Vitamin D Receptor and Analogs

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In chronic kidney disease (CKD), high circulating levels of parathyroid hormone (PTH) cause osteitis fibrosa, bone loss, and cardiovascular complications that increase morbidity and mortality. Impaired production of 1,25-dihydroxyvitamin D (calcitriol), the hormonal form of vitamin D, is a major contributor to the generation and maintenance of parathyroid hyperplasia and increased synthesis and secretion of PTH. Calcitriol inhibits PTH gene transcription and ameliorates parathyroid hyperplasia by suppressing the expression of and growth signals from the autocrine transforming growth factor α (TGFα)/epidermal growth factor receptor (EGFR)-growth loop, a main determinant of parathyroid cell proliferation. Calcitriol reduction of parathyroid hyperplasia and serum PTH levels demands a functional vitamin D receptor (VDR). Although VDR is normal in CKD, parathyroid VDR content is reduced markedly. Furthermore, VDR function, as a transcriptional regulator of vitamin D responsive genes, is impaired by several factors including hypocalcemia, hyperphosphatemia, accumulation of uremic toxins, and reduction in cellular levels of the VDR partner, retinoid X receptor. Therapy with calcitriol analogs can overcome the antagonism on calcitriol-VDR actions induced by CKD. Although not all analog formulations are equally effective, they offer a wider therapeutic window in counteracting vitamin D resistance and survival advantage over exclusive calcitriol therapy.

IN CHRONIC KIDNEY disease (CKD) stage 3, impaired renal production of 1,25-dihydroxyvitamin D (calcitriol), the hormonal form of vitamin D, is a major contributor to the generation and maintenance of secondary hyperparathyroidism, a disorder characterized by parathyroid hyperplasia and increased synthesis and secretion of parathyroid hormone (PTH).[1] High circulating levels of PTH cause osteitis fibrosa, bone loss, and a variety of systemic defects, including cardiovascular complications that increase morbidity and mortality in renal failure patients.

Calcitriol directly represses both parathyroid cell proliferation and PTH synthesis.[2] Thus, its deficiency in renal failure causes high serum PTH levels and parathyroid gland enlargement. Calcitriol deficiency also leads to secondary hyperparathyroidism indirectly through decreasing intestinal calcium absorption. The resulting hypocalcemia is the most potent stimulus for rapid increases of PTH synthesis and secretion directed to normalize serum calcium.[1,2] Prolonged hypocalcemia also induces hyperplastic parathyroid growth.

The efficacy of calcitriol in inhibiting parathyroid cell growth and PTH synthesis renders calcitriol replacement therapy a valuable tool to treat renal hyperparathyroidism. However, calcitriol actions in the parathyroid gland demand a functional vitamin D receptor (VDR),[2] and although the VDR is normal in patients with CKD, parathyroid VDR content is reduced markedly in hyperplastic human parathyroid glands, particularly in areas of more aggressive growth.[3] Vitamin D resistance in advanced kidney disease (CKD stage 5) limits the efficacy of vitamin D replacement therapy.

This review presents the current understanding on mechanisms responsible for: (1) calcitriol/VDR-control of PTH synthesis and parathyroid cell growth; (2) resistance to the control of parathyroid function by the calcitriol/VDR complex in renal failure, and (3) the advantage of analog therapies in overcoming vitamin D resistance. In addition, it presents recent evidence on the relative efficacy of the vitamin D replacement therapy available today in controlling secondary hyperparathyroidism, bone disease, and morbidity and mortality in patients with CKD.

