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Vitamin D receptor: Mechanisms for vitamin D resistance in renal failure

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Vitamin D receptor: Mechanisms for vitamin D resistance in renal failure. 1,25-dihydroxyvitamin D [1,25$(OH)_2D_3$], the hormonal form of vitamin D, controls serum levels of parathyroid hormone (PTH) and parathyroid hyperplasia. Both 1,25$(OH)_2D_3$ actions involve regulation of gene transcription by the 1,25$(OH)_2D_3$/vitamin D receptor (VDR) complex. In advanced renal failure, in addition to low serum 1,25$(OH)_2D_3$ and reduced parathyroid vitamin D receptor content, several mechanisms downstream from 1,25$(OH)_2D_3$/VDR complex formation contribute to the impairment of 1,25$(OH)_2D_3$ action, including reduced levels of the retinoid X receptor, RXR, with the consequent reduction in VDR/RXR heterodimer formation, and accumulation of uremic toxins and increases in nuclear levels of calreticulin, two processes that impair the binding of the VDR/RXR complex to vitamin D responsive elements in vitamin D-regulated genes. VDR/RXR-heterodimer formation and its binding to DNA is critical for 1,25$(OH)_2D_3$ regulation of gene transcription. Early interventions with 1,25$(OH)_2D_3$ could delay the onset of vitamin D resistance by preventing both 1,25$(OH)_2D_3$ deficiency and its critical consequence, reduction in VDR content. Once established, vitamin D resistance could be counteracted by vitamin D analogs. While their less calcemic properties make higher dosing safer, their specificity to recruit co-activator molecules to the transcriptional pre-initiation complex could compensate for reduced 1,25$(OH)_2D_3$/VDR by potentiating VDR-transactivation/transrepression of genes critical for normal PTH synthesis and parathyroid cell growth.

Secondary hyperparathyroidism (HPT) is a frequent complication in chronic renal failure characterized by parathyroid hyperplasia and enhanced synthesis and secretion of PTH. 1,25$(OH)_2D_3$ is critical in controlling PTH synthesis and parathyroid cell proliferation [1]. Similar to most 1,25$(OH)_2D_3$ biological responses, its actions in the parathyroid glands demand a functional vitamin D receptor (VDR). This review presents our current understanding of the molecular mechanisms that cause vitamin D resistance in renal failure and insights into potential new avenues for therapy.

Key words: calcitriol, gene transcription, hyperparathyroidism, hyperplasia.

CURRENT MODEL OF 1,25$(OH)_2D_3$–VDR ACTION

The 1,25$(OH)_2D_3$ synthesized in the kidney by mitochondrial 1α-hydroxylase is transported in the blood by carrier proteins. Although vitamin D binding-protein (DBP) is the main carrier, 1,25$(OH)_2D_3$ also binds albumin and lipoproteins [2]. Recently, reports showing that the free form of 1,25$(OH)_2D_3$ triggers biological responses after entering target cells by simple diffusion have been challenged by the demonstration that, in renal proximal tubular cells, 25-hydroxyvitamin D uptake occurs through receptor (megalin)-mediated endocytosis of the 25(OH)D bound to plasma DBP [3]. A similar endocytosis could mediate the cellular uptake of 1,25$(OH)_2D_3$ bound to DBP or lipoproteins. Uremia-induced reduction in megalin expression may constitute a mechanism for vitamin D resistance independent of abnormalities in VDR content or function. Once inside the cell, 1,25$(OH)_2D_3$ can be inactivated by mitochondrial 24-hydroxylase or it can bind the VDR. Ligand binding activates the VDR to translocate from the cytosol to the nucleus, where it heterodimerizes with its partner, the retinoid X receptor, RXR. The VDR/RXR complex binds specific sequences in the promoter region of target genes, called vitamin D response elements (VDRE), and recruits basal transcription factors and co-regulator molecules to either increase or suppress the rate of gene transcription by RNA-polymerase II. Numerous genes, transcriptionally induced or suppressed by the 1,25D/VDR complex, are relevant for the efficacy of 1,25$(OH)_2D_3$ therapy in renal failure. The biological actions resulting from vitamin D-regulation of their expression include: (a) the classic vitamin D control of calcium homeostasis in bone, intestine, and the kidney; (b) regulation of the rates of 1,25$(OH)_2D_3$ synthesis and catabolism; (c) suppression of PTH synthesis; (d) modulation of immune responses; and (e) suppression of cell proliferation [2]. Several mechanisms have been identified as being responsible for the reduced efficacy of vitamin D in controlling the expression of these genes in renal failure.

