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Review

Vitamin D Analogs and Coactivators

GUY EELEN[1], LIEVE VERLINDEN[1], PIERRE DE CLERCQ[2], MAURITS VANDEWALLE[2], ROGER BOUILLON[1] and ANNEMIEKE VERSTUYF[1]

[1]*Laboratorium voor Experimentele Geneeskunde en Endocrinologie, Katholieke Universiteit Leuven, Gasthuisberg, Herestraat 49, B-3000 Leuven;*
[2]*Vakgroep voor Organische Chemie, Universiteit Gent, B-9000 Gent, Belgium*

Abstract. *The secosteroid hormone 1α,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$] has potent antiproliferative and prodifferentiating actions on a wide variety of normal as well as malignant cell types. Strong calcemic effects obstruct the actual application of 1,25-$(OH)_2D_3$ for the treatment of hyperproliferative disorders such as cancer. To overcome this problem, structural analogs of 1,25-$(OH)_2D_3$ have been designed with a clear dissociation between antiproliferative and calcemic effects. This review focuses on the molecular mode of action of different 1,25-$(OH)_2D_3$ analogs and, in particular, on the recruitment of cofactor molecules to the vitamin D receptor by these analogs.*

1α,25-Dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$], the biologically active form of vitamin D, plays a crucial role in bone metabolism and in mineral homeostasis through complex interactions with parathyroid hormone (PTH) and calcium and phosphate levels. In addition to this classic effect, 1,25-$(OH)_2D_3$ has a powerful antiproliferative and prodifferentiating action on various normal and malignant cell types (1). This potent growth-inhibitory effect, combined with the presence of the vitamin D receptor (VDR) in a wide variety of cells, makes 1,25-$(OH)_2D_3$ an ideal compound to treat hyperproliferative disorders such as cancer. Nevertheless, major calcemic 'side'-effects (*e.g.,* hypercalcemia, hypercalciuria and increased bone resorption) at the required pharmacological doses have severely hampered the therapeutic application of 1,25-$(OH)_2D_3$. A way to overcome this hindrance is to design structural analogs of 1,25-$(OH)_2D_3$ with the same or even amplified antiproliferative and prodifferentiating capacity and with reduced undesired effects on calcium and bone metabolism. Two analogs that meet this criteria are the 14-epi-analogs 19-nor-14-epi-23-yne-1,25-$(OH)_2D_3$ (TX522) and 19-nor-14,20-bisepi-23-yne-1,25-$(OH)_2D_3$ (TX527). The present works briefly reviews the molecular mode of action of 1,25-$(OH)_2D_3$ in general and of the above two analogs, as well as of a number of other 1,25-$(OH)_2D_3$-analogs.

Correspondence to: Roger Bouillon, MD, Ph.D., Laboratorium voor Experimentele Geneeskunde en Endocrinologie, Onderwijs en Navorsing, 9th floor, Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium. Tel: +32 16 345970, Fax: +32 16 345934, e-mail: roger.bouillon@med.kuleuven.be

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Genomic actions of 1,25-$(OH)_2D_3$

The genomic action of 1,25-$(OH)_2D_3$ is mediated by nuclear VDR, a member of the superfamily of steroid/thyroid hormone receptors (2). Upon binding of 1,25-$(OH)_2D_3$, VDR recruits its preferred dimerization partner retinoid X receptor (RXR), the receptor for 9-cis-retinoic acid (3-5) (Figure 1). Binding of VDR-RXR heterodimers on the vitamin D response elements (VDREs) in the promoter region of target genes induces DNA-bending, which facilitates transcription complex assembly (6). To contact the basal transcription complex, VDR-RXR releases corepressors and recruits coactivator proteins. Ligand-binding in the ligand-binding pocket (LBP) of VDR causes helix 12 (H12) to close off the LBP and to expose its activation function 2 (AF2), to which these coactivators can bind through a conserved LXXLL motif in their amino acid sequence.