CALCITRIOL/VDR-CONTROL OF PTH SYNTHESIS AND PARATHYROID CELL GROWTH

The ability of calcitriol to inhibit PTH synthesis and arrest parathyroid cell growth in vivo and in vitro has been known for many years.[2] The mechanisms mediating calcitriol-transcriptional repres-

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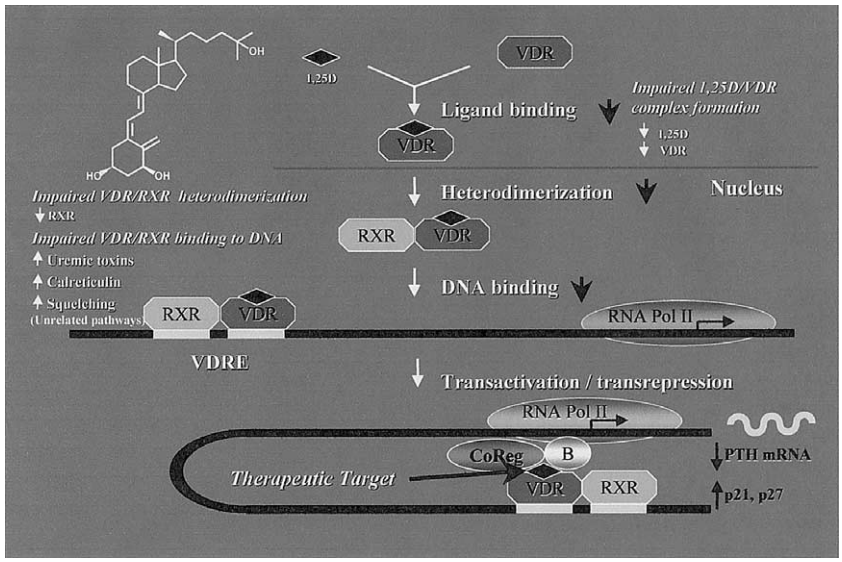

Fig 1. Mechanisms for impaired calcitriol/VDR regulation of gene expression in kidney disease. Calcitriol (1,25D) binding activates the VDR to interact with nuclear RXR, basal transcription factors (B), and co-regulator molecules (Coreg) to activate (p21, p27) or repress (PTH) gene transcription by RNA polymerase II. Uremia induced mechanisms to impair 1,25D/VDR complex formation, VDR/RXR heterodimerization, and DNA binding of VDR/RXR to VDREs in the promoter of calcitriol-regulated genes.

sion of PTH gene are depicted in Figure 1. In contrast, direct characterization of the pathogenic mechanisms underlying both induction of parathyroid cell proliferation by kidney disease and its suppression by calcitriol has been difficult because of a lack of an appropriate parathyroid cell line and the rapid dedifferentiation of primary cultures of hyperplastic parathyroid cells. Recent studies in our laboratory have implicated increases in parathyroid co-expression of the growth promoter, transforming growth factor-α (TGFα) and its receptor, the epidermal growth factor receptor (EGFR), in uremia-induced enhancement of proliferative activity and gland size.[4] Similar to secondary hyperparathyroidism in humans,[5] TGFα expression is higher in uremic rats compared with normal controls. More importantly, when uremia-induced hyperplasia is worsened, either by a high phosphorus (P)-[4] or a low calcium intake,[6] increases in parathyroid TGFα and EGFR expression directly correlate with high proliferating activity and gland enlargement. In normal and transformed tissues, enhanced co-expression of TGFα and EGFR is associated with aggressive growth. Using highly specific inhibitors of EGFR-tyrosine kinase, which abolish growth signals from ligand-activated EGFR, we have shown in vivo that enhanced parathyroid co-expression of TGFα and EGFR is a major contributor to the growth of the parathyroid glands in experimental renal failure.[7] In early stages of kidney disease in rats, prophylactic vitamin D administration (calcitriol or its analog 19-nor1,25-dihydroxyvitamin D_2 [19-nor]) counteracts parathyroid hyperplasia by preventing uremia-induced increases in parathyroid TGFα and EGFR expression, which occur within the first week after inducing kidney failure by 5/6 nephrectomy.[6]

