The Vitamin D – Fibroblast Growth Factor 23 – Klotho Axis and Progression of Chronic Kidney Disease
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Chapter 2

Vitamin D in Chronic Kidney Disease: New Potential for Intervention

Katarina Mirković, Jacob van den Born, Gerjan Navis and Martin H. de Borst

ABSTRACT

Prevention of progressive renal function loss and its complications remains the main challenge in clinical nephrology. Although current therapeutic strategies aiming at reduction of blood pressure and proteinuria often slow down deterioration of renal function, still many patients progress to end-stage renal disease. The development of novel pharmacological approaches for treatment of chronic kidney disease (CKD) is therefore instrumental. Here we review the renoprotective potential of vitamin D and its analogues. In CKD patients, vitamin D deficiency is common and progression of CKD is associated with low (active) vitamin D levels. Moreover, in animal models of CKD, treatment with vitamin D (analogues) alone or in combination with renin-angiotensin-aldosterone system (RAAS) blockade reduces proteinuria, glomerulosclerosis and tubulointerstitial fibrosis. Potential underlying mechanisms include suppression of the RAAS, modulation of immune cell function and direct protective effects on renal cells such as podocytes. Whether vitamin D analogues could further optimize existing therapies in human renal disease is currently under investigation.
**2.1 Introduction**

Prevention of progressive deterioration of renal function and its complications remains the main challenge in clinical nephrology. According to Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines, chronic kidney disease (CKD) is defined either by reduced kidney function or by the presence or absence of kidney damage - irrespective of etiology. Severity of CKD can be classified in 5 stages - according to the level of glomerular filtration rate and the presence of markers of kidney damage such as (micro-)albuminuria. Progression of CKD may lead to end-stage renal disease (ESRD), requiring renal replacement therapy such as dialysis or transplantation. The prevalence of CKD, estimated from data in NHANES III (Third National Health and Nutrition Examination Survey) is about 11% of the general population in the United States. These data are comparable between United States and European countries.

Therapeutic strategies aiming at reduction of blood pressure and proteinuria by blockade of the renin-angiotensin-aldosterone system (RAAS) combined with adequate control of volume status (i.e. low sodium diet or diuretics) have been shown beneficial in slowing down the progression of CKD. However, in many patients RAAS blockade only delays ESRD but does not prevent it. Therefore, in spite of good therapy response, reflected by reduction of proteinuria and blood pressure, the protection against ongoing renal damage is incomplete. Consequently, the prevalence of ESRD is still growing. Resistance to therapy may be explained by adverse effects of RAAS-blockade such as a reactive rise in renin and aldosterone levels eliciting pro-inflammatory and pro-fibrotic effects. Moreover, efficacy of antiproteinuric therapy is determined by the extent of pre-treatment renal damage. Novel treatment strategies in CKD, especially strategies to overcome resistance to RAAS-related therapy, are warranted.

This chapter will address the potential role of active vitamin D (analogues) as (additional) therapy in CKD. Until recently, active vitamin D was used mainly for correction of secondary hyperparathyroidism in CKD patients. However, besides the classical view on this hormone as a regulator of mineral metabolism, recent evidence point to other important functions in different target organs including the renal and cardiovascular system, and regulation of the immune response.

This chapter first gives a brief overview of physiology and biological functions of vitamin D. Mechanisms and pathogenic consequences of vitamin D deficiency in CKD will also be discussed. The main focus, however, is on the renoprotective effects of active vitamin D and its analogues, possible underlying mechanisms, and their potential to overcome therapy resistance.

**2.2 Vitamin D Physiology**

### 2.2.1 Production and Metabolism

Vitamin D is a steroid prohormone produced in the skin from cholesterol-derived precursors (i.e. 7-dehydrocholes-terol) through a photochemical process. Alternatively, vitamin D can be obtained from dietary sources such as fortified dairy products, fish oils and mushrooms (Figure 2.1). Two native, biologically inactive, forms of vitamin D are vitamin D₃ (cholecalciferol) derived from the skin and animal products and vitamin D₂ (ergocal-
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Figure 2.1: Vitamin D production and metabolism. Vitamin D is produced photochemically from its precursors in the presence of solar ultraviolet B radiation. Alternatively, it can be absorbed by the intestine from a variety of dietary sources. Conversion into biologically active form takes place in the liver and the kidney, via two hydroxylation steps. Enzyme 1α-hydroxylase is expressed in many other cells besides the proximal tubular epithelial cells of the kidney. Active vitamin D is inactivated by addition of another hydroxyl group at position C24. This ultimately leads to a production of water-soluble calcitroic acid, excreted by the kidney.

ciferol) of non-animal origin. It has been suggested that these forms are not bioequivalent due to the differences in their side chains. However, a recent study showed that both vitamin D2 and D3 are equally effective in maintaining circulating concentrations of 25-hydroxy vitamin D and exert identical biological roles.