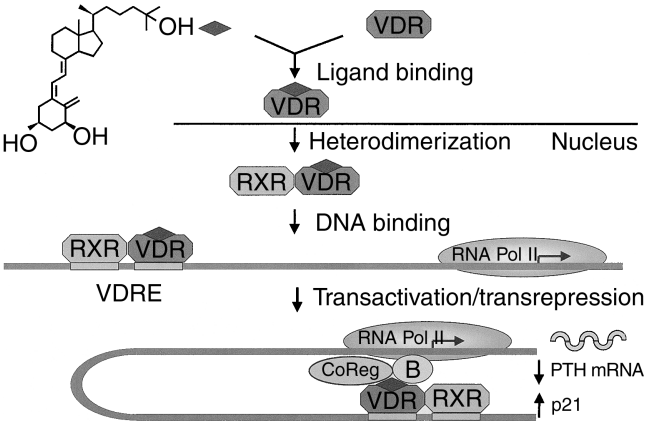

Fig. 1. $1,25(OH)_2D_3$ regulation of gene transcription. $1,25(OH)_2D_3$ binding activates the VDR to interact with nuclear RXR, basal transcription factors, and co-regulator molecules to activate p21 or repress PTH gene transcription by RNA polymerase II. B is basal transcription factor.

MECHANISMS FOR IMPAIRED $1,25(OH)_2D_3$/VDR ACTION IN RENAL FAILURE

It can be easily inferred from the diagram depicting $1,25(OH)_2D_3$/VDR control of gene transcription (Fig. 1) that, in the parathyroid glands, the magnitude of $1,25(OH)_2D_3$/VDR inhibition of PTH synthesis and/or parathyroid growth arrest (induction of p21) is determined mainly by the intracellular levels of both $1,25(OH)_2D_3$ and the VDR. It is well known that in renal failure, serum $1,25(OH)_2D_3$ decreases with the progressive reduction in glomerular filtration rates as renal function deteriorates. In addition to low serum $1,25(OH)_2D_3$, the immunohistochemical study by Fukuda et al [4] of nodular and diffuse hyperplastic human parathyroid glands demonstrate a marked reduction in VDR content, particularly in areas of more aggressive nodular growth. One reason for reduced VDR levels is the low serum $1,25(OH)_2D_3$ since the sterol increases VDR mRNA levels and protein stability, the latter by preventing VDR degradation by the proteasome complex, thus prolonging the half life of $1,25(OH)_2D_3$-bound VDR over that of the apo-VDR (unbound receptor) [5]. The actual contribution of $1,25(OH)_2D_3$ levels to VDR content in vivo was demonstrated in uremic rats by a strong correlation between serum $1,25(OH)_2D_3$ and parathyroid VDR content [6]. This suggests a potential for $1,25(OH)_2D_3$ therapy to correct the reduced parathyroid VDR content in renal failure patients. In fact, the reduced VDR content in the parathyroid glands of uremic rats could be increased to the levels in normal animals by administration of either $1,25(OH)_2D_3$ or its analog, 22-oxa-calcitriol [6].

In addition to impaired formation of the $1,25(OH)_2D_3$/VDR complex resulting from the combination of decreases in $1,25(OH)_2D_3$ synthesis and parathyroid VDR content, abnormalities in steps downstream from ligand binding to the VDR were demonstrated by the studies shown in Figure 2, which compares $1,25(OH)_2D_3$ action in peripheral blood monocytes from normal individuals and hemodialysis patients. In the presence of a similar VDR content, the binding of endogenous VDR/RXR complex to DNA is markedly impaired in uremia, leading to an 80% inhibition of the ability of exogenous $1,25(OH)_2D_3$ to induce 24-hydroxylase gene transcription. Other factors contribute to impairing $1,25(OH)_2D_3$/VDR control of gene transcription.