The efficacy of analog therapy (19-nor and 22-oxacalcitriol [OCT]) in preventing further enlargement of the parathyroid gland in established secondary hyperparathyroidism (high TGFα/EGFR overexpression) suggested that calcitriol antiproliferative properties could involve down-regulation of the potent TGFα/EGFR growth-promoting signals. In fact, in normal and carcinogenic cell lines, overexpressing TGFα and EGFR, the potent vitamin D antiproliferative actions involve inhibition of EGFR growth-promoting signals from the plasma membrane as well as EGFR transactivation of the cyclin D1 gene.[8] Increased expression of parathyroid cyclin D1 is a common feature in secondary hyperparathyroidism in humans.[9]

The time of exposure to calcitriol required for the sterol to suppress parathyroid TGFα and EGFR expression in vivo and/or TGFα/EGFR growth signals in vitro[8] discards rapid nongenomic vitamin D actions and suggests a VDR-mediated effect. It is unclear at present whether calcitriol exerts transcriptional suppression of the TGFα and EGFR genes in parathyroid cells or on the unknown genes responsible for its anti-EGFR properties.

In addition to the strong inhibition of the expression and signaling of the TGFα/EGFR growth loop, calcitriol antiproliferative actions in hyper-

plastic parathyroid glands involve induction of the cyclin-dependent kinase inhibitors p21 and p27.[6,10] Both genes are induced transcriptionally by the calcitriol/VDR complex.

Calcitriol-VDR Regulation of Gene Expression

Figure 1 depicts the current model for calcitriol/VDR action. Calcitriol, a lipid-soluble molecule, is transported in the blood by carrier proteins, mainly vitamin D binding protein (DBP) and, in a lesser degree, albumin and lipoproteins.[2] In renal proximal tubular cells, 25-hydroxyvitamin D uptake occurs through receptor (megalin)-mediated endocytosis of the 25-hydroxyvitamin D bound to plasma DBP.[11] This surprising finding raised the possibility that the cellular uptake of circulating calcitriol takes place through an endocytic process similar to that of 25-hydroxyvitamin D rather than simple diffusion through the cell membrane. If megalin-mediated endocytosis is an important contributor to parathyroid calcitriol uptake, uremia-induced reduction in megalin expression in the parathyroid glands could constitute a mechanism for vitamin D resistance in addition to reduced VDR content.

Once inside the cell, calcitriol binding to the VDR activates its receptor to translocate from the cytosol to the nucleus where it heterodimerizes with its partner the retinoid X receptor (RXR). The VDR/RXR complex then binds specific sequences in the promoter region of vitamin D responsive 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.[2] In calcitriol/VDR transactivation, that is, calcitriol induction of gene expression, such as that of the promoter of growth arrest p21, the binding of the VDR/RXR heterodimer to the VDRE of this gene initiates the recruitment of co-activator molecules. These co-activators act synergistically with the VDR to markedly amplify calcitriol-transactivating potency.[12-14] The VDR-nuclear co-activators (SRC-1 and CBP/p300) possess histone acetyl transferase activity, which unfold and expose the DNA. This allows the recruitment of a second complement of transcriptional coactivators (DRIP205 and TRIP) that favor the assembly of the pre-initiation complex to potentiate calcitriol/VDR–induction of gene expression.

In transcriptional repression, such as that of the PTH gene, binding of the VDR/RXR complex to negative VDRE, recruits co-repressors of the family of histone deacetylases. These molecules prevent DNA exposure and, consequently, the binding of proteins (TATA binding protein) mandatory to initiate transcription.[12]

Both the transcriptional suppression of the PTH gene and the induction of the expression of the genes for the cyclin-dependent kinase inhibitors p21 and p27 by the 1,25D/VDR complex are of relevance for the efficacy of calcitriol therapy in renal hyperparathyroidism. The mechanisms mediating calcitriol/VDR suppression of parathyroid TGFα and EGFR expression and inhibition of membrane and nuclear EGFR growth signaling await identification.