The main plasma carrier for vitamin D metabolites is vitamin D-binding protein (VDBP). VDBP has the highest affinity for 25-hydroxy vitamin D [25(OH)D], and virtually all plasma 25(OH)D is bound to VDBP. VDBP also functions to maintain stable serum stores of vitamin D metabolites and modulate their bioavailability, thereby influencing responsiveness of target cells to vitamin D and its analogues. VDBP-bound vitamin D metabolites have limited access to target cells and longer half-life in the circulation which might have important clinical implications given the fact that vitamin D analogues differ in their VDBP-binding capacity. In healthy individuals, the VDBP-vitamin D complex is filtered by the glomerulus in the kidney, and fully reabsorbed by proximal tubular cells via receptor-mediated uptake by megalin. Another protein involved in this process is cubulin which facilitates endocytosis by sequestering 25(OH)D-VDBP complex to the cell surface before internalization by megalin.

The biologically active form of vitamin D is 1α, 25-dihydroxyvitamin D [1,25(OH)2D] or calcitriol. Conversion into the active form consists of two hydroxylation steps catalyzed by mitochondrial and/or microsomal cytochrome P450 (CYP) isoforms. In the liver,
pro-vitamin D is converted to 25-hydroxyvitamin D or calcidiol by the 25-hydroxylases (CYP3A4, CYP2R1, CYP2J3 and CYP27A1) 23. These enzymes differ in their affinity and specificity for the substrate. The physiological relevance of different 25-hydroxylases in vitamin D metabolism is not fully elucidated. The first hydroxylation step is mainly substrate dependent and 25(OH)D is the main circulating form of vitamin D; consequently, serum concentration of 25(OH)D serves as an indicator of vitamin D status 24. In the second hydroxylation step, 25(OH)D is converted into the biologically active form 1,25(OH)2D. The key enzyme in this process is 25-hydroxyvitamin D−1α−droxylase (1α-hydroxylase, CYP27B1), expressed primarily in proximal tubular epithelial cells of the kidney 25. This enzyme is expressed in other parts of the kidney and extra-renal tissues and cells as well 26. In these tissues, via local activation by 1α-hydroxylase, vitamin D functions as a paracrine or autocrine factor mediating different cellular processes. The final step in the vitamin D metabolic pathway is its inactivation, a process catalyzed by 24-hydroxylase (CYP24A1) that catabolizes both 1,25(OH)2D and 25(OH)D into 1,24,25(OH)3D and ultimately water-soluble calcitroic acid and inactive blood metabolite 24,25(OH)3D 27,28.

Proximal tubular cells of the kidney are the main site of 1,25(OH)2D synthesis for systemic needs and their circulating levels are tightly regulated via several feedback mechanisms (Figure 2.2). First, 1,25(OH)2D negatively regulates its own levels by induction of CYP24A1 in target cells, thereby preventing toxicity. Evidence from CYP24A1 knockout mice supports this 29. On the other hand, renal 1α-hydroxylase also has an important role in regulation of circulating 1,25(OH)2D levels. Expression and activity of this enzyme is positively regulated by parathyroid hormone (PTH) and calcitonin 30,31, depending on the serum calcium levels. Active vitamin D itself negatively regulates expression of 1α-hydroxylase, either directly via vitamin D receptor mediated repression of 1α-hydroxylase gene transcription 32 or through downregulation of PTH. In addition, 1,25(OH)2D induces the production of FGF23 (fibroblast growth factor 23) in osteocytes which acts through FGFR1-Klotho complex to negatively regulate renal expression of 1α-hydroxylase and influences its own synthesis 33−35.

### 2.2.2 Mechanisms of Action

Active vitamin D mediates its biological effects by binding to vitamin D receptor (VDR). The VDR is a ligand-dependant transcriptional factor, predominantly located in the nucleus. However, unliganded VDR may be partitioned between the cytoplasm and nucleus and 1,25(OH)2D induces its translocation to the nucleus 36. The VDR is a member of the nuclear receptor superfamily and exhibits the same modular structure as other members of the superfamily 37. VDR regulates transcription of target genes through heterodimerization with retinoid X receptors (RXRs). VDR/RXR heterodimers interact with specific sequences generally in the promoter region of the target genes. These sequences are known as vitamin D response elements (VDREs) and binding of vitamin D-VDR complexes to these sequences leads to induction of gene expression. Alternatively, the VDR may also repress the expression of target genes, via negative vitamin D response elements and interaction with corepressors. Genes like CYP27B1 32 and PTH 38 are thought to be regulated by negative VDREs. Analysis of VDR target genes identified over 200 primary 1,25(OH)2D -responding genes involved in different cellular processes such as cell
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Figure 2.2: Regulation of 1α-hydroxylase and circulating levels of vitamin D. Circulating levels of active vitamin D depend both on its synthesis from precursors and the rate of its degradation to a non-functional metabolite, catalyzed by enzymes 1α-hydroxylase (CYP27B1) and 24-hydroxylase (CYP24A1) respectively. 1α-hydroxylase is upregulated by parathyroid hormone (PTH) and calcitonin and downregulated by fibroblast growth factor 23 (FGF23). Active vitamin D regulates 1α-hydroxylase either by direct suppression of 1α-hydroxylase gene transcription or by feedback mechanisms via PTH and FGF23. Also, active vitamin D is the most potent inducer of its own degrading enzyme 24-hydroxylase.