The coactivators that interact with the VDR are the CBP/p300 and p160 family of proteins, such as SRC-1, GRIP1/TIF2 and ACTR. These cofactors are known to recruit histone acetyl-transferase (HAT) activity; they acetylate histone tails and create a permissive chromatin surrounding for gene transcription (for review see 7). The multimeric vitamin D receptor interacting proteins (DRIP)-complex, of which the 205 kDa subunit (DRIP205) interacts directly with the VDR-RXR heterodimer, constitutes another class of coactivators; the DRIP-

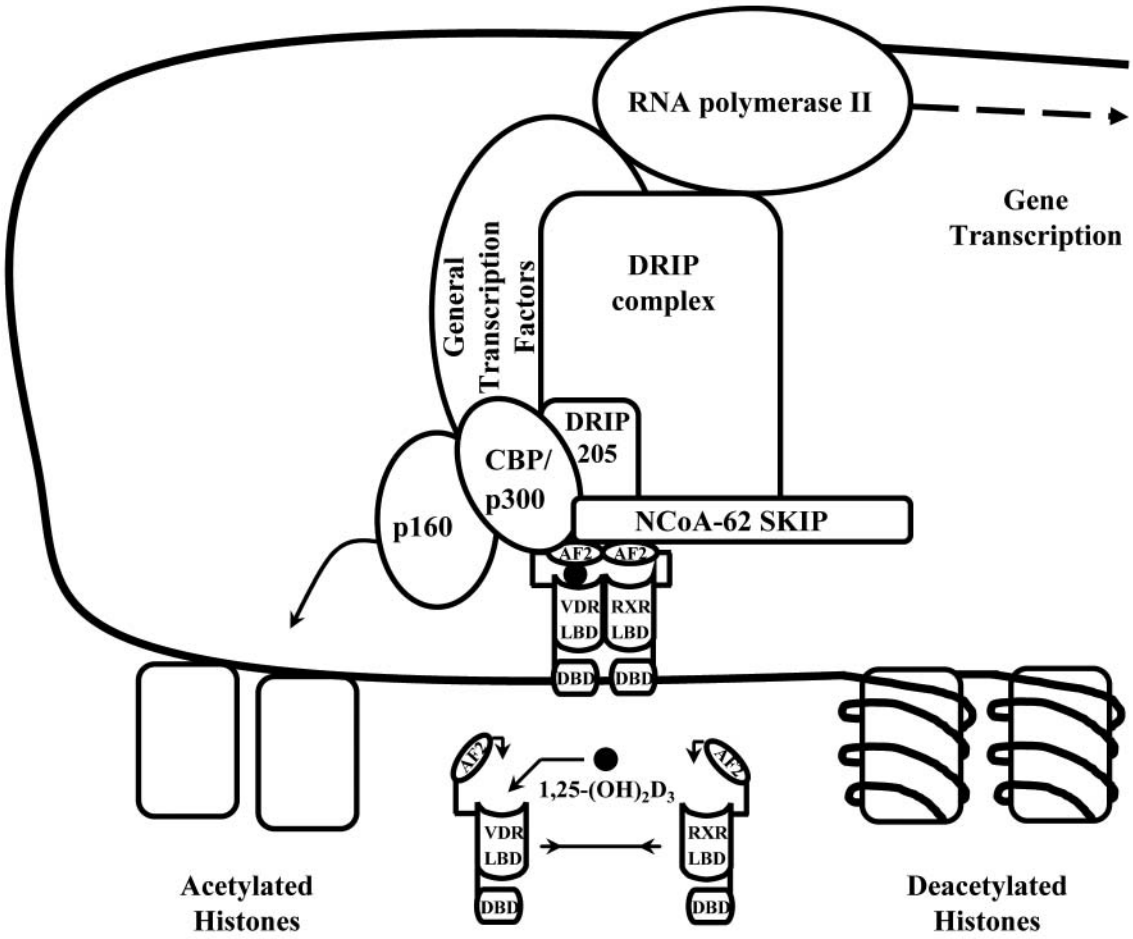

Figure 1. *Simplified scheme for gene transcription by 1,25-$(OH)_2D_3$. Ligand-bound vitamin D receptor (VDR) forms a heterodimer with retinoid X receptor (RXR) and binds to target vitamin D response element (VDRE) sequences. Upon binding of 1,25-$(OH)_2D_3$, VDR releases corepressors (not in figure) and recruits different coactivators (represented here by p160, CBP/p300, DRIP and NCoA-62/SKIP). The coactivators that recruit histone acetyl transferase (HAT)-activity acetylate histones create a permissive chromatin surrounding.*

complex is not known to be associated with HAT activity, but recruits RNA polymerase II, the key enzyme needed for gene transcription (8).