Mechanisms for Impaired $1,25(OH)_2D_3$/VDR Action in Renal Failure

Figure 1 also summarizes the abnormalities in calcitriol/VDR regulation of gene transcription associated with kidney disease. Intracellular levels of calcitriol (1,25D) and the VDR determine the magnitude of 1,25D/VDR inhibition of PTH synthesis and/or parathyroid growth arrest (induction of p21 and p27). In CKD, serum calcitriol decreases as renal function deteriorates, starting at stage 3. Because calcitriol increases VDR messenger RNA levels and protein stability, the latter by preventing VDR degradation,[15] low serum calcitriol is in itself partially responsible for reduced parathyroid VDR content. In fact, a strong direct correlation exists between serum calcitriol and parathyroid VDR content in 5/6 nephrectomized rats.[16] More importantly, prophylactic administration of either calcitriol or its analog, 22-oxa-calcitriol, prevents the marked reduction in parathyroid VDR content induced by renal failure and worsen by high dietary P.[16] Thus, in kidney disease, impaired formation of the calcitriol/VDR complex, the first step in calcitriol action, results from the combination of decreases in renal calcitriol synthesis and parathyroid VDR content. Thus, early therapeutic interventions with oral calcitriol (0.25 daily) or its analogs, in CKD stage 3, could delay the onset of vitamin D resistance by preventing calcitriol deficiency and its critical consequence, the reduction in VDR content.

Studies in our laboratory in peripheral blood monocytes from normal individuals and hemodial-

ysis patients showed an impaired response to exogenous calcitriol (10^{-8} mol/L) despite a similar VDR. Thus, CKD induces abnormalities in VDR actions downstream from the formation of the calcitriol/VDR complex. Specifically, calcitriol/VDR-transcriptional activation of the 24-hydroxylase gene was inhibited by 80% in monocytes from hemodialysis patients. Several laboratories have identified factors contributing to impaired heterodimerization and VDR/RXR-binding to VDRE, including (1) reduced RXR. Studies in unilaterally nephrectomized rats showed a reduction in the content of a 50-kd RXR isoform in cell extracts from the remnant kidney. This decrease in RXR resulted in decreased endogenous VDR/RXR heterodimer formation and binding to VDRE (mouse osteopontin promoter). A similar reduction of RXR content in the parathyroid glands in these rats, even in the presence of normal parathyroid VDR, could explain their enhanced serum PTH levels in the absence of hypocalcemia or hyperphosphatemia.[17] (2) Accumulation of uremic toxins. Ultrafiltrate from uremic plasma causes a dose-dependent inhibition of VDR/RXR binding to VDRE and, consequently, in calcitriol potency to regulate gene expression in transfected JEG-3 cells.[18] (3) 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 sometimes presents in CKD stages 3 to 5 and could be caused either by decreased $1,25(OH)_2D_3$ or hyperphosphatemia. In rats, hypocalcemia enhances nuclear levels of parathyroid calreticulin. In vitro studies have shown that increases in calreticulin inhibit VDR/RXR binding to VDRE in a dose-dependent manner and totally abolish calcitriol suppression of PTH gene transcription.[19] High P^- induction of nuclear calreticulin in the parathyroid glands could mediate the well-known resistance to calcitriol action in hyperphosphatemic states. (4) 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. γ interferon activation 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.[20] Thus, the higher levels of inflammatory cytokines after hemodialysis could contribute to vitamin D resistance. Clearly, adequate prevention of hypocalcemia, hyperphosphatemia, accumulation of uremic toxins, and activation of the signaling pathways of inflammatory cytokines should improve VDR/RXR binding to the VDRE of target genes and the response to exogenous administration of calcitriol (or its less calcemic analogs) despite the reduced parathyroid VDR.

Little is known at present on how renal failure affects transactivation/transrepression, the most critical step in calcitriol/VDR-mediated regulation of gene expression. The numerous protein-protein-DNA interactions of the VDR/RXR with VDRE and nuclear co-regulator molecules[12-14] suggest that, in uremia, vitamin D resistance also could result from a decreased expression of essential coactivator- or co-repressor molecules or from defective recruitment of these molecules by the VDR. Alternatively, uremia-induced activation of VDR-unrelated signaling pathways also could interfere with the recruitment of co-regulator molecules to the VDR-transcriptional pre-initiation complex.