metabolism, growth and differentiation and control of bone formation and inflammation.

Besides genomic actions, 1,25(OH)2D may also exert so-called rapid or non-genomic responses, mediated via a membrane form of VDR associated with caveolae of the plasma membrane. The membrane-associated VDR is probably the same as the classical VDR, expressed in various tissues of different species. Non-genomic effects occur within seconds and are independent of transcription or translation. These effects include changes in calcium flux through interaction with calcium channels, release of calcium from intra-cellular stores (i.e. endoplasmatic reticulum), induction of second messenger system and activation of cytosolic kinases. Involvement of the VDR in these pathways is still under debate, as rapid responses of 1,25(OH)2D may be induced in the cells lacking a VDR. In addition, non-genomic actions of 1,25(OH)2D may modulate VDR-dependent gene transcription and this in turn may influence cell-specific biological responses to 1,25(OH)2D.

The VDR is expressed in over 30 different tissues including the kidney. In the normal human kidney, VDR is expressed in proximal and distal tubular epithelial cells,
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glomerular parietal cells and collecting duct cells \(^45\). Moreover, treatment of cultured mesangial cells \(^46\), podocytes and juxtaglomerular cells \(^47\) with active vitamin D or its 24, 25(OH)\(_2\)D analogues, modulates expression of target genes, suggesting the presence of a functional VDR in these cells. Analysis of gene expression profile in the kidney of VDR knockout mice revealed 95 differentially expressed genes when compared to the kidney of the wild-type mice \(^48\). These genes are involved in vitamin D metabolism, mineral and volume homeostasis, cellular signaling, cell adhesion, and immune response. However, the question remains if all of these genes are primarily responsive or their altered expression may be associated with VDR inactivation. Nevertheless, this indicates that active vitamin D has at least some local renal effects.

2.2.3 Biological Functions of Vitamin D

The best-defined physiological function of 1,25(OH)\(_2\)D is regulation of calcium and phosphate homeostasis and promotion of bone mineralization and is reviewed elsewhere \(^49\). In short, 1,25(OH)\(_2\)D stimulates active absorption of calcium and phosphate from the intestine and their reabsorption by the kidney, suppresses parathyroid hormone synthesis and cell growth and controls bone remodeling to maintain calcium levels within normal extracellular limits. Vitamin D deficiency results in increased levels of PTH and increased bone demineralization which ultimately leads to development of rickets in children and osteomalacia in adults \(^50\).

The widespread presence of VDR and vitamin D metabolizing enzymes as well as large numbers of responsive genes extend the role of this hormone beyond the classical target tissues and control of mineral metabolism. Non-classical functions include regulation of cell proliferation and differentiation, apoptosis, regulation of immune responses and inflammatory pathways and other cell and tissue specific effects (Figure 2.3). Conversely, 1,25(OH)\(_2\)D and its analogues attract much attention as potential therapeutic agents in a number of human diseases such as cancer, autoimmune diseases and recently CKD.

The biological functions of 1,25(OH)\(_2\)D and the effects of vitamin D deficiency can be studied by vitamin D metabolic pathway knockout mice (Table 2.1) VDR \(^51,52\) and 1\(\alpha\)-hydroxylase \(^53,54\) knockout mice develop severe vitamin D deficiency, osteomalacia, hypocalcemia and hyper-parathyroidism. In addition, they have an impaired immune system and are more prone to autoimmune diseases and tumors. Both VDR-and 1\(\alpha\)-hydroxylase-deficient mice have increased renin expression leading to high plasma angiotensin II levels, hypertension and finally cardiac hyper-trophy. VDR null mice also develop more severe nephropathy after streptozotocin-induced diabetes characterized by overactivation of the RAAS, increased proteinuria and decreased podocyte number. The relevance of active vitamin D as a negative regulator of the RAAS and its effects on modulation of immune response in terms of renal protection are discussed below in more detail. Due to the disruption of vitamin D catabolic pathway, CYP24A1 knockout mice develop lethal hypercalcemia secondary to hypervitaminosis D and impaired bone mineralization \(^29\). Chronic exposure to 1,25(OH)\(_2\)D induces abnormal kidney histology, consistent with hypervitaminosis D. This phenotype can be rescued by crossing CYP24A1 knockout mice with mice carrying targeted mutation in the VDR. On the other hand, transgenic rats constitutively expressing CYP24A1 show somewhat unexpected phenotype with low levels of circulating 25(OH)D and 24, 25(OH)\(_2\)D , hyperlipidemia and albuminuria \(^55\). A
possible explanation for the observed low 25(OH)D levels is that excreted albumin competes with 25(OH)D-VDBP complexes for binding to megalin resulting in a urinary loss of 25(OH)D. Plasma levels of 1,25(OH)\textsubscript{2}D remained unchanged probably due to observed upregulation of renal 1α-hydroxylase in transgenic rats. Interestingly, aged transgenic animals develop tubular injury, probably as a consequence of proteinuria.