The existence of these two functionally different types of coactivator raises questions as to whether they act simultaneously or one after the other to mediate gene transcription. Evidence for the latter option comes from chromatin immunoprecipitation (ChIP) assays on estrogen and thyroid hormone response elements. Apparently, the transcription complex is first entered by p160 coactivators, which remodel and open the chromatin template by acetylation of histones; this event allows subsequent entry of the DRIP-complex (9, 10). In a recent study, Kim and colleagues used ChIP assays to determine 1,25-$(OH)_2D_3$-induced recruitment of VDR, RXR and coactivators to the VDREs in the promoters of the 24-hydroxylase (*Cyp24*)-gene and the osteopontin (*Opn*)-gene. Here too, the entry of DRIP205 into the transcriptional complex seemed to follow that of the p160 type of coactivators (11).

Two other types of coactivators include WINAC and NCoA-62/ski-interacting protein (SKIP). The former interacts with VDR through the Williams' syndrome transcription factor (WSTF) and displays ATP-dependent chromatin-remodeling activity (12), whereas the latter is thought to link transcriptional activation by nuclear receptors with mRNA splicing (13, 14). In addition, VDR also interacts with components of the basal transcription apparatus, such as transcription factor IIB (TFIIB) and TAF_{II}-17, a subunit of the general transcription factor TFIID (15, 16). A recent study showed that the peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α) can serve as a coactivator for VDR as well (17).

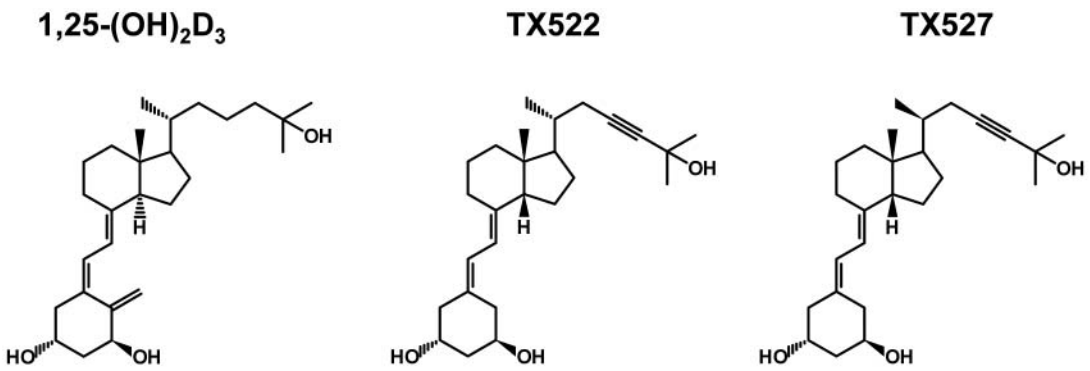

Figure 2. *Chemical structures of 1,25-$(OH)_2D_3$ and the two 14-epi-analogs, 19-nor-14-epi-23-yne-1,25-$(OH)_2D_3$ (TX522) and 19-nor-14,20-bisepi-23-yne-1,25-$(OH)_2D_3$ (TX527).*

The effects of 1,25-$(OH)_2D_3$ analogs on coactivator recruitment

The two 14-epi-analogs, 19-nor-14,20-bisepi-23-yne-1, 25-$(OH)_2D_3$ (TX527) and 19-nor-14-epi-23-yne-1,25-$(OH)_2D_3$ (TX522), have a strongly enhanced antiproliferative action (at least 10-fold) and are 50 to 400 times less calcemic than the parent compound 1,25-$(OH)_2D_3$; a feature that makes these two analogs suited for therapeutic application (18) (Figure 2). To determine the basis for their 'superagonistic' action, the activity of the two analogs was studied at different steps of the pretranscriptional complex. No differences were found between the 14-epi-analogs and 1,25-$(OH)_2D_3$ at the level of binding to VDR, at the level of interaction between the ligand-bound VDR and RXR, nor at the level of interaction of the ligand-VDR-RXR-complex with VDREs (19). However, both TX522 and TX527 induced stronger interactions between the VDR and the coactivator proteins TIF2, SRC-1 and DRIP205 than did the parent compound; this indicates that differences at the level of VDR – coactivator interactions underlie the superagonistic profile of TX522 and TX527. Moreover, assays with VID400 (a selective inhibitor of CYP24 (20, 21)) showed that the increased potency of the two analogs to induce VDR-coactivator interactions is not merely due to an increased resistance to CYP24-mediated catabolism (22). The superagonistic analog 2-methylene-19-nor-(20S)-1,25-$(OH)_2D_3$ was also demonstrated to be significantly more potent in inducing the interaction between VDR and the coactivators SRC-1 and DRIP205 (23). For the superagonistic 22-oxa-1, 25-$(OH)_2D_3$ analog OCT, a stronger interaction between VDR and TIF2 was seen (24). In contrast, a 25-carboxylic ester analog of 1,25-$(OH)_2D_3$, ZK159222, was unable to induce an interaction between VDR and the coactivators RAC3, SRC-1 and TIF2 (25).

