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Nonclassical Aspects of Differential Vitamin D Receptor Activation

Implications for Survival in Patients With Chronic Kidney Disease

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Abstract

The 'classical' effects of vitamin D receptor activator or agonist (VDRA) therapy for the treatment of secondary hyperparathyroidism in patients with chronic kidney disease primarily involves suppressive effects on the parathyroid gland, and regulation of calcium and phosphorus absorption in the intestine and mobilisation in bone. Observational studies in haemodialysis patients report improved cardiovascular and all-cause survival among those receiving VDRA therapy compared with those not on VDRA therapy. Among VDRAs, the selective VDRA paricalcitol has been associated with greater survival than nonselective VDRAs, such as calcitriol (1,25-dihydroxyvitamin D_3). The survival benefits of paricalcitol appear to be linked, at least in part, to 'nonclassical' actions of VDRAs, possibly through VDRA-mediated modulation of gene expression. In cardiovascular tissues, VDRAs are reported to have beneficial effects such as anti-inflammatory and antithrombotic effects, inhibition of vascular smooth muscle cell proliferation, inhibition of vascular calcification and stiffening, and regression of left ventricular hypertrophy. VDRAs are also reported to negatively regulate the renin-angiotensin system, which plays a key role in hypertension, myocardial infarction and stroke. The selective VDRAs, paricalcitol and maxacalcitol, are associated with direct protective effects on glomerular architecture and antiproteinuric effects in response to renal damage. Paricalcitol regulates several cardiovascular and renal parameters more favourably than nonselective VDRAs. Complex nonclassical effects, which are not clearly understood, possibly contribute to the improved survival seen with VDRAs, especially paricalcitol.

Chronic kidney disease (CKD) is associated with metabolic and pathophysiological changes, which, if not adequately treated, result in increased cardiovascular risk and mortality.[1,2] An analysis of a national database of >40 000 haemodialysis patients revealed that disordered calcium and phosphorus metabolism, and hyperparathyroidism are independently associated with cardiovascular morbidity and mortality. Optimal management of the metabolic disorders accompanying CKD is essential for slowing disease progression and improving patient outcomes.

Active vitamin D (1,25-dihydroxyvitamin D_3) insufficiency in patients with CKD results in reduced intestinal absorption of calcium, serum calcium levels and parathyroid vitamin D receptor

(VDR) content, which triggers a compensatory release of parathyroid hormone (PTH) that may ultimately result in parathyroid hyperplasia. The elevated PTH levels that characterise secondary hyperparathyroidism (SHPT) in patients with CKD result in the mobilisation of calcium from bone and a reduction in bone mineral density.[3]

Decreased serum 1,25-dihdroxyvitamin D_3 levels not only characterise SHPT, but also play a central role in its development, and in the development of mineral bone disease and other important sequelae of CKD. Recent evidence shows that serum 1,25-dihdroxyvitamin D_3 levels decline earlier during the course of CKD than previously realised, in the presence of normal calcium and phosphorus levels.[4] Patients with predialysis CKD exhibit skeletal changes, evidenced by a reduction in bone mineral density, and changes in biochemical markers of bone formation and resorption. SHPT and reduced levels of serum 1,25-dihdroxyvitamin D_3 are evident in patients with both moderate and severe CKD (glomerular filtration rate 6–70 mL/min),[5] and 1,25-dihdroxyvitamin D_3 levels continue to fall as renal function declines.[6] Thus, early initiation of, and continued treatment with, a selective vitamin D receptor activator or agonist (VDRA) should be considered as very important in preventing the onset and delaying the progression of SHPT, and improving long-term outcomes in patients with CKD without deleterious effects in calcium and phosphorus.

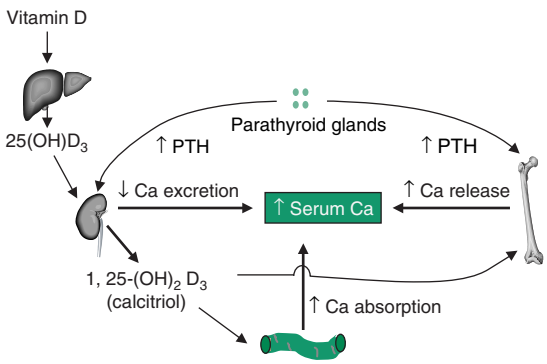


Fig. 1. 'Classical' actions of vitamin D receptor activation. Vitamin D is hydroxylated in the liver to 25-hydroxyvitamin D_3, which is then hydroxylated in the kidney to 1,25-dihydroxyvitamin D_3 (calcitriol; active vitamin D). 1,25-Dihydroxyvitamin D_3 enhances intestinal calcium and phosphorus absorption, and induces osteoclast maturation, which in turn stimulates calcium release from bone.[7] **PTH** = parathyroid hormone.

