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Journal of Steroid Biochemistry & Molecular Biology 97 (2005) 195–202
The Journal of Steroid Biochemistry & Molecular Biology
www.elsevier.com/locate/jsbmb

Vitamin D compounds in leukemia

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Abstract

The biologically active form of vitamin D, 1,25-dihydroxyvitamin D3 [1,25$(OH)_2$D3,] possess in vitro multiple anti-cancer activities including growth arrest, induction of apoptosis and differentiation of a variety of different types of malignant cells. However, its use as a therapeutic agent is hindered by its calcemic effects. Analogs of 1,25$(OH)_2$D3 have enhanced anti-tumor activity, with reduced calcemic effects. However, limited clinical studies using vitamin D compounds have not yet achieved major clinical success. Nevertheless, pre-clinical studies suggest that the combination of either 1,25$(OH)_2$D3 or its analogs with other agents can have additive or synergistic anti-cancer activities, suggesting future clinical studies.

Keywords: Vitamin D analogs; Anti-leukemic action; Leukemia

1. Introduction

Vitamin D3 is produced in skin and is sequentially metabolized by the liver and kidney to the biologically active form 1,25-dihydroxyvitamin D3 [1,25$(OH)_2$D3]. It is a seco-steroid hormone that regulates calcium homeostasis within the body. The genomic actions of 1,25$(OH)_2$D3 are modulated through its vitamin D receptor (VDR) [1]. VDR belongs to a superfamily of nuclear receptors that transduce hormonal signals from the immediate environment and transactivate genes in response to these signals. Target genes contain hormone response elements (VDREs) in their promoters to which heterodimers of VDR and retinoid X receptors (RXR) can bind and transactivate expression of the target genes [2]. The VDR is expressed in at least 30 different target tissues including bone, kidney, blood, breast, prostate, gut, activated B- and T-lymphocytes, monocytes and keratinocytes [3,4]. Perhaps every dividing cell type, normal and malignant, can express VDR and therefore can respond to 1,25$(OH)_2$D3.

2. 1,25$(OH)_2$D3 in normal hematopoiesis

Hematopoiesis is the formation of specialized circulating blood cells from pluripotent progenitor stem cells residing in the bone marrow. These stem cells are primitive blood cells that have the ability to either self-replicate or differentiate. The feedback system that regulates these stem cells is affected by bone marrow depletion, infection, hemorrhage, and stress. More mature, 'committed' stem cells proliferate and differentiate down different lineages acquiring specific functional properties such as the ability to fight infection (granulocytes and monocytes), stop and prevent bleeding (platelets) or carrying oxygen to the tissues (erythrocytes) [5]. Expression of VDR is detected in various normal hematopoietic and leukemic cells. Constitutive expression is found in monocytes, certain subsets of thymocytes, and after in vitro activation of T- and B-lymphocytes [6].

Mice whose VDR has been genetically deleted (knock-out, KO) have normal numbers of white cells (granulocytes, monocytes, and lymphocytes), platelets, and red cells compared to wild type (wt) mice [7]. However, addition of 1,25$(OH)_2$D3 to an in vitro clonogenic assay, dramatically increased the number of committed hematopoietic stem cells of the bone marrow that differentiated to macrophages from the wt, but not the KO mice. This suggests that 1,25$(OH)_2$D3

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doi:10.1016/j.jsbmb.2005.06.017

is not required for normal terminal differentiation of hematopoietic cells including macrophages. Thus, although $1,25(OH)_2D3$ has a profound effect in vitro on macrophage development, its role in differentiation of these cells in vivo is unclear. The proportion of T- and B-lymphocytes from peripheral blood was normal in the VDR KO mice, but their antigen-stimulated spleen cells produced more interleukin 4 (IL4) and less gamma-interferon (IFNγ) than those of wt mice, indicating impaired Th1 differentiation [5,7].