### 2.3 Vitamin D System in Renal Disease

The kidney plays a central role in vitamin D metabolism and regulation of circulating levels of this hormone. Therefore, impaired renal function may lead to vitamin D deficiency, as seen in patients with CKD.
Vitamin D deficiency is common in patients with CKD, is observed even at the early stages of the disease, and is more pronounced than in general population. Thus, it is important to identify specific factors that may cause vitamin D deficiency in CKD patients. This probably results from multiple factors. A major determinant of low circulating levels of 1,25(OH)2D in CKD patients is reduced activity of 1α-hydroxylase resulting from the reduction in renal mass and tubular dysfunction. Another interesting hypothesis is that urinary loss of 25(OH)D-VDBP associated with proteinuria and reduced megalin-mediated uptake might result in vitamin D deficiency. Megalin knockout mice develop low molecular weight proteinuria and lose large amounts of vitamin D-VDBP complexes in their urine which results in severe vitamin D deficiency and skeletal defects. Kidney-specific megalin knockout mice display a similar phenotype. Decrease in megalin expression may occur early in the progression of chronic kidney disease, as it has been demonstrated in the model of partial nephrectomy. However, so far there is no evidence of reduced megalin expression in patients with renal disease. Alternatively, reduced levels of 25(OH)D might be a result of compromised endogenous pre-vitamin D production in the skin due to uremia.

Beside its important role in vitamin D metabolism, the kidney is also one of the primary target organs for both endocrine and paracrine actions of this hormone. Thus, it can be assumed that as decline in renal function leads to vitamin D deficiency, deficiency itself may exacerbate the disease. Indeed, several studies have demonstrated an independent inverse association between circulating (active) vitamin D levels and progression of renal function loss. Data from the NHANES III demonstrated an association between vitamin D deficiency and increased risk of albuminuria.

2.4 Renoprotective Effects of Vitamin D and Its Analogues

Emerging evidence from clinical and experimental studies suggests that vitamin D and its analogues may have beneficial effects in patients with CKD that are beyond the control of secondary hyperparathyroidism. Vitamin D and vitamin D receptor analogue (VDRA) therapy has shown to be beneficial and associated with improved survival in hemodialysis as well as non-dialysis patients. Recent clinical studies demonstrated antiproteinuric effects of paricalcitol which were independent of concomitant use of RAAS blockers and possibly PTH independent as well. Large, randomized, controlled clinical studies to assess the renoprotective potential of VDR activation are currently underway.

Results from animal experiments demonstrated renoprotective effects of both active vitamin D and its analogues (Table 2.2). These include direct antiproteinuric effect via protection of podocytes, suppression of the renin-angiotensin-aldosteron system, and immunomodulatory and anti-inflammatory effects. To our knowledge, Schwarz et al. were the first to report reduction of albuminuria and suppression of glomerulosclerosis in subtotal nephrectomized rats following treatment with calcitriol. In addition, calcitriol treatment reduced vascular and tubular TGF-β (transforming growth factor beta) expression. A similar study showed that vitamin D analogue 22-oxa-calcitriol also reduced albuminuria and glomerulosclerosis. Tubular injury markers such as NAG (N-acetyl-beta-D-glycosaminidase) and β2M (beta(2)-microglobulin) were unaffected. In a rat model of acute
Table 2.2: Summary of Studies Investigating the Role of Vitamin D and VDRA in Models of Renal Diseases.

