

The Vitamin D Axis in Chronic Kidney Disease – State of the Art and Future Perspectives

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Abstract

All the components of the vitamin D axis participate in the pathogenesis of chronic kidney disease (CKD) and this knowledge can be exploited to devise targeted therapeutic strategies. The vitamin D axis includes vitamin D, vitamin D receptor and vitamin D-binding protein (VDBP), which is the precursor of a potent macrophage activating factor, group-specific component (Gc)-protein-derived macrophage activating factor (GcMAF), a protein that is being extensively studied after it was demonstrated that it eradicates advanced cancer and human immunodeficiency virus (HIV) infection. Thanks to its effects on the immune system and angiogenesis, it also appears to be a promising candidate for targeted therapy of CKD. In this article we shall summarise the state of the art on vitamin D and CKD, and then we shall focus on the perspectives opened by increasing knowledge of other components of the axis, in particular VDBP and GcMAF.

Keywords

Chronic kidney disease, vitamin D, vitamin D receptor, vitamin D-binding protein, group-specific component globulin-protein-derived macrophage activating factor (GcMAF), polymorphism, angiogenesis

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There has been much recent interest in the role of the vitamin D axis in chronic kidney disease (CKD). The vitamin D axis includes vitamin D, vitamin D receptor (VDR) and vitamin D-binding protein (VDBP; also known as group-specific component [Gc] globulin), which is the precursor of Gc-macrophage activating factor (GcMAF), probably the most potent macrophage activator.¹ This interest is demonstrated by the number of publications on these compounds in relationship with CKD, even though it is evident that some components of the axis have not yet received enough attention. Therefore, a search of PubMed (accessed on 24 October 2010) for 'vitamin D and chronic kidney disease' yielded 2,586 papers, 750 of which are reviews and 365 free full text. The search for 'vitamin D receptor and chronic kidney disease' yielded 248 papers with 128 reviews and 42 free full texts. 'Vitamin D-binding protein and chronic kidney disease' yielded 19 papers, six reviews and three free full texts. However, a search for 'GcMAF and chronic kidney disease' resulted in 'No items found', even though there are 35 papers published on GcMAF. The uneven distribution of the number of papers published on each component of the vitamin D axis reflects the progress of knowledge on each topic and indicates the future perspectives as well as the windows of opportunity for original research. In this article we will summarise the state of the art on vitamin D and CKD, and then we shall focus on the perspectives opened by increasing knowledge on the role of the other components of the axis, in particular VDBP and GcMAF.

Vitamin D and Chronic Kidney Disease

Vitamin D physiology has gained more importance and publicity than any other component of the vitamin D axis, including its counterparts in water- and fat-soluble vitamin groups combined. This is partly because vitamin D deficiency is still widely prevalent in the developed world and the beneficial effects are thought to extend beyond the regulation of calcium and phosphorus homeostasis alone.² In fact, the biologically active metabolite of vitamin D, 1,25(OH)₂D³ – known for centuries to affect mineral homeostasis – has numerous other diverse physiological functions including effects on the growth of cancer cells and protection against certain immune disorders.³ Interventional studies revealed the protective effect of vitamin D against cancers, intermediate markers of cardiovascular risk, epidemic influenza, albuminuria and even risk of fall. Further activities of vitamin D relate to defence of microbial infections, e.g. tuberculosis, contractility of muscle cells and counteraction of congestive heart failure. Cross-sectional studies have demonstrated that vitamin D deficiency in humans is associated with elevated blood pressure and propagation of atherogenesis, and vitamin D supplementation in adults may be regarded as a simple means with few potential side effects to prevent atherogenesis or halt its progression and combat arterial hypertension.⁴

It is immediately evident how these features of vitamin D are related to the progression of CKD and how vitamin D supplementation is an

