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Vitamin D: a new hope for chronic kidney disease?

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Vitamin D analogs may slow the progression of chronic kidney disease. Tan *et al.* report that paricalcitol has additive effects with an angiotensin-converting enzyme inhibitor (ACEI) in suppressing extracellular matrix expression, interstitial inflammation, and myofibroblast activation in obstructive nephropathy. Paricalcitol inhibited renin transcription, countering the compensatory ACEI-induced rise in renin, thus achieving a more complete blockade of the renin–angiotensin system (RAS). Vitamin D analogs may enhance the renoprotective effects of RAS inhibitors.

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Chronic kidney disease (CKD) is characterized by a progressive loss of renal function that often leads to end-stage kidney disease and high mortality. There is extensive evidence that the renin–angiotensin system (RAS) is a major mediator of progressive renal injury in CKD. Drugs that target the RAS, including angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin II type 1 receptor blockers (ARBs), have been shown to slow the progression of glomerulosclerosis, tubulointerstitial fibrosis, and proteinuria.[1,2] Intrarenal angiotensin II (Ang II) and angiotensin-converting enzyme activity exert multiple effects on the kidney that promote progression of renal injury. These effects include an increase in glomerular capillary pressure, induction of profibrotic and proinflammatory cytokines, promotion of inflammatory-cell infiltration, stimulation of cell proliferation and hypertrophy, and upregulation of extracellular matrix (ECM) synthesis. The hormonal form of vitamin D, 1,25–dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), is a negative endocrine regulator of the RAS.[3,4] $1,25(OH)_2D_3$ suppresses renin biosynthesis, and homozygous mutant mice lacking the vitamin D receptor (VDR) gene develop high renin hypertension, cardiac hypertrophy, and increased thrombogenicity. Recent studies have shown that VDR-deficient diabetic mice develop a more severe nephropathy than wild-type mice,[5,6] suggesting that vitamin D plays a protective role against hyperglycemia-induced renal injury. These observations provide the rationale for studies exploring whether co-treatment with low-calcemic vitamin D analogs and ACEIs/ARBs has additive or synergistic therapeutic effects on the progression of proteinuric kidney disease.

RAS inhibitors, including ACEIs and ARBs, are widely used in the therapy of renal and cardiovascular diseases. The major limitation of these drugs is the compensatory rise in renin levels due to the disruption of the feedback inhibition of renin production.[7,8] High renin buildup increases the risk of Ang II-dependent and -independent organ damage, which may limit the efficacy of RAS inhibition and may be the cause of the resistance to therapy sometimes seen with the use of ACEIs and/or ARBs. Because low-calcemic vitamin D analogs are able to inhibit renin expression in animals,[9] Tan *et al.*[10] (this issue) reasoned that combining a vitamin D analog with a RAS inhibitor to suppress the reactive renin increase should generate additive therapeutic effects and ameliorate renal interstitial fibrosis and inflammation. To test this concept, they administered a combination of trandolapril, an ACEI, and paricalcitol (19–nor-1,25–dihydroxyvitamin D_2) to mice with unilateral ureteral obstruction (UUO). Paricalcitol is an activated vitamin D analog that stimulates the VDR at low concentrations and suppresses renin expression in mice with the same potency as calcitriol but without inducing hypercalcemia. The combination resulted in reductions in the expression of proteins or mRNAs for ECM components, tubular epithelial-to-mesenchymal transition markers, and proinflammatory cytokines, as well as a reduction in the invasion by $CD3^+$ T cells and macrophages (Figure 1). The authors' data suggest that the combination of an ACEI and a vitamin D analog markedly reduces renal injury, presumably because of the more complete inhibition of the RAS within the kidney.

The expression of the VDR in virtually all tissues in the body, including the kidney, is the basis for postulating broad non-calcemic actions for vitamin D beyond the regulation of calcium and phosphorus homoeostasis. Recent studies using a variety of experimental models, including UUO, have shown that vitamin D analogs may be renoprotective by reducing proteinuria, renal inflammation, and fibrosis.[11–13] The most prominent feature of fibrosis is the increased accumulation of ECM in the interstitial space. The UUO model is a well-established, although aggressive, model of acute tubulointerstitial fibrosis with high inflammatory response. In the obstructed kidney, paricalcitol suppressed the induction of ECM proteins, including fibronectin and type I and type III collagens, as well as α-smooth muscle actin, a marker for EMT.[10] Not surprisingly, the profibrotic transforming growth factor-β pathway was also repressed by paricalcitol. In stark contrast,