3. Molecualr targets of $1,25(OH)_2D3$

A screen of the human genome for VDREs and their corresponding genes, performed by Wang et al. [8], identified antimicrobial immune response genes, cathelicidin antimicrobial peptide, and defensin β2, to contain these response elements and to be induced by $1,25(OH)_2D3$. Functional VDREs have also been found in the promoters of many genes that regulate the cell cycle including p21, Rb, cyclin-dependent kinases (CDKs), p53, and PTEN [9–13]. Responsive genes of $1,25(OH)_2D3$ required for differentiation include p38 kinase and protein kinase C (PKC). A few of the other genes that are regulated by $1,25(OH)_2D3$ are BRCA1, early growth response gene-1, MAPK, and insulin-like growth factor-binding protein [14–18]. Furthermore, C/EBPβ and Rb are up-regulated as HL60 myeloblasts differentiate to macrophages when cultured with $1,25(OH)_2D3$. Some of the genes regulated by vitamin D compounds in leukemia are given in Table 1; in general, growth stimulating genes are down-regulated in their expression while those genes that limit growth and/or enhance differentiation are up-regulated.

Table 1
Molecular targets of vitamin D compounds in leukemia

Class	Genes	Regulation
Cell cycle/apoptosis	Cyclin D1, A, E	↓
	Cdk-2,-4,-6	↓
	Hypophosphorylated Rb	↑
	$p21^{Waf1}$, $p27^{Kip1}$	↑
	Bcl-2	↓
	Bax-mitochondrial translocation	↑
	Telomerase	↓
Oncogenes	*c-myc*	↓
	Dek1	↓
	Fli	↓
Tumor suppressors	PTEN	↑
	p53	↑
Kinases	PI3-K, p38, ERK activities	↑
	PKC levels	↑
Differentiation markers	Macrophage phenotype	↑

4. $1,25(OH)_2D3$ and leukemia

A brief overview of the use in vitro and in vivo of vitamin D compounds in cancer is presented in Table 2. The earliest findings were in murine and human myeloid leukemic cell lines showing that $1,25(OH)_2D3$ inhibited their proliferation and promoted their differentiation towards monocytes/macrophages [4]. Many studies that followed, have demonstrated that treatment with $1,25(OH)_2D3$ resulted in growth arrest, induction of monocytic differentiation and apoptosis in a variety of AML cell lines including HL-60, U937, NB4 and THP-1 [4,5,19,20].

5. Effects of vitamin D3 analogs in leukemia

The hypercalcemic action of $1,25(OH)_2D3$ is a serious toxicity, preventing pharmacological doses of the hormone to be given. Therefore, highly potent analogs of $1,25(OH)_2D3$ with little calcemic activity, have been synthesized [21–26]. The chemical structure of several of these analogs is presented in Fig. 1. The analogs have decreased binding affinity to the vitamin D binding protein which may increase their availability to enter cells. These compounds may have an

Table 2
A brief history of vitamin D compounds in cancer therapy

Compound	Tumor	First used	Ref.
$1,25(OH)_2D3$	Murine leukemia cell line, M1	1981	[68]
	Human leukemia cell line, HL-60		[69]
	Myeloid leukemia and myelodysplastic syndrome (MDS)		[70,71]
$1,25(OH)_2D3$ analogs	Hematopoietic and solid malignancies (in vitro studies)	1989	[72,73]
OCT (Chugai Pharmaceutical Company)	Rat osteosarcoma; breast cancer (in vitro and in vivo); prostate cancer; colon cancer (in vivo); pancreatic cancer (in vivo); thyroid carcinoma cells (in vitro)	1991	[74–79]
EB1089	Breast and prostate cancers (in vitro and in vivo)	1992	[80–83]
(Leo Pharmaceutical)	Breast and colon cancers (phase I study); pancreatic cancer (phase II study)	1998, 2002	[84,85]
	Liver cancer (clinical studies)	2001, 2003	[86,87]
Vitamin D2 analogs	Hematopoietic and solid tumors (in vitro and in vivo)	1998	[88]
High Dose $1,25(OH)_2D_3$	Prostate cancer	2003	[89]
Paricalcitol (Abbott Laboratories)	MDS, prostate cancer	2003–2004	[33,46,90]