VDRA treatment is the standard approach for suppressing elevated PTH levels in patients with SHPT. The so-called 'classical' effects of VDRAs primarily involve regulation of parathyroid function, and calcium and phosphorus homeostasis (figure 1).[7] Animal studies have shown that this occurs by direct effects on the parathyroid gland, increasing VDR expression on parathyroid cells[8] and decreasing PTH production.[9] In addition to direct effects on the parathyroid gland, VDRAs act on the gastrointestinal tract, increasing intestinal absorption of calcium and phosphorus, and, in concert with PTH, mobilising calcium and phosphorus stores from bone to maintain serum calcium and phosphorus levels adequate for bone health and metabolic functions.[7]

However, excessive mobilisation of calcium and phosphorus, resulting in hypercalcaemia and hyperphosphataemia, are potentially serious problems associated with worsened outcomes that can accompany treatment with nonselective VDRAs such as calcitriol (synthetic 1,25-dihydroxyvitamin D_3).[10,11] This is particularly evident when calcitriol is used with calcium-based phosphate binders.[12] These undesirable mineral effects are likely to be the result of a combination of enhanced intestinal absorption and bone resorption. Therefore, a primary therapeutic goal in SHPT is to maintain desirable effects on PTH levels with minimal impact on calcium and phosphorus levels. This goal can be realised through the use of selective VDRAs that suppress PTH secretion and the development of parathyroid hyperplasia, while exerting lesser effects on mineral metabolism.

Currently, several VDRAs are used for the treatment or prevention of SHPT in patients with CKD (figure 2). Calcitriol was the first commercially available VDRA. Other VDRAs include doxercalciferol and alphacalcidol (αD or 1α), maxacalcitol (22-oxacalcitriol) and paricalcitol.[12,13] Both doxercalciferol and alphacalcidol require 25-hydroxylation in the liver to produce the active moieties, whereas all other VDRAs are not prodrugs

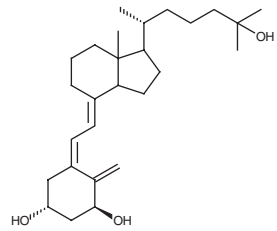
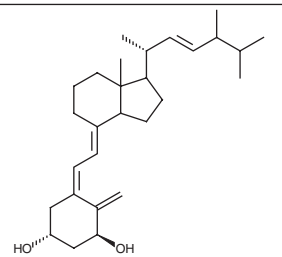
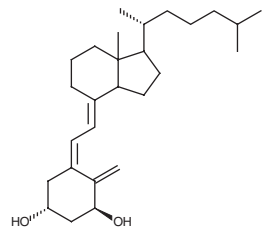
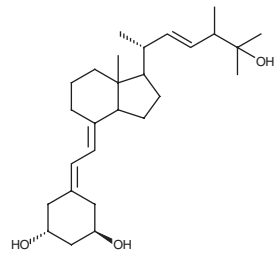
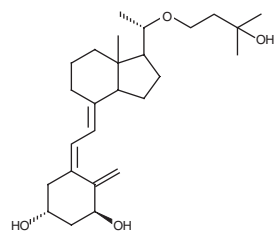
Name	Compound	Structure	Features
Nonselective			
Calcitriol	$1\alpha 25(OH)_2D_3$		Active Nonselective Suppresses PTH Associated with calcaemic and phosphataemic effects
Doxercalciferol	1α-$(OH)D_2$		Requires activation by liver Nonselective Suppresses PTH Similar to alphacalcidol in calcaemic and phosphataemic effects
Alphacalcidol*	1α-$(OH)D_3$		Requires activation by liver Nonselective Suppresses PTH Similar to doxercalciferol in calcaemic and phosphataemic effects
Selective			
Paricalcitol	19-nor- 1α-$25(OH)_2D_2$		Active Selective Suppresses PTH Less calcemic than calcitriol, doxercalciferol, and alphacalcidol
Maxacalcitol*	22-oxa- 1α-$25(OH)_2D_3$		Active Selective Suppresses PTH Less calcaemic and phosphataemic than calcitriol

Fig. 2. Currently marketed VDRAs for treatment in chronic kidney disease. **PTH** = parathyroid hormone; ***** indicates not approved by US FDA.[12,13,15]

and do not require the additional step of enzymatic activation.[12] These VDRAs differ in their functional selectivity at the VDR. The mechanisms and evidence for selectivity among VDRAs in the gut and bone are outlined briefly in this article, and are reviewed in greater detail by Brancaccio et al.[14] in an accompanying article in this issue of *Drugs*.

Induction of VDRA-mediated transcription requires heterodimerisation of VDR with the retinoid X receptor and the recruitment of comodulator proteins.[16] Through differential recruitment of transcriptional coregulators, as well as through differences in their ability to induce VDR homo- or heterodimerisation, nonselective and selective VDRAs can induce different patterns of gene expression,[17-19] which, as discussed in section 2, are paralleled by functional differences at the level of cells and tissues.