<table>
<thead>
<tr>
<th>Animal Model</th>
<th>Intervention</th>
<th>Effects on Renal Function and Structure</th>
<th>Other Findings</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subtotally nephrectomized rats, with and without parathyroidectomy</td>
<td>1,25(OH)₂D</td>
<td>Reduction of glomerulosclerosis and glomerular volume, reduction of albuminuria</td>
<td>Decreased tubular and vascular expression of TGF-β in vitamin D treated group</td>
<td>[78]</td>
</tr>
<tr>
<td>Subtotally nephrectomized rats</td>
<td>22-oxa-calcitriol</td>
<td>Reduction of albuminuria, amelioration of glomerulosclerosis and glomerular hypertrophy, tendency towards prevention of tubular dilatation</td>
<td>No significant effect on tubular dysfunction markers (NAG and β2M)</td>
<td>[79]</td>
</tr>
<tr>
<td>Anti-Thy1 glomerulonephritis</td>
<td>22-oxa-calcitriol and 1,25(OH)₂D</td>
<td>Amelioration of glomerular cell proliferation, significantly lower albumin to creatinin ratio</td>
<td>Vitamin D treatment reduces expression of type I and IV collagens, α-smooth muscle actine and TGF-β</td>
<td>[80]</td>
</tr>
<tr>
<td>Anti-Thy1 glomerulonephritis</td>
<td>1,25(OH)₂D</td>
<td>Reduction of proteinuria, abrogation of podocytes injury</td>
<td>Preserved expression of nephrin and zonula occludens-1 protein</td>
<td>[81]</td>
</tr>
<tr>
<td>Anti-Thy1 glomerulonephritis</td>
<td>1,25(OH)₂D</td>
<td>Reduction in urinary protein and IL-6 excretion, reduction of glomerular hypercellularity and inflammatory cell infiltration</td>
<td>Restoration of VDR in the obstructed kidney; inhibition of TGF-β and its receptor type I</td>
<td>[82]</td>
</tr>
<tr>
<td>Unilateral ureteral obstruction</td>
<td>Paricalcitol</td>
<td>Reduction of interstitial fibrosis and tubular EMT</td>
<td>Reduced mRNA expression of angiotensinogen, renin, renin receptor and vascular endothelial growth factor in paricalcitol treated groups as well as renal expression of TGF-β</td>
<td>[83]</td>
</tr>
<tr>
<td>5/6 nephrectomy</td>
<td>Paricalcitol</td>
<td>Development of proteinuria markedly reduced, reduced glomerular sclerosis and tubulo-interstitial damage in paricalcitol treated groups</td>
<td></td>
<td>[84]</td>
</tr>
<tr>
<td>Diabetic VDR knockout mice</td>
<td>Diabetic VDR⁻/⁻ mice</td>
<td>Diabetic VDR⁻/⁻ mice developed more severe albuminuria and glomerulosclerosis than wild type counterparts</td>
<td>Higher renal renin, angiotensinogen and AT1R expression in the VDR null mice, increased glomerular basement membrane thickening and podocyte effacement</td>
<td>[47]</td>
</tr>
<tr>
<td>Subtotally nephrectomized rats</td>
<td>1,25(OH)₂D</td>
<td>Less pronounced albuminuria and glomerulosclerosis, decreased podocyte loss and abrogation of podocyte hypertrophy</td>
<td>Less expression of desmin, PCNA and p27, suggesting less podocyte injury and activation of cyclin cascade in treated group</td>
<td>[93]</td>
</tr>
<tr>
<td>Puromycine aminonucleoside nephrosis model</td>
<td>1,25(OH)₂D or 22-oxa-calcitriol</td>
<td>Reduction of proteinuria, preventive effects on podocyte injury</td>
<td>Renal 1α-hydroxylase and 24-hydroxylase decreased and increased respectively before the onset of proteinuria</td>
<td>[94]</td>
</tr>
<tr>
<td>Puromycine aminonucleoside nephrosis model</td>
<td>1,25(OH)₂D</td>
<td>Amelioration of podocyte damage and proteinuria induced by PAN</td>
<td>Changes in nephrin expression reversed by vitamin D treatment, modulation of TGF-β/BMP-7 signaling</td>
<td>[95]</td>
</tr>
</tbody>
</table>