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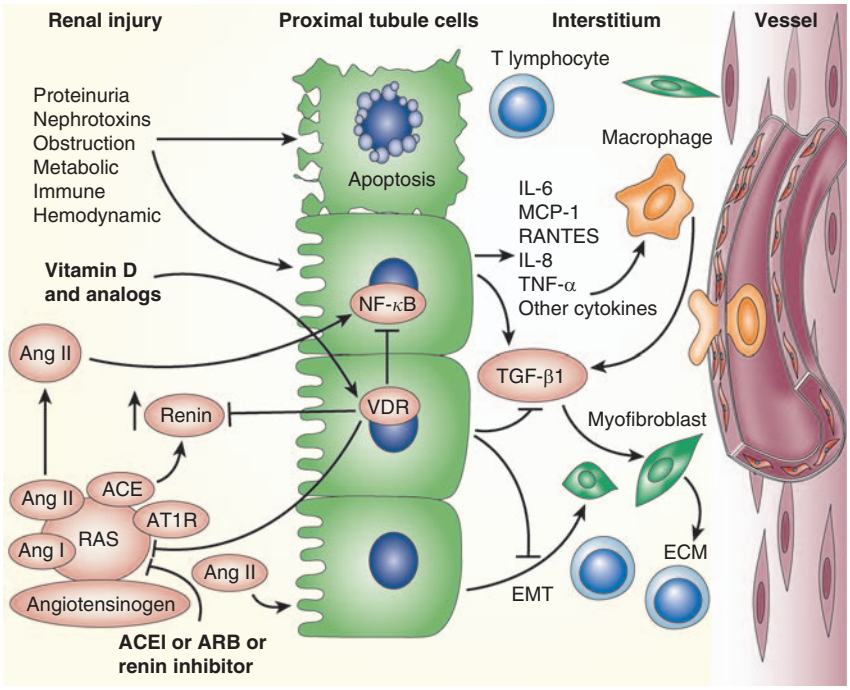

Figure 1 | Renoprotective effects of combination therapy with renin–angiotensin system inhibitors and vitamin D receptor activators in chronic kidney disease. Various injuries to the kidney stimulate the intrarenal renin–angiotensin system (RAS) and lead to proinflammatory/inflammatory cytokine production, infiltration of inflammatory T cells and macrophages, and induction of epithelial-to-mesenchymal transition (EMT). The activation of the RAS results in suppression of renin gene expression. When the system is inhibited with an angiotensin-converting enzyme inhibitor (ACEI), angiotensin II type 1 receptor blocker (ARB), or renin enzymatic inhibitor, this feedback loop is disrupted, leading to overexpression of renin. The compensatory renin overproduction will increase angiotensin II (Ang II) conversion, thus decreasing the efficacy of inhibition of the RAS. Activation of the RAS and the transcription factor nuclear factor-κB (NF-κB) results in increased production of cytokines, including transforming growth factor-β1 (TGF-β1), which plays pivotal roles in causing renal damage. Vitamin D and its analogs bind to the vitamin D receptor (VDR) and transcriptionally inhibit renin expression, counterbalance extracellular matrix (ECM) synthesis by inhibiting TGF-β1, and suppress NF-κB activation. An active vitamin D analog combined with a RAS inhibitor (ACEI, ARB, or renin inhibitor) will block the compensatory expression of renin and, therefore, synergistically achieve a more complete RAS inhibition. AT1R, angiotensin II type 1 receptor; IL, interleukin; MCP-1, monocyte chemoattractant protein-1; RANTES, regulated on activation normal T cell expressed and secreted; TNF -α, tumor necrosis factor-α.

VDR-null genetic mutant mice developed a more severe interstitial fibrosis with the UUO model. The present report by Tan *et al.*[10] clearly shows an additive or synergistic effect of paricalcitol and trandolapril co-treatment on renal fibrosis and inflammation in the mouse UUO model.

The local intrarenal RAS is an important determinant of kidney tissue injury, contributing to inflammation and the progression of renal disease. Inhibition of the actions of the RAS can preserve renal function in CKD. The non-calcemic actions of vitamin D analogs that may help protect the kidney include regulation of the local RAS and the nuclear factor-κB (NF-κB) pathway, which promote renal damage and the progression of kidney disease characterized by proteinuria, glomerulosclerosis, and tubulointerstitial fibrosis.[14] Kidney cells have the capacity to synthesize all components of the RAS, which are highly inducible by an initial insult. In CKD, the intrarenal interstitial Ang II levels can reach as much as 1000 times higher than those in plasma. Currently, ACEIs, ARBs, and the renin inhibitor aliskiren remain first-line treatments for the progression of CKD; however, not all patients respond to these treatments, in many cases because of incomplete blockade of the RAS. Tan *et al.*[10] have now demonstrated that blocking the compensatory increase of renin expression with vitamin D analogs dramatically increases the therapeutic efficacy of the conventional RAS inhibitors in the UUO model. Whether vitamin D analogs have additional advantages over the selective renin inhibitor aliskiren is not addressed in this study. However, aliskiren, while it reduces renin activity, may increase plasma renin concentrations, which may be a major limitation of this drug.[15] In contrast, the inhibitory effect of vitamin D analogs on renin transcription and biosynthesis may be a significant advantage.

