun (38,39). Accumulation of putative second messengers such as either protein kinase C or intracellular Ca^{++} levels also occur early after exposure of these cells to $1\alpha,25(OH)_2D_3$ (40,41). However, the precise association between the above gene products and the classical VDR-VDRE pathway of $1\alpha,25(OH)_2D_3$ remains to be clarified (42). Why $1\alpha,25(OH)_2D_3$ preferentially inhibits the proliferation of leukemic cells, but not normal myeloid progenitor cells in vitro is not clear. Differences in the potential for activation of a set of genes in leukemic cells that may differ from those activated in normal cells, may provide an explanation.

Induction of differentiation of fresh leukemic cells by $1\alpha,25(OH)_2D_3$ in vitro

Leukemic cells from acute myelocytic leukemia (AML) patients can be induced to undergo monocytic differentiation when cultured with $1\alpha,25(OH)_2D_3$, but they are less sensitive than HL-60 cells to be induced to differentiate by the seco-steroid in vitro (29,32,43). Peripheral blood myeloblasts from AML patients formed 47% differentiated monocyte-macrophage colonies in soft agar cultures containing $1\alpha,25(OH)_2D_3$ (10^{-8}M) compared with 18% in control plates (27). Myeloid leukemic cells from nine patients showed significant growth inhibition when grown in liquid culture with $1\alpha,25(OH)_2D_3$ (10^{-7}M) for 6 days (43). The percentage of mature monocytes also increased from 5% in control flasks to 40% in the presence of the hormone. The blast cells from phenotypically more mature AML subtypes (M2,M4) showed greater ability to differentiate than those of an immature subtype (e.g., M1). Differentiation as assessed by cellular morphology, superoxide production and phagocytic abilities were significantly increased by exposure to 10^{-7}M $1\alpha,25(OH)_2D_3$, but not by lower concentrations of the seco-steroid.

Additional studies of the growth inhibitory effects of $1\alpha,25(OH)_2D_3$ on cells from 14 patients with AML revealed a spectrum of drug sensitivities (32). Leukemic clonogenic cells from 5 of 14 patients were inhibited $\geq$ 50% (ED_{50}) by 5×10^{-9} M; and all but one patient had 50% or greater inhibition of leukemic clonal growth at 5×10^{-7} M $1\alpha,25(OH)_2D_3$. Clonal proliferation of bone marrow myeloid progenitor cells from leukemia patients in clinical remission was not inhibited by $1\alpha,25(OH)_2D_3$.

Adminstration of $1,25(OH)_2D3$ to patients with myelodysplastic syndrome (MDS)

As a prelude to clinical studies, mice inoculated with syngeneic murine myeloid leukemic cells and treated with $1\alpha,25(OH)_2D_3$, were found to live significantly longer than control mice (44). Clinical investigations of the efficiency of this seco-steroid in MDS were performed by us and several other groups (43,45,46). The MDS patients were chosen because the tempo of their disease allows scrutiny of therapeutic maneuvers. These patients demonstrated ineffective hematopoiesis in vivo with anemia, thrombocytopenia, leukopenia and often increased number of marrow leukemic blasts (47). One trial treated seven MDS patients with $1\alpha,25(OH)_2D_3$ (2.5 ug/day) for a minimum of eight weeks (46).

No improvement in either peripheral blood cytopenia or bone marrow blast numbers occurred in any patient. In our trial, eighteen MDS patients were given escalating doses of $1\alpha,25(OH)_2D_3$, up to 2 ug/day, for a median duration of 12 weeks (43). Eight patients experienced a significant improvement in at least one hematologic parameter for more than 4 weeks. Nevertheless, no patient had improvement in either peripheral blood or bone marrow blast counts at the end of the study (at least 12 weeks). In addition, seven patients developed AML while on the trial. The major dose-limiting toxicity was hypercalcemia which developed in nine patients (43). When $1\alpha,25(OH)_2D_3$ was administered at 2 ug/day, serum drug levels reach only 2×10^{-10} M, which is an inadequate concentration either to induce myeloid differentiation or inhibit leukemic cells in vitro; therefore, the failure of vitamin D_3 therapy in MDS patients may in part be attributed to dose-limiting hypercalcemia.

Development of new vitamin D_3 analogs

Because of the dose-limiting toxicity of hypercalcemia, investigators have focused on the development of novel, chemically modified vitamin D_3 analogs with increased potency to induce differentiation and inhibit growth of leukemic cells with reduced ability to cause hypercalcemia (24,27,32,44,48-60). We have surveyed approximately 230 vitamin D_3 analogs and identified twenty-one that possess either equal or greater potency than $1\alpha,25(OH)_2D_3$ in their induction of differentiation and inhibition of proliferation of leukemic cells in vitro; and yet, these analogs are less potent than $1,25(OH)_2D_3$ in inducing hypercalcemia in vivo (51-56). Effects on calcium metabolism in vivo were assessed by their ability to mediate chick intestinal calcium absorption (ICA) and bone calcium mobilization (BCM) (61).