Animal studies have demonstrated that paricalcitol is less calcaemic and less phosphataemic than both doxercalciferol and calcitriol.[20,21] These differences result in a wider therapeutic window for the treatment of SHPT with paricalcitol. The lower calcaemic effect of paricalcitol appears to be due, in part, to differences from nonselective VDRAs in effects on intestinal absorption. In *ex vivo* and *in vitro* models, paricalcitol showed no appreciable effects on intestinal calcium transport, whereas calcitriol and doxercalciferol significantly increased transport.[22]

Maxacalcitol has also been shown to have increased selectivity in suppressing PTH in animals models with a therapeutic window that was 6-fold wider than that of calcitriol in uraemic rats.[23] Maxacalcitol was associated with less calcification than calcitriol in uraemic rats.[24] The increased selectivity of maxacalcitol is possibly due to its low affinity for the serum vitamin D binding protein and its pharmacokinetic profile. Half the haemodialysis patients achieved 30% reduction in PTH after 1 year of treatment with maxacalcitol; however, there were 51 hypercalcaemic episodes in 41 patients.[25] Another study that compared intravenous maxacalcitol with oral calcitriol for 6 months reported that maxacalcitol was as effective as calcitriol, but not superior.[26] Maxacalcitol is available only in Japan for the treatment of SHPT.

Although the bone resorptive capacity of calcitriol has been established,[27] recent data from genetically modified mice indicate that calcitriol, when tested in a background depleted of both endogenous 1,25-dihydroxyvitamin D_3 and PTH, also has anabolic activity in bone.[28] These data suggest that it may be possible for VDRAs to exert selectively beneficial anabolic effects on bone with decreased resorptive activity. Significant differences between selective and nonselective VDRAs on skeletal calcium mobilisation have been noted in animals: paricalcitol or maxacalcitol treatment had no significant effect on calcium release from mouse bone, whereas doxercalciferol induced a significant dose-dependent release.[29,30] Paricalcitol has also demonstrated reduced resorptive capacity in humans, as measured by phosphorus mobilisation, compared with doxercalciferol.[31]

Going beyond the primary therapeutic goal of PTH suppression, the results of several large observational studies of haemodialysis patients have demonstrated improved survival with VDRA therapy compared with no VDRA treatment.[32-35] Moreover, some of these studies have noted improved survival with paricalcitol compared with 1,25-dihydroxyvitamin D_3 or no VDRA therapy.[32,35] In contrast, Tentori et al.[36] reported that doxercalciferol (5.7 μg/week) and paricalcitol (7.5 μg/week) are associated with similar survival rates and equivalent effects on serum calcium, phosphorus and PTH levels. However, the authors noted that the differences in survival observed between patients treated with calcitriol (1.6 μg/week) and those treated with either doxercalciferol (5.7 μg/week) or paricalcitol (7.5 μg/week) were smaller than previously reported. Furthermore, a smaller sample size of only 7731 total patients distributed among three groups is not large enough to distinguish a 12–16% difference in survival. The study by Teng et al.[35] demonstrated a 16% survival benefit with paricalcitol compared with calcitriol in a larger sample size of 67 399 patients. These survival benefits and the differential effects of selective VDRAs

can be linked to the nonclassical effects of VDRAs, which are distinct from their effects on the parathyroid gland, gut and bone, and are the focus of this review.

1. Survival Benefits Associated With Vitamin D Receptor Activator (VDRA) Treatment

Low levels of 25-hydroxyvitamin D_3 and 1,25-dihydroxyvitamin D_3 are common in haemodialysis patients, and are associated with increased mortality among those not receiving injectable VDRA therapy. Interestingly, among the patients who had low vitamin D levels, those who received intravenous paricalcitol had an improved survival benefit compared with those who had not received this selective VDRA.[37] However, further research using prospective, randomised, controlled trials is required to better understand survival benefits associated with VDRA treatment.

In a historical cohort study involving 51 037 haemodialysis patients, 2-year survival was 75.6% in the group that received an injectable VDRA, compared with 58.7% in the group that did not receive a VDRA ($p < 0.001$). After adjustment for potential confounders, the analysis revealed that patients who received any form of injectable VDRA had a 20% lower mortality compared with those who did not receive a VDRA (figure 3a).[32] These investigators also compared the 36-month survival rate among patients undergoing long-term haemodialysis treated with paricalcitol (n = 29 021) versus calcitriol (n = 38 378).[35] In an analysis adjusted for period of study entry, the mortality rate was 16% lower among paricalcitol-treated patients compared with calcitriol-treated patients. A 2-year survival rate of 73% was observed within a subset of patients who switched from calcitriol to paricalcitol therapy, compared with 64% among patients who switched from paricalcitol to calcitriol therapy ($p = 0.04$ in favour of paricalcitol) [figure 3b].