Figure 1 points to the ligand bound to the VDR as the therapeutic target to overcome the numerous inhibitory interactions triggered by uremia to induce vitamin D resistance in secondary hyperparathyroidism. Less calcemic vitamin D analogs offer a wider therapeutic window. In addition, an important new finding allows a better understanding of tissue-specific actions (selectivity) and potency of vitamin D analogs. Takeyama et al[21] showed that the ligand (calcitriol or its less calcemic analogs) bound to the VDR dictates critical changes in VDR tridimensional conformation that favor a selective recruitment of nuclear co-regulator molecules. Thus, in a target cell expressing the co-regulators required by either calcitriol or its analog, both vitamin D metabolites will elicit similar efficacy. Conversely, in cells in which the co-regulator required by an analog is limiting or absent, the analog will elicit weaker potency than the parent hormone. More important for therapy, analog recruitment to the VDR-transcription initiation complex of a co-activator more potent than that recruited by calcitriol could result in higher potency of the analog in eliciting a biologic response. This is not theoretical speculation. Studies by Takeyama et al[22] showed that analog-specific recruitment of co-repressor molecules mediate their

differential transrepression potency on the human PTH gene. Extensive research is mandatory before extrapolating these findings in vitro to VDR transcriptional potency in the parathyroid glands in vivo. Certainly, a better understanding of the role of nuclear co-activator/repressors in VDR-mediated transactivation should help design better VDR ligands in recruiting the most effective co-regulator molecules to maximize vitamin D efficacy in suppressing serum PTH, parathyroid cell growth, and, ultimately, regressing hyperplastic gland size through induction of parathyroid cell apoptosis.

ADVANTAGES OF THE VITAMIN D REPLACEMENT THERAPY CURRENTLY AVAILABLE

Intravenous, oral, and intraperitoneal calcitriol therapy has been used successfully in the United States for treatment of secondary hyperparathyroidism for almost 2 decades. However, the use of calcitriol at doses that effectively suppress serum PTH is limited by the development of hypercalcemia and hyperphosphatemia, which result from the potent effects of the sterol in increasing intestinal absorption and bone mobilization of calcium and phosphate.[2] In advanced kidney disease, the hypercalcemic toxicity of calcitriol is aggravated further by the concomitant use of large doses of calcium containing phosphate-binding antacids. To minimize the toxicities of calcitriol therapy, structural modifications in the vitamin D molecule led to the development of pro-hormone and calcitriol analogs. These compounds retain the capacity to suppress parathyroid function with lesser effects on kidney and bone. Their relative potencies in suppressing parathyroid function, calcemic activity, and mechanisms for selectivity are summarized later.

1α-hydroxyvitamin D_2 (1α-D_2, Hectorol, Bone Care Int, Middleton, WI) is a pro-hormone that requires hydroxylation in the C25 position in the liver to become active 1,25-dihydroxyvitamin D_2. The mechanisms for the selective actions of this pro-hormone are unclear. In experimental animals, 1α-D_2 is less toxic than its D_3 counterpart despite similar stimulation of calcium and phosphate transport.[23,24] A potential mechanism is the conversion of high levels of 1α-D_2 to 1,24-dihydroxyD_2, a compound with lower calcemic activity than calcitriol.[25,26] Oral and intravenous 1α-D_2 have been approved and used effectively for the treatment of secondary hyperparathyroidism in the United States,[27,28] although little evidence exists that this pro-hormone is less calcemic or hyperphosphatemic than its D_3 counterpart.

Three calcitriol analogs, falecalcitriol (Sunitomo Pharmaceutical, Osaka, Japan), 22-oxacalcitriol (OCT, Oxarol, Chugai Pharmaceuticals, Tokyo, Japan), and 19-nor-1,25-dihydroxyvitaminD2 (19-nor, Zemplar, Abbot Pharmaceuticals, Chicago, IL) have been approved for the treatment of secondary hyperparathyroidism.