Furthermore, vitamin D analogs may also have significant anti-inflammatory properties that may make them uniquely advantageous over more selective renin inhibitors. A major anti-inflammatory effect of paricalcitol seems to be due to its stabilizing effect on NF-κB. The NF-κB nuclear transcription pathway is a master regulator of immune responses and is pivotal in the regulation of inflammatory cytokines and fibrogenic molecules that are involved in the development of kidney disease. Numerous studies have demonstrated the suppression of NF-κB activation by vitamin D analogs, partly through blockade of nuclear translocation of NF-κB DNA. Paricalcitol, the vitamin D analog used by Tan *et al.*,[10] was previously shown by the same authors to reduce renal inflammation by blocking NF-κB activity in UUO mice.[13] Although paricalcitol or trandolapril alone can directly suppress the induction of regulated on activation normal T cell expressed and secreted (RANTES) and tumor necrosis factor-α expression after obstructive injury, the current study by Tan and colleagues[10] showed that co-treatment with paricalcitol and trandolapril achieved a better inhibition of these cytokines and renal infiltration of inflammatory T cells and macrophages *in vivo*. Because RANTES and tumor necrosis factor-α are upregulated by Ang II, the suppression of these factors by co-treatment is likely mediated by the blockade of both Ang II accumulation and NF-κB activation (Figure 1).

The molecular basis underlying the impressive antiproteinuric and antisclerotic effects of the combination therapy is the blockade of RAS activation within the kidney and simultaneous achievement of anti-inflammatory effects. The effect of

paricalcitol was seen even in subjects already treated with ACEIs or ARBs, indicating that the vitamin D analog has additive or synergistic effects with ACEIs/ARBs in reducing proteinuria. In a randomized double-blind pilot trial, Alborzi *et al.*[16] reported that paricalcitol treatment for 1 month significantly reduced albuminuria and inflammatory status in the drug-treated subjects, and these effects were independent of its effects on hemodynamics and parathyroid hormone suppression. The anti-albuminuric effect of vitamin D analogs is of particular significance, as albuminuria is a major risk factor for the progressive decline of renal function and is believed to harbinger an inevitable progression to overt proteinuria and renal failure. Thus, reduction of albuminuria is a convincing and favorable effect during renoprotective therapy.

Trandolapril treatment markedly increased renin levels in the kidney. Yet combination treatment with trandolapril and paricalcitol markedly attenuated the induction of renin and Ang II. Thus, in the combination therapy, paricalcitol blocked the increase in renin, minimizing the Ang II-independent activation of the prorenin/renin receptor, which may also contribute to renal damage. Together these actions of the combination therapy lead to an effective strategy to counter the development of CKD.

The renoprotective effect of paricalcitol, however, appears to be not limited to the regulation of the RAS. The anti-inflammatory properties of vitamin D and its analogs may further enhance their renoprotective effects in kidney disease. This study offers new insights into a novel therapeutic approach for further clinical studies. Given the limitations of animal models, whether this therapeutic strategy also works in humans requires further clinical investigations.

DISCLOSURE
The authors declared no competing interests.

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Expanding the spectrum of *NPHS1*-associated disease

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The nephrin gene *NPHS1* was cloned in 1998. Studies in families with congenital nephrotic syndrome led to the identification of this critical component of the glomerular slit diaphragm. Studies such as the new one by Santín *et al.* are expanding our understanding of the spectrum of disease associated with *NPHS1* mutations.

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Gene identification via positional cloning represents a form of deliberate ascertainment bias. Of necessity, researchers study families with extreme forms of disease to maximize the ability of these studies to succeed. In studies of dominantly inherited phenotypes, for example, it is typically through the study of large, multigenerational pedigrees with high penetrance that geneticists can home in on a narrowly defined genetic locus, and ultimately a single mutant gene. Although recessive families typically do not present as multigenerational, extended pedigrees (except in the presence of significant inbreeding), it is similarly typical for the initial success in a

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