One criticism of the previous analyses of the impact of VDRA therapy on survival is that associations between survival and baseline values were made without fully accounting for variations in clinical and laboratory values over the study period. In an elegantly designed study, Kalantar-Zadeh et al.[33] collected data prospectively and used a time-dependent Cox model with repeated measures and a fixed-covariate Cox model with only baseline values to examine associations between survival, quarterly laboratory values and administered paricalcitol in a 2-year cohort of 58 058 patients on maintenance haemodialysis. Administration of any dose of paricalcitol, based on average dose administered within

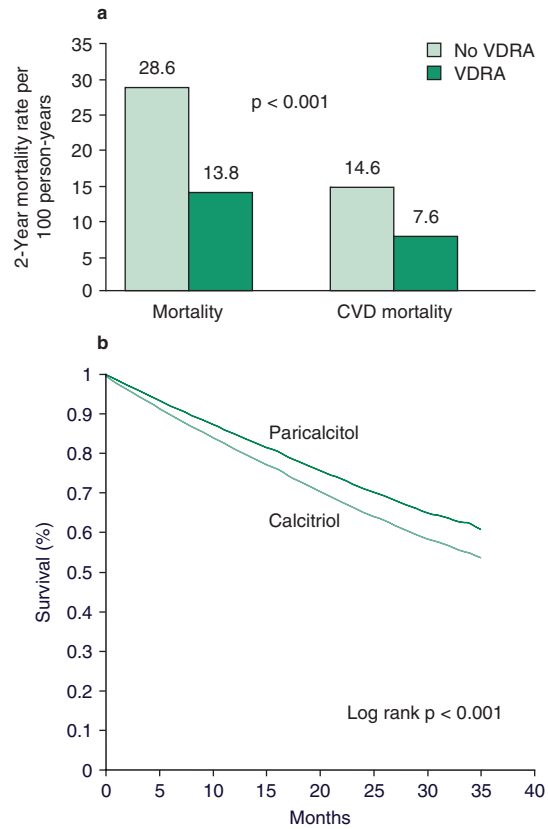


Fig. 3. (**a**) Therapy with an injectable vitamin D receptor activator (VDRA) is associated with a significant reduction in all-cause (52%) and cardiovascular (48%) mortality compared with no VDRA therapy over a 2-year period in patients with stage 5 chronic kidney disease. p-Value relates to both mortality and cardiovascular disease (CVD) mortality.[32] (**b**) Kaplan-Meier survival curves for selective VDRA therapy with paricalcitol vs nonselective VDRA therapy with calcitriol; survival at end of the 36-month follow-up period are 59% and 51%, respectively (reproduced from Teng et al.,[35] with permission. Copyright © *[2003] Massachusetts Medical Society. All rights reserved*).

each calendar quarter, was associated with improved survival in the time-varying models. As was noted in the previous studies that used non-time-dependent approaches, higher serum calcium and phosphorus levels were consistently associated with increased mortality risk, although a higher serum calcium threshold was noted using a time-dependent model compared with the non-time-dependent models (>10.5 mg/dL compared with >8.5 mg/dL, respectively). Survival rate was greatest within the normal range of serum calcium. In another observational study, haemodialysis patients with 25-hydroxyvitamin D_3 levels ≥10 ng/mL or 1,25-dihydroxyvitamin D_3 levels ≥15 pg/mL who received an injectable VDRA showed significantly reduced risk of early mortality compared with patients who were untreated and had 25-hydroxyvitamin D_3 and 1,25-dihydroxyvitamin D_3 levels below these respective values.[37]

2. VDRA Treatment and Cardiovascular Effects

Cardiovascular disease is the leading cause of death in patients with stage 5 CKD. From 2002 to 2004, approximately one-half of deaths among dialysis patients were attributable to cardiovascular disease or related causes.[38] Therefore, it is rational that an investigation of the mechanisms underlying the beneficial effects of VDRAs on survival should begin by considering the cardiovascular effects of these agents. A number of potential mechanisms underlie the increased cardiovascular morbidity and mortality risk in patients with stage 5 CKD. Excessive growth of vascular smooth muscle cells (VSMCs), vascular calcification, arterial stiffness and acute-phase inflammatory markers are each present to a greater degree in patients with CKD than in the general population, and each is strongly correlated with the presence of atherosclerotic cardiovascular disease,[39] including coronary artery disease,[40] peripheral artery disease,[41] left ventricular (LV) hypertrophy[42] and mortality.[41,43-45] VDRAs have been shown to have a variety of effects on these interrelated processes in cardiovascular tissues that may underlie their cardioprotection. Experimental evidence for cardioprotective effects and for differential effects of selective and nonselective VDRAs is presented in sections 2.1–2.4.