From these findings, it can be deducted that an analog's ability to induce interaction between VDR and coactivators corresponds well with the analog's superagonistic or antagonistic profile. The results of our recent study, in which the analogs TX522, TX527, MC903 (1,24-dihydroxy-22-ene-24-cyclopropyl-vitamin D_3), BL314 (9,11-bisnor-16a-homo-20-epi-1,25-$(OH)_2D_3$), KH1060 (20-epi-22-oxa-24a,26a,27a-trihomo-1,25-$(OH)_2D_3$) and Ro24-5531 (1,25 $(OH)_2$-16-ene-23-yne-26,27-hexafluorocholecalci-ferol) were used, demonstrated a strong correlation for these analogs between their potency to inhibit the proliferation of human breast cancer MCF-7 cells and their ability to induce VDR – TIF2 interaction (26).

Enhanced coactivator recruitment by superagonistic analogs might be explained by the way the analogs dock into the VDR-LBP and the possible conformational changes at H12 (to which the coactivator can bind) of the VDR-LBP that result from that event. However, crystallographic studies of VDR complexed to 1,25-$(OH)_2D_3$ and the superagonistic 20-epi-analogs MC1288 and KH1060 have shown that there is almost no difference in conformation between VDR-LBD with the parent compound and VDR-LBD with MC1288 or KH1060 (27, 28); these findings corroborate the hypothesis that VDR-LBP has one single agonistic conformation to which the different ligands adapt. The superagonistic action of the analogs (*e.g.,* the above-mentioned 20-epi-analogs) more likely originates from stronger and more numerous contact points with VDR-LBP in comparison to 1,25-$(OH)_2D_3$. Co-crystallization of the VDR in complex with the 14-epi-analog TX522 demonstrated that this analog, in comparison with 1,25-$(OH)_2D_3$, has closer contacts with residues Ile268 and Val300 of VDR-LBP (22). How exactly these closer contacts lead to enhanced VDR-coactivator interaction remains unclear. Possibly, the closer contacts result in a more stable and energetically more favorable VDR-analog complex and, thus, in a longer half-life of the complex. In turn, this could lead to

more potent coactivator recruitment. However reasonable this hypothesis might seem, it conflicts with the finding that TX522 leaves the VDR faster, or in other words has a higher VDR-dissociation-rate than 1,25-$(OH)_2D_3$ (19).

Conclusion

Rational design of 1,25-$(OH)_2D_3$ analogs, with the aim of dissociating the antiproliferative from the calcemic effects, has yielded several thousands of analogs, a number of which have selective action on malignant tumors. Although the molecular mode of action of these 'superagonistic' analogs remains largely unknown, the difference between 1,25-$(OH)_2D_3$ and its analogs is generally most pronounced at the level of interaction between VDR and different coactivator proteins. For the 14-epi-analogs TX522 and TX527, clearly stronger interactions between VDR and the coactivators TIF2, SRC-1 and DRIP205 were detected. Although crystallographic studies point towards a single agonistic conformation of the VDR-LBP to which the different ligands adapt, subtle differences in ligand docking translated into altered contacts with residues of LBP might underlie the enhanced coactivator recruitment by several analogs. Co-crystallization studies of superagonistic analogs in complex with VDR and a coactivator molecule might be the most appropriate way to investigate why certain analogs induce stronger VDR-coactivator interactions.

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