glomerulonephritis induced by anti-Thy1, administration of both active vitamin D and 22-oxa-calcitriol lowered proteinuria and degree of glomerulosclerosis via antiproliferative, antifibrotic and anti-inflammatory effects. It has also been shown that active vitamin D and VDRA can ameliorate renal interstitial fibrosis. In a mouse model of tubulointerstitial fibrosis induced by unilateral ureteral obstruction paricalcitol reduced interstitial fibrosis and restored VDR expression in the obstructed kidney. Moreover, paricalcitol directly inhibited endothelial-to-mesenchymal transition of tubular cells, by direct suppression of TGF-β and its receptor. Recent studies, in both subtotally nephrectomized rats and diabetic VDR knockout mice provided evidence that renoprotective effects of active vitamin D and its analogues are, to some extent mediated via suppression of the RAAS. An overview of renoprotective effects of active vitamin D is shown on Figure 2.4.
Figure 2.4: Renoprotective effects of active vitamin D and vitamin D analogues. Vitamin D negatively regulates renin-angiotensin-aldosterone system (RAAS) by inhibition of renin gene in juxtaglomerular cells, directly preventing its pro-fibrotic effects. Anti-inflammatory effects of vitamin D are mediated mostly via downregulation of NF-\(\kappa\)B pathways in tubular epithelial cells and macrophages. T-helper-cell responses and maturation and differentiation of dendritic cells are also affected and modified by active vitamin D. In podocytes, vitamin D exerts protective effects evidenced as upregulation of nephrin and podocin and decrease in podocyte injury markers such as desmin. Permission obtained from Nature Publishing Group Ltd © Doorenbos CRC, van den Born J, Navis G, de Borst MH, Possible renoprotection by vitamin D in chronic renal disease: beyond mineral metabolism Nat. Rev. Nephrol. 5, 691-700 (December 2009).

2.4.1 Direct Antiproteinuric Effects of Active Vitamin D

The podocyte, a highly specialized cell involved in maintenance of glomerular filtration barrier\textsuperscript{85}, has been recognized to mediate progression of glomerular damage and chronic kidney disease\textsuperscript{85–87}. Moreover, podocyte injury may be the initial step in the pathogenesis of CKD. Disruption of the integrity of the filtration barrier due to apoptosis or detachment of podocytes leads to glomerulosclerosis, tubulointerstitial fibrosis and proteinuria\textsuperscript{86,88,89}. Conversely, proteinuria itself can induce tubular damage. Underlying mechanisms of this process are beyond the scope of current review. In short, ultrafiltrated protein load promotes infiltration of inflammatory cells and fibrosis via induction of expression of tubular chemokines and intrarenal activation of the complement system\textsuperscript{90}. Preventing podocyte damage and resulting proteinuria in kidney disease would be a meaningful therapeutic strategy.

Recent studies provide evidence for direct antiproteinuric effect of active vitamin D through maintenance of structural and functional integrity of podocytes. Active vitamin D induces expression of nephrin (an important slit diaphragm protein) in cultured podocytes.
possibly in cooperation with the retinoic acid receptor \(^92\), suggesting active vitamin D to be a differentiation factor for podocytes.

In subtotally nephrectomized rats, Kuhlman et al.\(^93\), showed that treatment with active vitamin D markedly decreased podocyte loss and ameliorated morphological and immunohistochromical markers of podocyte damage such as desmin but whether this is a direct effect remains inconclusive. On the other hand, in the puromycin aminonucleoside nephrosis model, characterized by podocyte loss and massive proteinuria without mesangial proliferation and matrix accumulation, treatment with active vitamin D or its analogue 22-oxacalcitriol demonstrated a clear antiproteinuric effect. Moreover, administration of vitamin D partially restored expression of nephrin and podocin (molecule associated with the slit diaphragm) and decreased expression of desmin (podocyte injury marker) \(^94\),\(^95\). Taken together, these studies suggest that active vitamin D exerts direct protective effect on podocytes, which contributes to its overall renoprotection. However, the possibility of indirect effects through interference with the RAAS cannot be excluded. In support of this, it should be mentioned that transgenic rats overexpressing angiotensin II receptor type I (AT1R) on podocytes develop proteinuria and structural podocyte damage that results in glomerulosclerosis \(^96\).

### 2.4.2 RAAS-Related Effects of Vitamin D

The RAAS plays a major role in the pathophysiology of kidney disease \(^97\). Regardless the primary cause, initial renal injury may result in progressive loss of nephrons and hyperfiltration and glomerular hypertension, which induces local stimulation of the RAAS. The main effector molecule of this system is angiotensin II (AngII). Ang II acts as a vasoconstrictor and exerts profibrotic and proinflammatory effects. In the kidney, AngII increases intraglomerular pressure and induces pro-inflammatory and pro-fibrotic cytokines and growth factors like TGF-\(\beta\) \(^98\), plasminogen activator inhibitor-1 (PAI-1) \(^99\) and monocyte chemotactic protein-1 (MCP-1) \(^100\). These events result in proteinuria, glomerulosclerosis and tubulointerstitial fibrosis and promote further deterioration of renal function and ultimately renal failure.