2.1 Effects of VDRAs on Vascular Smooth Muscle Cells

Vitamin D deficiency and low 1,25-dihydroxyvitamin D_3 levels are associated with arteriosclerosis and endothelial dysfunction in dialysis patients.[46] A current hypothesis to explain the development of vascular thickening, calcification and atherosclerosis in patients with CKD focuses on the effects of high phosphate levels and uraemia[47-49] on VSMCs on the medial arterial wall. These effects appear to be mediated by changes in expression of genes, including core binding factor α1, within the VSMC that induce osteoblast-like phenotypic changes, which promote growth and calcification.[50,51]

Vitamin D receptors are expressed in VSMCs[52] as well as endothelial cells,[53] and 1,25-dihydroxyvitamin D_3 has been shown to modulate VSMC proliferation.[52] The effects of nonselective and selective VDRA treatment with calcitriol and paricalcitol on proliferation of VSMCs were compared in primary rat aortic cultures.[54,55] Calcitriol induced a dose-dependent increase in VSMC proliferation, measured by bromodeoxyuridine labelling, in quiescent cells and in cells stimulated by fetal bovine serum to grow *in vitro*, as well as in aortic rings *ex vivo*. The effect was mediated by an increase in vascular endothelial growth factor A expression. In contrast, paricalcitol did not have a proliferative effect on VSMC (figure 4a).

The impact of a nonselective therapy (calcitriol) and a selective therapy (paricalcitol) on vascular calcification was also compared in rat aortic VSMCs cultured in calcification media, as well as in intact rat aorta in nephrectomised rats.[57] Calcitriol induced a significant increase in calcium incorporation in VSMC, measured by ^{45}Ca uptake, compared with cells cultured in medium alone, whereas paricalcitol had no appreciable effects on calcification. Similarly, whereas calcitriol markedly increased aortic calcification *in vivo*, imaged with von Kossa staining, only sporadic calcification was seen with

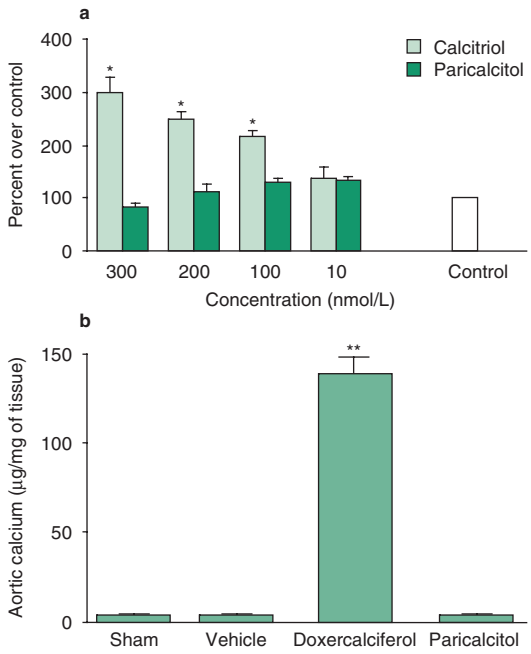


Fig. 4. (**a**) Paricalcitol induced dose-dependent and significantly lower levels of vascular smooth muscle cell (VSMC) proliferation than calcitriol, as measured by bromodeoxyuridine incorporation *in vitro*. Control was quiescent rat aoxtic VSMC (reproduced from Cardus et al.,[54] with permission). (**b**) Calcium content in the aorta of uraemic rats treated for 40 days with doxercalciferol or paricalcitol. Following treatment, the aortas were harvested and tested for ^{45}Ca uptake *ex vivo* using liquid scintillation measurement of radioactivity (reproduced from Wu-Wong et al.,[56] with permission). * $p < 0.01$, ** $p < 0.01$.

paricalcitol treatment, even under conditions of similar serum calcium levels. Increased vascular calcification was also differentially associated with increased pulse pressure in this system. The differential effect on calcification may be the result of differences in gene activation: only cells incubated with calcitriol exhibited increased expression of nuclear factor-κB ligand (RANKL), whereas expression of control genes was similar with calcitriol and paricalcitol treatment.[57] In another study, paricalcitol treatment of uraemic rats was associated with significantly reduced aortic calcium *in vivo* compared with doxercalciferol (figure 4b).[56] In this study, calcification was dependent on elevated phosphate levels, consistent with the calcification hypothesis mentioned earlier in this section. These results suggest that a decreased proliferative and calcifying propensity of paricalcitol may provide additional advantages over nonselective VDRA therapy, along with its lower calcaemic and phosphataemic potential.