A novel vitamin D_3 analog, $1\alpha,25(OH)_2$-16ene-23yne-D_3 (compound V) was identified to be about 1000-fold and 40- fold more potent than $1\alpha,25(OH)_2D_3$ in inhibition of growth and induction of differentiation of HL-60 cells, respectively (51,56). In contrast, this analog stimulated 97% less ICA and 50-fold less BCM than $1\alpha,25(OH)_2D_3$. The therapeutic potency of this analog (V) was assessed using a murine myeloid leukemic model (62). Mice injected with lethal numbers of syngeneic WEHI-3BD$^+$ myeloid leukemic cells had significantly prolonged survival when given V compound (1.6 ug every other day) compared with those given either $1\alpha,25(OH)_2D_3$ or placebo. When lower number of leukemic cells were injected, V compound increased the percentage of mice surviving over 180 days, from 13% in placebo-treated mice to 43% in the drug-treated ones. The doses of drug adminstered, were the maximal doses tolerated by BALB/c mice without the development of hypercalcemia.

Another analog, $1\alpha,25(OH)_2$-16ene-D_3 (compound HM), is slightly more potent than compound V in growth inhibition of HL-60 cells and has less ability to stimulate BCM and ICA (56). This analog is unique because it binds to VDRs of HL-60 and chick cells about 1.5 fold more avidly than $1\alpha,25(OH)_2D_3$, and has 50-fold lower affinity for the D-binding protein, which therefore probably increases the availability of the compound for target tissues.

The differentiation induction and growth inhibition activities of these new analogs against fresh myeloid leukemic cells from patients also paralleled their potency against HL-

60 cells in vitro (51). Several additional analogs [1α,24$(OH)_2$-22ene-24cyclo-propyl-D_3, 1α,25$(OH)_2$-22oxa-D_3, 1α,25$(OH)_2$-24homo-D_3] have also been shown to be potent inducers of leukemic cell differentiation with little ability to cause hypercalcemic (57-60). Thus, these analogs may provide new therapeutic compounds in differentiation therapy for MDS/leukemia patients as well as other cancers and possibly certain dermatologic disorders such as psoarsis.

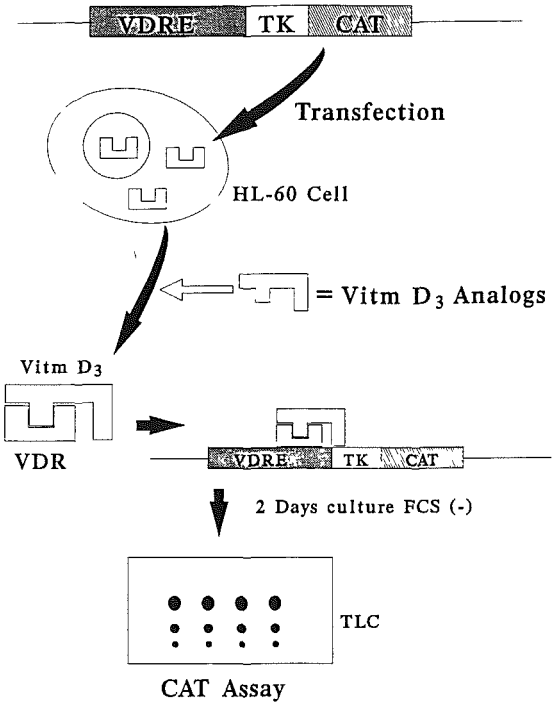

Fig 2. Schema of assay of transcriptional activity of vitamin D_3 analogs using transient transfection system. HL-60 cells were transfected with VDRE-tk-CAT reporter plasmid by electropolation, cultured with or without vitamin D_3 analogs in serum-free conditions for 48 hours, harvested and CAT activity measured by thin layer chromatography and autoradiography.

Mechanism of actions of new vitamin D_3 analogs

The precise mechanism to explain the range of activities of the new analogs is yet to be clarified (53,54,63-65). We have analyzed the relative effectiveness of binding of the analogs to the VDRs of both HL-60 and chick intestinal mucosa cells (51-56,63,64). The affinities of the analogs for VDRs of intestinal cells were very similar to those for

HL-60 cells suggesting that the specificity of the ligand binding domain of the VDRs are similar in both cell types. The metabolic half-time of $1\alpha,25(OH)_2$-22oxa-D_3 is short compared to $1\alpha,25(OH)_2D_3$, which might explain why this compound has less hypercalcemic potential as compared to the parental compound (58,66). Likewise, many of the analogs have less avidity to D-binding protein in serum which might enhance their release from the plasma transport protein in order to interact with various target organs.