An inverse relationship between 1,25(OH)\(_2\)D levels and high blood pressure and renin activity has been documented two decades ago \(^101\)−\(^103\). Whether VDRA exert antihypertensive effects is still debatable \(^104\) and beyond the scope of this paper. However, emerging evidence points to active vitamin D as negative regulator of the RAAS. Both VDR knockout and 1α-hydroxylase knockout mice display increased renal renin mRNA and protein levels \(^105\),\(^106\). Subsequent treatment of 1α-hydroxylase knockout mice with active vitamin D restores normal renin levels. This effect appears to be independent of calcium and PTH \(^107\). Normal mice with induced vitamin D deficiency also have increased renin levels and treatment with active vitamin D results in renin suppression \(^105\). In addition, VDR knockout mice develop hypertension, probably because of RAAS activation. AngII levels are elevated as a consequence of high renin levels because there is no difference in angiotensinogen expression in the liver between knockout and wild type mice. Together, these results suggest that vitamin D directly suppresses renin expression \textit{in vivo}. Treatment of VDR knockout mice with angiotensin-converting enzyme inhibitors (ACEi) or/and AT1R blockers (ARB) reverses high blood pressure phenotype but renin levels remain elevated \(^108\). This indicates that repression of renin expression by active vitamin D
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is independent of the AngII feedback loop. At the molecular level, active vitamin D negatively regulates renin expression at the transcriptional level by binding to the promoter region of the renin gene and blocking the activity of the cAMP response element in the promoter region. It has been shown that vitamin D, at least in adipocytes, down-regulates the expression of AT1R in a dose-dependent manner. At this time, there is no evidence to suggest the existence of similar interactions in renal cells.

RAAS blockade by ACEi and ARB is currently therapy of choice for patients with CKD. The beneficial effect of RAAS blockade on blood pressure and proteinuria can be potentiated by volume-depleting measures such as low sodium diet and/or administration of diuretics. Higher doses and combined blockade of ACE and AT1R were found effective to reduce proteinuria. However, the limits of further improvement of renoprotection by RAAS blockade based therapy might have been reached, whereas in many patients residual proteinuria and progression towards end-stage renal disease remains.

Due to multiple feedback loops and alternative routes within the RAAS, inhibition of this system at one level leads to compensatory responses at other levels resulting in suboptimal therapeutic efficacy. Besides AngII, also aldosterone and renin, the latter possibly through the recently discovered (pro)-renin receptor (PPR), also exert proinflammatory and pro-fibrotic effects. In addition, results from animal studies showed dissociation between improvement of blood pressure and proteinuria during intensified RAAS blockade on the one hand, and worsening of interstitial fibrosis on the other hand. This may be due to the elevated levels of renin during intensified RAAS blockade. Interestingly, reactive rise of renin is followed by de novo expression of the (pro)-renin receptor in interstitial fibrotic areas. These data suggest that any intervention that blunts or prevents reactive rise in renin during RAAS blockade may improve renoprotection. Several recent studies indicated that addition of aliskiren to conventional RAAS blockade enhances renoprotection but long-term effects of this therapeutic approach remain to be established. It should be mentioned that while direct renin inhibitors such as aliskiren, inhibit only enzymatic activity of renin without affecting its production or its interaction with the PPR, active vitamin D directly inhibits renin production by repressing renin gene transcription. This implicates that addition of a VDRA to RAAS blockade may indeed improve its efficacy by blocking the reactive rise in renin (and downstream aldosterone). This would allow potentiation of therapeutic effects of the RAAS blockade without a reactive rise in renin. Several studies addressed the effects of combined treatment of VDRA and RAAS blockade in both diabetic and non-diabetic models of renal injury. Mizobuchi et al. showed that combination of paricalcitol and ACEi reduced renal damage more effectively than either paricalcitol or ACEi alone. This treatment also reduced glomerulosclerosis and suppressed the TGF-β pathway. Similarly, in a model of diabetic nephropathy, combination therapy of paricalcitol and ARB markedly reduced renal injury due to blockade of the compensatory increase in renin levels. Therefore, inhibition of local renin production by VDRA may have therapeutic potential for patients with CKD.

Given the pleiotropic actions of active vitamin D, however, its renoprotective effect may not be limited to regulation of the RAAS. Anti-inflammatory properties of vitamin D may also be important for its renoprotective effect in addition to down regulation of the RAAS, as inflammation has been shown to be a determinant of RAAS blockade resistance.
2.4.3 Anti-Inflammatory and Immuno-Modulatory Properties of Vitamin D

The concept of active vitamin D as an immuno-modulatory molecule was first postulated in the 1980s. Underlying mechanisms and the relevance of these effects on the progression and treatment of renal disease have been uncertain for a long time. Recent studies have provided important information on the anti-inflammatory properties and mechanisms of action of vitamin D. Several studies have demonstrated the role of active vitamin D in the modulation of renal inflammation. In a recent study, Zehnder et al. reported that plasma active vitamin D status is inversely associated with renal inflammation in several types of kidney disease.