Reduced RXR

Studies in unilaterally nephrectomized rats demonstrated a reduction in the content of a 50 kD RXR isoform in cell extracts from the remnant kidney. This decrease in RXR results in a reduction of the binding of the endogenous VDR/RXR heterodimer to the VDRE of the mouse osteopontin promoter. A similar reduction of RXR content in the parathyroid glands in these rats could explain their enhanced serum PTH levels in the absence of hypocalcemia or hyperphosphatemia [7].

Accumulation of uremic toxins

Ultrafiltrate from uremic plasma causes a dose-dependent inhibition of VDR/RXR binding to VDRE and $1,25(OH)_2D_3$/VDR-transactivating function [8].

Increases in parathyroid calreticulin

Calreticulin is a cytosolic protein that binds integrins in the plasma membrane and the DNA-binding domain of nuclear receptors, including the VDR, thus interfering with receptor mediated transactivation. Hypocalcemia, commonly present in renal failure and caused by both low $1,25(OH)_2D_3$ or hyperphosphatemia, enhances nuclear levels of parathyroid calreticulin. In vitro studies demonstrate that increases in calreticulin inhibit VDR/RXR binding to VDRE in a dose-dependent manner and totally abolish $1,25(OH)_2D_3$ suppression of PTH gene transcription [9].

Activation of VDR-unrelated pathways interfering with $1,25(OH)_2D_3$ signaling

In human monocytes and macrophages, cytokine activation markedly inhibits $1,25(OH)_2D_3$-VDR gene transcription. Activation by the cytokine gamma interferon of its signaling molecule, Stat1, induces physical interactions between Stat1 and the DNA binding domain of the VDR, thus impairing VDR/RXR binding to VDRE and gene transcription [10]. The higher levels of inflammatory cytokines following hemodialysis could contribute to vitamin D resistance.

Little is known at present about how renal failure affects the last and most critical step in $1,25(OH)_2D_3$/VDR-mediated transactivation or transrepression. Bind-

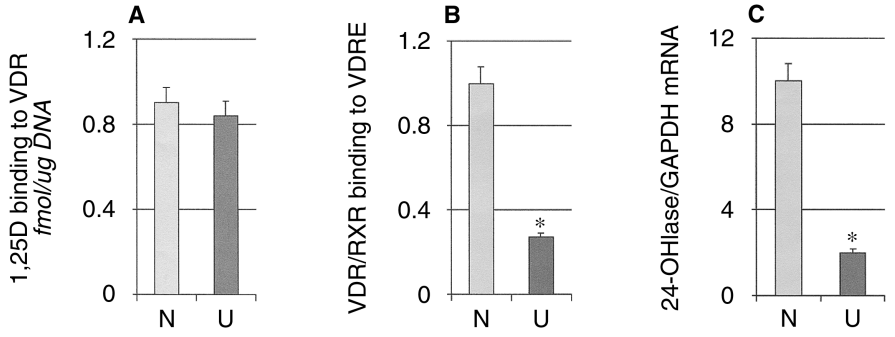

Fig. 2. Vitamin D resistance unrelated to reduced VDR. In spite of similar VDR content (left panel), endogenous VDR/RXR binding to human 24-hydroxylase VDRE is markedly impaired in monocytes from hemodialysis patients compared to normal volunteers (middle panel), thus resulting in almost complete inhibition of the ability of exogenous $1,25(OH)_2D_3$ to induce 24-hydroxylase gene transcription (right panel). U is hemodialysis patients.