2.2 Effects of VDRAs on Inflammatory, Atherogenic and Thrombotic Processes

The uraemic state is associated with chronic inflammation and atherogenesis, as evidenced by elevated levels of a number of inflammatory markers, such as C-reactive protein, and proinflammatory cytokines such as interleukin (IL)-1 and IL-6.[58] Expression of these markers is associated with increased cardiovascular risk and increased cardiovascular and all-cause mortality in patients with stage 5 CKD.[45] In a study of otherwise healthy adults, nutritional vitamin D insufficiency was associated with elevated levels of inflammatory mediators associated with atherosclerosis, tissue matrix metalloproteinases 2 and 9, as well as C-reactive protein. VDRA supplementation reduced levels of atherosclerosis-associated inflammatory markers in these patients.[59] A recent 2-year prospective study involving patients on haemodialysis used computed tomography imaging to demonstrate that elevated C-reactive protein was a strong independent predictor of progression of coronary artery calcification, even after adjusting for baseline calcification.[60]

A number of potential inducers of calcification have been identified in cell culture models. These include, but are not limited to: (i) the transcription factor core binding factor α1 (Cbfα1), which upregulates expression of type I collagen and other matrix components that serve as scaffold structures for the calcification process;[61,62] (ii) inflammatory cytokines IL-1β, IL-6 and tumour necrosis factor (TNF)-α;[63,64] (iii) tumour growth factor (TGF)-β1;[65] and (iv) bone morphogenetic protein (BMP)-2.[66] Inhibitors of calcification have also been identified using genetically modified mice and cell culture models. These include matrix γ-carboxyglutamic acid protein (MGP),[67] osteopontin[68] and type IV collagen.[62]

In addition to modifying the expression of inflammatory mediators, VDR activation in osteoblas-

tic cells has been shown to inhibit the synthesis of inducers of calcification, such as type I collagen.[69] VDRA-mediated inhibition of expression of Cbfα1,[70] IL-1β,[71,72] IL-6,[72] TNFα[71] and BMP-2,[73] as well as increased cellular responsiveness to TGFβ,[74] has also been demonstrated. Similarly, VDR activation is associated with upregulation of inhibitors of calcification, including MGP,[75] osteopontin[69] and type IV collagen.

Differential effects of selective and nonselective VDRAs on factors involved in calcification have been demonstrated in five of six nephrectomised rats.[76] After 1 month of three times a week administration, both calcitriol (10ng) and doxercalciferol (40ng) significantly increased vascular calcification, assessed by von Kossa staining, as well as Cbfα1 and osteocalcin messenger RNA (mRNA) expression. However, the selective VDRA paricalcitol (40ng) did not have significant effects on any of these parameters. To determine whether the increase in aortic calcification was the result of only the increase in calcium-phosphorus product or a differential effect of the nonselective and selective VDRAs on vascular calcification, the study was repeated with doxercalciferol at a dose of 25ng. This dose did not significantly increase the calcium-phosphorus product, but it did induce aortic and myocardial calcification. This suggests that the differential effect between paricalcitol and the nonselective VDRAs on vascular calcification was independent of effects on the calcium-phosphorus product, indicating differential mechanisms of action between these agents.

VDRA activation also has potentially beneficial effects on platelet aggregation and thrombogenesis, which are critical components of inflammation and the development of atherosclerotic disease. VDRAs up-regulated the expression of the anticoagulant protein thrombomodulin and down-regulated expression of tissue factor, a coagulation factor in monocytic cell cultures.[77] VDR knockout mice rendered normocalcaemic through a high-calcium diet exhibited significantly enhanced platelet aggregation compared with wild-type mice and hypocalcaemic knockout mice, suggesting that VDR activation has a suppressive effect on platelet aggregation.[78] VDRAs also modulate plasminogen activator inhibitor (PAI)-1 expression in endothelial cells,[79] which is an established marker of inflammation and cardiovascular risk.[80] Differential effects of selective and nonselective VDR activation were also noted in this system: PAI-1 levels were markedly reduced in paricalcitol- versus calcitriol-treated cells at concentrations of paricalcitol that were similar to those observed in the sera of haemodialysis patients treated with this agent.[79]

2.3 Arterial Stiffening and Left Ventricular Hypertrophy

Arterial wall thickening, calcification and stiffening in patients with CKD has a deleterious effect on aortic pulse wave velocity (PWV), which increases LV load and, ultimately, increases the risk of LV hypertrophy.[81,82] Aortic stiffness and insensitivity of aortic PWV is an independent predictor of cardiovascular mortality in patients with stage 5 CKD.[83] LV hypertrophy is also an independent risk factor for mortality in these patients and regression of LV mass has been associated with survival benefits in these patients.[84]

A recent study conducted in uraemic rats found that chronic treatment with calcitriol or paricalcitol suppressed PTH with similar efficacy. However, calcitriol, but not paricalcitol (equivalent doses), was associated with aortic calcification and increased PWV.[85] In a prospective, controlled study of patients undergoing a short, daily haemodialysis regimen, paricalcitol, despite high doses, was associated with less vascular calcification at 12 months compared with a control.[86] Paricalcitol was also associated with LV mass reduction, which was greater in patients receiving paricalcitol compared with calcitriol.[87]