Recent studies have reported "rapid" (within several minutes) generation of biological responses by vitamin D_3 compounds, which may be generated via non-genomic pathways, perhaps either by changes in phospholipid of the cell membrane or by opening of Ca^{2+} channels in various tissues (67-72). This putative membrane receptor for opening of Ca^{2+} channels may have a distinctly different ligand preference from that of the classical vitamin D_3 nuclear receptor (59). For example, an analog [25(OH)-16ene-23yne] was found to be quite effective at stimulating the opening of Ca^{2+} channels in intestinal mucosa cells without possessing any ability to bind to the classic nuclear/cytosolic VDR (73). Nevertheless, we have developed hematopoietic cell lines from patients with vitamin D deficient rickets, type II. These cells do not have functional VDRs and no longer respond to $1\alpha,25(OH)_2D_3$ reaffirming the pivotal importance of classic nuclear VDRs in hematopoietic cells (35). The possibility and relevance of nongenomic pathways in target tissue needs to be clarified in terms of physiological conditions.

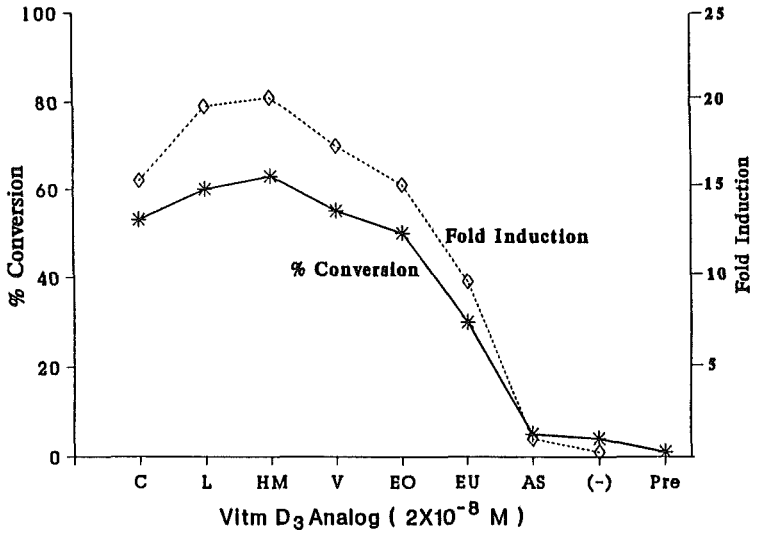

Fig 3. Transcriptional activation of vitamin D_3 analogs. Data represent % conversion of CAT and fold-induction of CAT compared to the activity without vitamin D_3. Assays were done at the concentration of 2 x 10^{-8} M. C: $1\alpha,25(OH)_2D_3$; L: $1\alpha,25(OH)_2$-23yne-D_3; HM: $1\alpha,25(OH)_2$-16yne-D_3; V: $1\alpha,25(OH)_2$-16ene-23yne-D_3; EO: $1\alpha,25(OH)_2$-16ene-23yne-26, 27-F6-D_3; EU: 22-Oxa-$1\alpha,25(OH)_2D_3$; AS: $24R,25(OH)_2D_3$.

Classically, vitamin D_3 analogs interact with their VDRs and critical VDRE and perhaps this interaction in intestinal and bone cells differs in comparison to hematopoietic cells. This area of investigation is relatively unexplored (74). In preliminary experiments, we have transfected HL-60 cells with the human osteocalcin VDRE sequences hooked to the thymidine kinase promoter attached to a reporter gene [chloramphenicol acetyl transferase, (CAT)] and cultured these cells with several new analogs which are very potent inducers of differentaition of leukemic cells (Fig. 2). None of the analogs including $1\alpha,25(OH)_2$-16ene-23yne-D_3 (V) and $1\alpha,25(OH)_2$-16ene-D_3 (HM) were more potent than $1\alpha,25(OH)_2D_3$ in activating the reporter gene (Fig. 3). In contrast, AT compound, which possesses extremely low binding affinity to VDR and has neglible biological effects in HL-60 cells, showed almost no increased CAT activity (Fig. 4). The results suggest that $1\alpha,25(OH)_2D_3$ and the new vitamin D_3 analogs exert at least in part, their effects through the VDR-VDRE pathway; however, this does not explain the relative effectiveness of these analogs. The possibility that analogs interact differently with VDRE of certain hematopoietic and/or differentiation specific genes than they interact with the osteocalcin VDRE remains to be explored.