Anti-inflammatory properties of active vitamin D and its analogues may be attributed to their ability to suppress the NF-κB pathway. The NF-κB pathway plays an important role in the progression of renal disease by promoting both inflammation and fibrogenesis via regulation of inflammatory cytokines and chemokines (MCP-1, TNFα, PAI-1). Different stimuli, such as bacterial LPS (lipopolysaccharide), IL-1β (interleukin-1 beta) and TNF-α (tumor necrosis factor alpha) have been known to activate NF-κB pathway in the kidney. In addition, both angiotensin II and angiotensin degradation products were shown to activate NF-κB. Treatment of cultured proximal epithelial cells with paricalcitol induces formation of complex consisting of VDR and NF-κB p65 component thus reducing its binding to promoter regions of the target genes. This results in decreased activation of genes such as CCL5 [chemokine (C-C motif) ligand 5, RANTES] and subsequently in amelioration of renal inflammation in vivo. Similarly, in mesangial cells administration of active vitamin D inhibits induction and activity of MCP-1 (CCL2) through increase or stabilization of the NF-κB inhibitory unit IκB thus preventing nuclear translocation of p65 NF-κB unit. The reason behind the discrepancy in mechanisms of downregulation of NF-κB signalling pathway remains uncertain and can be attributed to cell-type specificity.

In addition to processing in the liver and the kidneys, vitamin D can be metabolized by the cells of the immune system. Active T-cells, macrophages/monocytes and some dendritic cells express VDR and vitamin D metabolizing enzymes, 1α-hydroxylase and 24-hydroxylase. In contrast to its regulation in the kidney, activity of extrarenal 1α-hydroxylase is mostly substrate dependent. Locally produced active vitamin D may act on immune cells in an autocrine or paracrine manner. In macrophages, both 1,25(OH)₂D₃ and its less calcaemic analogue 1,25(OH)₂D suppress NF-κB activity by up-regulating expression of IκB through stabilization of IκB-mRNA and reduction of its phosphorylation. This results in inhibition of inflammatory genes such as TNFα. Active vitamin D exerts direct effect on T-helper-1 (Th1) and T-helper-2 (Th2) cells by inhibiting their proliferation and production of cytokines. Key cytokines of Th1-cells, IFN-γ (interferon gamma) and IL-2 (interleukin 2) are direct targets of active vitamin D. Active vitamin D can modulate Th2-cell responses both indirectly, through suppression of IFN-γ and IL-2 by Th1-cells and directly by influencing expression of Th2 cytokines such as IL-4 (interleukin 4). Moreover, it has been shown that active vitamin D inhibits...
expression of Fas ligand by activated T-cells \(^{145}\). Active vitamin D affects T-cell responses also by direct inhibition of maturation, differentiation and activation of dendritic cells \(^{146}\). This leads to down-regulation of MHC class II and costimulatory molecules and decrease of dendritic cell dependant T-cell activation. In addition, vitamin D may have an important role in promotion of dendritic cell tolerogenicity \(^{147,148}\). The role of dendritic cells in the kidney and their contribution to progression of kidney disease is not fully elucidated. However, a recent study demonstrated their importance in the progression of glomerulonephritis after glomerular injury \(^{149}\). Since vitamin D inhibits maturation and differentiation of dendritic cells, it might be expected that treatment with vitamin D or its analogues may reduce the immune response.

Together, these findings indicate that vitamin D or its analogues might be useful for the treatment of inflammatory and autoimmune diseases including inflammatory kidney diseases such as autoimmune nephritis. Moreover, given its anti-inflammatory and immuno-modulatory properties, vitamin D could also have important role in patients with non-autoimmune CKD.

### 2.5 Summary and Conclusions

Prevention of renal function loss and its complications remains the main challenge in clinical nephrology. Current therapy options (i.e. blockade of the RAAS) aim to reduce hypertension and proteinuria; nevertheless, many patients develop end-stage renal disease on the long term. Resistance to the therapy may be due to inappropriate effects of the RAAS blockade, such as reactive rise in renin and aldosterone with possible profibrotic effects. Therefore, there is an increased interest in finding novel treatments that are able to halt progression of renal function loss or even improve renal function. Growing evidence indicates that vitamin D may have therapeutic potential for patients with CKD that extends beyond its classical role in maintenance of mineral homeostasis and the present use of active vitamin D for the treatment of secondary hyperparathyroidism in CKD. Moreover, vitamin D deficiency is common in CKD patients and in fact may contribute to deterioration of the kidney function. Renoprotective effects of vitamin D and its analogues include suppression of the RAAS and reduction of proteinuria, either directly through the protection of podocytes or via negative regulation of the RAAS. In addition, vitamin D exerts anti-inflammatory and immuno-modulatory effects. Therefore, addition of vitamin D to conventional therapy may present a promising treatment modality. Results of randomized, controlled trials addressing renoprotective potential of this approach are expected in the near future.
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