2.4 Effects on the Renin-Angiotensin System

It is well established that inappropriate activation of the renin-angiotensin system (RAS) plays an important role in the risk of hypertension, myocardial infarction and stroke. The RAS may be involved in the beneficial effects of VDRAs on cardiac hyper-

trophy. Several lines of evidence from animal studies indicate that VDRAs act as a direct negative endocrine regulator of the RAS. In VDR knockout mice, renin expression and angiotensin II production were increased several fold compared with wild-type mice.[88] However, expression of the angiotensin II precursor, angiotensinogen, was normal in the VDR knockout mice, indicating that the increase in angiotensin II was mainly the result of an increase in renin activity. Mutant mice exhibited hypertension, cardiac hypertrophy and increased water intake. Further studies indicated that cardiac hypertrophy in VDR knockout mice is a consequence of activation of both the systemic RAS and cardiac RAS, supporting the notion that VDR activation not only has systemic effects but also important local tissue effects that regulate cardiovascular and renal health.[89]

Both calcitriol and paricalcitol suppressed renin mRNA expression in similar dose-dependent fashions in As4.1 cells transiently transfected with the human VDR complementary DNA.[15,90] Paricalcitol was as potent a renin suppressor as calcitriol (concentration that produces a 50% effective response = 3.3 vs 3.5 nmol/L) despite findings that paricalcitol is ≈4-fold less potent than calcitriol in PTH suppression and ≈10-fold less calcaemic. In vitamin D-deficient rats with elevated PTH levels and hypocalcaemia, paricalcitol suppressed renal renin mRNA expression, as well as PTH levels, at doses that had no significant effect on serum calcium levels.[91] These data suggest that paricalcitol may have a greater therapeutic window for providing beneficial effects on the RAS with less risk of hypercalcaemic effects, just as it does for PTH suppression. Gene mapping studies indicate that VDRA effects on renin expression are mediated at the level of renin gene transcription through blockage of nuclear protein binding to the cyclic adenosine monophosphate response element, which plays a crucial role in regulation of renin gene expression.[92] A recent animal study reported that in VDR knockout mice there is marked expansion of the juxtaglomerular apparatus, increased cardiac myocyte hypertrophy, and increased renin and angiotensin levels suggestive of inhibitory effects of vitamin D on RAS.[88]

The data reviewed in section 2 indicate that VDRAs have complex and interrelated actions that serve to modify mediators of inflammation, cardiovascular remodelling, calcification, thrombosis and the RAS. Moreover, it appears that many of these effects are potentially beneficial to patients with CKD and they may differ according to VDRA selectivity. As discussed in the section 3, direct glomerular and other renoprotective effects may also play a role in improved survival benefits of selective VDRAs.

3. VDRA Treatment and Renoprotection

3.1 Glomerular Effects

Renal fibrosis and progressive deterioration of renal function in response to metabolic, immune or haemodynamic insult is a complex process that involves renal inflammation, and alterations in the structure and function of the renal parenchyma, mediated by regulation of cell growth, death and differentiation. There is considerable evidence in the literature that VDRAs play a direct role in the regulation of renal cell growth and differentiation following renal damage.[93]

Studies in uninephrectomised animals, and *in vitro* studies using mesangial and tubular cell cultures, indicate that VDRAs have potentially beneficial antiproliferative effects.[94-96] In subtotally nephrectomised rats, calcitriol treatment was associated with a significant reduction in mean glomerular volume and glomerulosclerosis index compared with vehicle-treated animals. Animals treated with calcitriol also showed significantly lower renal levels of the sclerosis-promoting cytokine TGFβ. It was concluded that these findings were independent of VDRA-mediated suppression of PTH secretion.[97] These studies indicate that VDRA treatment can attenuate the glomerular growth and scarring seen in renal impairment in animal models.

Potential effects of paricalcitol on glomerular growth and scarring were evaluated in a mouse model of obstructive nephropathy induced by unilat-

eral ureteral obstruction, a model characterised by tubulointerstitial lesions.[98] Compared with vehicle treatment, paricalcitol attenuated renal interstitial fibrosis, demonstrated by reduced interstitial volume, decreased collagen deposition, and reduced expression of fibronectin and type I and III collagens. Paricalcitol also inhibited TGFβ expression and that of its type I receptor, and restored VDR expression in the obstructed kidney. The treatment appeared to preserve tubular epithelial integrity via suppression of the epithelial to mesenchymal transition. Differential effects between nonselective VDRA calcitriol and another VDRA, maxacalcitol, have been noted in a rat model of renal failure.[99] In rats with doxorubicin-induced progressive renal failure, calcitriol treatment was associated with increased kidney weight, glomerular sclerosis, tubular atrophy, interstitial volume and renal calcium deposits, whereas maxacalcitol attenuated these pathological changes compared with doxorubincin alone (i.e. controls) or with calcitriol treatment. Overall, the available data indicate that VDRA treatment exerts a number of direct, local renal effects under conditions of renal damage that serve to attenuate growth and scarring, and help to maintain glomerular architecture. These results support a beneficial effect of VDRAs on renal disease progression, but indicate the potential for differential effects among different VDRAs.