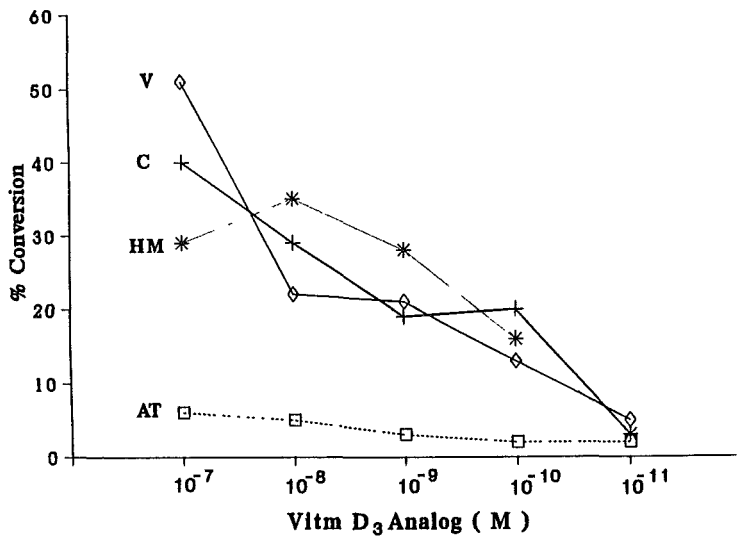

Fig 4. Dose-response effect of vitamin D_3 analogs on transcriptional activation.

CONCLUSIONS

The $1,25(OH)_2D_3$ and its analogs have regulatory effects on cell growth and differentiation in various kinds of tissues that extends beyond calcium and bone metabolism (1-3,75). These include growth inhibitory effects on tumor cell lines including melanoma, breast cancer and osteosarcoma (76-78); effects on proliferation and maturation of epidermal cells (79); functional effects on pancreas, cardiac muscle, pituitary, and thyroid glands (3). In several of these models, in vivo effects of these vitamin D_3 compounds have been demonstrated including their effects on mice inoculated with colon, lung and breast cancers, leukemia and melanoma cells (80-82). These observations support the concept that $1,25(OH)_2D_3$ have a wide-ranged of physiological roles. Further development and study of new vitamin D_3 analogs may identify potent therapeutic compounds for various human disease, perhaps including MDS/leukemia, low-grade non-Hodgkin's lymphoma (83), various solid tumors (82), psoriasis (84), osteoporosis (85), prevention of rejection of transplants and treatment of various autoimmune diseases such as rheumatoid arthritis (3). These investigations should facilitate our understanding of the mechanism of action of $1,25(OH)_2D_3$ and its analogs, as well as elucidate the mechanism contributing to the dichotomy between effects on calcium metabolism and other actions of these new compounds.

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Discussion - VITAMIN D_3 ANALOGS AND HEMATOPOIESIS

E. Dmitrovsky

Just a quick question, what is the nature of the defect of the receptor, is it the rickets class II patient population?

H.P. Koeffler

They actually have not been molecularly studied. Perhaps they are point mutations because the phenotype is somewhat heterogenous. Sometimes the receptor is detected by antibody but they are non-functional and sometimes the receptor protein is not identifiable at all.

I.B. Weinstein

We heard all about the complexity of the retinoid receptor system, could you give us a refresher on the vitamin D_3 receptor?

H.P. Koeffler

So far it does not seem to be as complex. It appears to be only one receptor, the consensus sequence for the receptor/ligand is a hexanucleotide separated by a spacer of three nucleotides. The vitamin D receptor can heterodimerize with RXR.

I.B. Weinstein

Has there been an intensive search for vitamin D_3 analogs since this might reveal receptor heterogeneity?

M. Oren

Does vitamin D or any of those analogs have any effect on the differentiation of bone precursors?

H.P. Koeffler

Yes, there are two major types of bone cells, osteoblasts and osteoclasts and

these are very hard to purify. An investigator in England can grow osteoblasts and their precursors on bone and claims that they can be induced to differentiate. Osteoblast cell lines, probably from osteosarcomas are readily available and those cells can be induced to differentiate. As far as osteoclasts, and again it is a murky area, they seem to be derived from macrophages. My data suggest that the myeloid precursors can be induced to differentiate towards macrophages in the presence of $1,25(OH)_2$ vitamin D_3. Some investigators suggest they can identify osteoclasts in the same system.

M. Oren

So I guess the next question is: have any of those analogs, or vitamin D itself, been tested for osteosarcoma or any malignancies related to bone differentiation?

H.P. Koeffler

No, but I think it is worth trying, both in vitro and in vivo.

H. De Thè

Is it known whether the vitamin D-3 receptor, like the oestrogen receptor, has two distinct transactivating domains and, if this were the case, could you imagine that your drugs would be like tamoxifen, that is, would antagonise one transactivating domain but leave the other intact?

H.P. Koeffler

To my knowledge there is only one transactivating domain.