Falecalcitriol, a calcitriol analog used in Japan, results from the substitution of hydrogens at C26 and C27 by fluorine atoms. Because of its decreased metabolic inactivation, falecalctriol has greater activity than alphacalcidol (1α-D_3) in suppressing PTH in patients with chronic renal failure.[29] Although serum calcium levels were similar in patients treated with either compound, the control of serum phosphorus was better with falecalcitriol treatment.[29]

OCT, a calcitriol analog available and used in Japan, differs from calcitriol by the substitution of an oxygen for C22. This structural modification reduces OCT affinity for both VDR and DBP.[30] The latter results in a rapid clearance of the sterol from the circulation. Marked differences in calcitriol and OCT pharmacokinetics could explain why OCT administration in vivo results in prolonged suppression of PTH secretion but short-lived effects on intestine and bone.[31,32] In addition to differences in metabolic half-life, the cell-specific recruitment of co-activator molecules by the OCT-VDR complex shown in vitro[21] could mediate OCT selectivity. Cell-specific expression of nuclear co-regulators required by the OCT-VDR complex in parathyroid, but not in intestinal or bone cells, could mediate OCT weaker calcemic activity.

In experimental animal models of kidney disease, OCT clearly was less calcemic than calcitriol.[32] Importantly, OCT was effective in reversing abnormalities in bone formation without affecting bone turnover.[33,34]

Paricalcitol, 19-nor, has a double bond at C22 and lacks the exocyclic carbon at position 19. Studies in uremic animal models showed that 19-nor is 10 times less active than calcitriol in mobilizing calcium from bone,[35] and 3 to 4 times less effective in suppressing serum PTH and parathyroid hyperplasia.[36,37] 19-nor is also approximately

10 times less calcemic and phosphatemic than $1\alpha D_2$ in normal and uremic rats.[38] Similar studies in hemodialysis patients with end-stage renal disease and on a low-calcium diet showed that 8 times more paricalcitol than calcitriol was required to achieve a similar increment in serum calcium levels, possibly mobilized from bone.[39] The mechanisms responsible for 19-nor selectivity are unknown at present.

Although 19-nor has less calcemic activity than calcitriol, hypercalcemia can occur during therapy. Hypercalcemic episodes were associated with overtreating as suggested by PTH suppression below the desired target range.[37] The widespread clinical use of 19-nor in the United States has allowed comparisons between therapy with this analog and calcitriol. Less severe hyperphosphatemia was shown in patients treated with paricalcitol compared with those receiving calcitriol.[40]

More importantly, up until 2002, there were no data to suggest one vitamin D formulation had a survival advantage compared with another. In a recent historic cohort study performed by investigators at Massachusetts General Hospital and Fresenius Medical Care (North America), a survival advantage was found among patients treated with paricalcitol compared with those treated with calcitriol.[41] In this study of over 60,000 chronic hemodialysis patients throughout the United States, patients naive to injectable vitamin D were followed-up after they were started on calcitriol or paricalcitol. Patients were censored when they switched formulations or received a renal transplant. During the 36-month follow-up period, patients treated with paricalcitol had a 16% survival advantage compared with those treated with calcitriol. This survival advantage accounted for baseline differences, including mortality rates of the different dialysis centers involved, duration on dialysis before starting injectable vitamin D, and baseline mineral levels.

Furthermore, patients who switched formulations also were studied. Those who switched from paricalcitol to calcitriol had a worse survival over the remaining follow-up period compared with those who switched from calcitriol to paricalcitol. Examination of serum levels of calcium, phosphate, and PTH revealed that the percent increase (from baseline) of serum calcium and phosphate was less and the percent decrease in serum PTH (from baseline) was more in the group treated with paricalcitol compared with those treated with calcitriol.

Although only a randomized trial can confirm these results, these compelling data suggest vitamin D therapy should be integral in the management of secondary hyperparathyroidism and that the formulations are not all equal.

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