3.2 Antiproteinuric Effects

In addition to being a marker of CKD and glomerular destruction, and a risk factor for renal disease progression, proteinuria is also predictive of cardiovascular disease and increased mortality.[100] Foley et al.[101] found that the mean serum albumin level during dialysis was strongly and negatively predictive of survival, even after controlling for baseline co-morbidities. Moreover, a large study in patients with diabetic nephropathy found that reduction of proteinuria was independently associated with improved cardiovascular and renal outcomes.[102]

A pooled analysis of three placebo-controlled studies evaluated the direct impact of selective VDRA therapy with paricalcitol capsules on proteinuria in patients with stages 3 and 4 CKD and SHPT.[103] At the end of a 24-week treatment period, paricalcitol-treated patients demonstrated 3.2-fold greater odds for reduction in proteinuria compared with the placebo-treated patients. The reduction was independent of concomitant use of agents that block

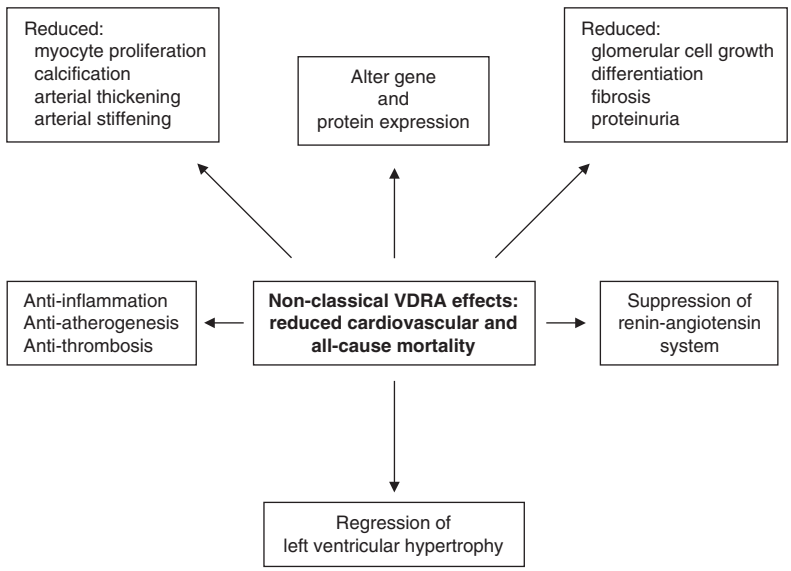


Fig. 5. Potential mechanisms involved in enhanced survival associated with vitamin D receptor activator (VDRA) therapy.

the RAS but may have been attributable, at least in part, to VDRA-induced renin suppression, as previously discussed in section 2.4.

4. Conclusions

VDRA therapy, particularly with a selective VDRA, is associated with improved cardiovascular and all-cause mortality outcomes in patients with stage 5 CKD. Recent and ongoing research is uncovering a variety of nonclassical VDRA effects in cardiovascular tissues relating to inflammation, vascular cell proliferation and vascular calcification that may underlie their cardioprotective effects (figure 5). Specifically, these include inhibitory effects on cardiac myocyte proliferation and hypertrophy, suppression of the RAS, and anti-inflammatory, antiatherogenic and antithrombotic effects. These effects are mediated largely through transcriptional regulation of numerous inflammatory mediators, matrix proteins and growth factors. Direct beneficial effects of VDRAs on glomerular remodelling and on proteinuria have also been documented, again relating to modulation of the expression of critical structural proteins, growth factors and cytokines. Newer vitamin D analogues, such as MC-1288, seocalcitol and falecalcitriol, are also being investigated for their efficacy, safety for treatment of SHTP, as well as for their pleiotropic effects.[104,105] Pleiotropic effects related to the modulation of gene expression have the potential to ameliorate the development and progression of cardiovascular disease, and reduce all-cause mortality in patients with stage 5 CKD.

The nonclassical effects of the selective VDRA paricalcitol appear to be more favourable than those of the nonselective VDRAs. Moreover, paricalcitol is associated with superior effects on survival. The interaction of these differential effects, the complexity of which we are only just beginning to understand, probably provides a mechanistic basis for the improved survival seen with selective VDRA therapy. An evaluation of both the classical and nonclassical effects of VDRAs in patients with CKD indicates that a selective VDRA should be the treatment of choice with regard to controlling PTH while minimising deleterious calcaemic and phosphataemic effects, as well as providing potential benefits on cardiovascular and renal disease progression, and ultimately survival.

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