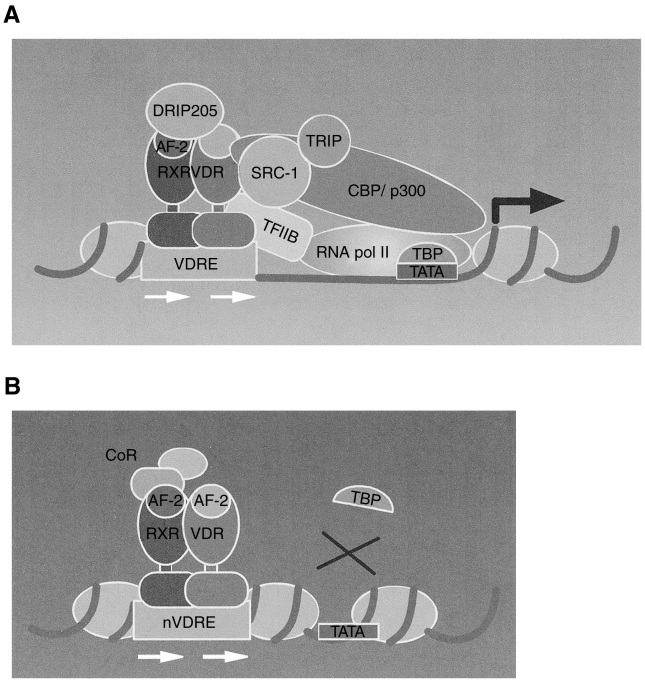

Fig. 3. Coregulator molecules in $1,25(OH)_2D_3$/VDR regulation of gene transcription. $1,25(OH)_2D_3$/VDR recruitment of coactivator (top) and/or corepressor (bottom) molecules to the pre-initiation complex modulates chromatin remodeling and gene transcription.

ing of the VDR/RXR heterodimer to the VDRE of genes induced by vitamin D (Fig. 3, top panel) begins the recruitment of coactivator molecules, which act synergistically with the VDR to markedly amplify $1,25(OH)_2D_3$ transactivation [11–13]. These coactivators possess histone acetyl transferase activity (SRC-1 and CBP/p300), which unfold and expose the DNA. The recruitment of a second complement of transcriptional coactivators, including DRIP205 and TRIP, favors the assembly of the pre-initiation complex and potentiates 1,25D/VDR induction of transcription. In transcriptional repression, the VDR/RXR complex bound to negative VDRE, that is, of genes that are suppressed by vitamin D, recruits co-repressors of the family of HDAC2, thus preventing DNA exposure and binding of TATA binding protein to initiate transcription (Fig. 3, bottom panel). The complex interactions of the VDR/RXR with VDRE and co-regulators in VDR transactivation or transrepression of vitamin D responsive genes suggests that, in uremia, vitamin D resistance may also result from a decreased expression of essential coactivator or corepressor molecules or from defective recruitment of these molecules by the VDR. Uremia-induced activation of VDR-unrelated signaling pathways could also interfere the recruitment of coregulator molecules to the VDR transcriptional pre-initiation complex.

In view of the numerous inhibitory interactions triggered by uremia, what are the mechanisms responsible for the efficacy of therapy with vitamin D or its less calcemic analogs? Studies by Takeyama et al [14] demonstrate that the ligand bound to the VDR dictates which coactivator is recruited by the VDR. This selective recruitment of coactivators has double implications for therapy. If a target cell expresses the coactivators required by either $1,25(OH)_2D_3$ or its analog, both vitamin D metabolites will elicit a similar efficacy. However, if the coactivator required by the analog is limiting or absent in a target tissue, the analog will elicit a weaker potency than the parent hormone. This mechanism could certainly contribute to analog selectivity. Furthermore, analog recruitment to the VDR transcription initiation complex of a coactivator more potent than that recruited by $1,25(OH)_2D_3$ could result in higher potency of the analog in eliciting a biological response. In fact, preliminary studies (abstract; Takeyama et al, *J Bone Min Res* 16: S433, 2001) [14] demonstrated that analog specific recruitment of corepressor molecules mediate their differential transrepression potency of the human PTH gene. Extensive research is mandatory before extrapolating these findings in vitro to VDR transcriptional potency in the parathyroid glands in vivo.

In summary, early therapeutic interventions with $1,25(OH)_2D_3$ or its analogs in renal failure result not only in prevention of $1,25(OH)_2D_3$ deficiency, but also in the reduction in cellular VDR content, thus improving formation of the 1,25D-VDR complex. Adequate prevention of hypocalcemia, hyperphosphatemia, and the accu-

mulation of uremic toxins will improve VDR/RXR binding to the VDRE of target genes. A better understanding of the role of nuclear coactivators/repressors in VDR-mediated transactivation should help design better VDR ligands in recruiting the most effective coregulator molecules, thus maximizing vitamin D efficacy in controlling parathyroid hyperfunction.

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