almost mandatory requirement for CKD therapy. In fact, CKD is considered one of the most powerful predictors of premature cardiovascular disease and emerging evidence suggests that the progression of CKD and many of the cardiovascular complications is linked to hypovitaminosis D. Patients with CKD have an exceptionally high rate of severe vitamin D deficiency that is further exacerbated by the reduced ability to convert 25-(OH)vitamin D into the active form, 1,25 dihydroxy-vitamin D. As new evidence has improved the understanding of the classic, as well as the non-classic, functions for vitamin D, it has become apparent that the autocrine role of vitamin D is an important modulator of several systems including the immune, renal and cardiovascular systems.⁵ In particular, vitamin D produced in the kidney is known to have classic endocrine phosphocalcic properties as well as autocrine and paracrine actions on cellular proliferation and differentiation, apoptosis, renin secretion and interleukin and bactericidal protein production.⁶ Epidemiological studies in CKD have demonstrated that vitamin D deficiency and absence of treatment with vitamin D is associated with increased cardiovascular mortality.⁷ Clinical studies have consistently shown that vitamin D supplementation in patients with its deficiency contributes to decreased frequency of bone disorders and cardiovascular incidents, lower risk of several malignancies and improvement of immune system response regardless of renal function.⁸

Several possible mechanisms may explain how vitamin D influences the development of cardiovascular complications in CKD. A number of studies indicate that treatment with vitamin D analogues reduces proteinuria, suppresses the renin-angiotensin-aldosterone system and exerts anti-inflammatory and immunomodulatory effects.⁹ Other studies suggest that vitamin D inhibits the proliferation of cardiomyoblasts by promoting cell-cycle arrest and enhances the formation of cardiomyotubes without inducing apoptosis. Vitamin D has also been shown to attenuate left ventricular dysfunction in animal models and humans.¹⁰ In addition, Vitamin D appears to be essential for the development and maintenance of a healthy arterial tree since it influences the migration, proliferation and gene expression of vascular smooth muscle cells and elastogenesis and immunomodulation – all processes involved in the pathogenesis of arterial disease.

Immunomodulation describes the intricate relationship between vitamin D and the immune system, a system frequently overactivated and/or deranged in CKD. From the immunological viewpoint, CKD is characterised by disorders of both the innate and adaptive systems, generating a complex and still not fully understood immune dysfunction. Markers of a chronically activated immune system are closely linked to several complications of CKD and represent powerful predictors for mortality in the CKD population. On the other hand, CKD patients show signs of immunodeficiency and respond poorly to vaccination and to challenges such as bacterial infection. Consequently, the main causes of death in patients with CKD are cardiovascular and infectious diseases, both being pathological processes closely linked to derangement of the immune function.¹¹ On one hand, vitamin D stimulates the innate immune system, facilitating the clearance of infections such as tuberculosis, and hypovitaminosis D has been associated with several autoimmune disorders, various malignancies and cardiovascular risk factors in a number of recent epidemiological reports. Based on these observational reports, vitamin D and its analogues are being evaluated for the prevention and treatment of a variety of conditions,

with early findings showing mixed results.¹² Conflicting results might be attributed to the complexity of the effects of vitamin D, which, on the other hand, acts as an immunosuppressant. In fact, in the skin where it is produced, vitamin D depresses the activity of Langerhans cells and inhibits the induction of T-helper type 1 (Th1) cells and the expression of major histocompatibility complex (MHC) class II proteins on antigen-presenting cells (APCs).¹³ The immunosuppressant effects of vitamin D in the skin can be evolutionary explained assuming that ultraviolet B (UVB)-induced immunosuppression through the synthesis of active vitamin D evolved to control the intensity of inflammation caused by UVB-provoked injury. However, as if to balance the compromise in host defence, vitamin D stimulates the synthesis of a potent antimicrobial peptide in skin and circulating phagocytic cells.¹³ Due to the complexity of the actions of vitamin D on the immune system, with both stimulatory and inhibitory effects reported, the term immunomodulation referred to vitamin D appears to be fully justified.¹⁴

All the considerations quoted above lead to the trivial conclusion that vitamin D supplementation is required in the management of CKD. Much less trivial is the answer to the logically consequent question: 'How much vitamin D has to be supplemented?' As we recently discussed in a major journal devoted to kidney research,¹⁵ the answer to that question does not depend solely on vitamin D serostatus, but it has to take into consideration the functionality of the receptor that is in turn influenced by the individual polymorphisms of the gene coding for the classic VDR. Now we know that also the other components of the vitamin D axis play a role in determining the adequacy of vitamin D supplementation, thus rendering the answer to that question even more complex. Below we shall focus on VDBP and GcMAF even though emerging evidence for non-classic vitamin D receptors leads us to suspect that the topic is destined to become all the more intriguing. In fact, plasma membrane-initiated signalling by steroid hormones is now widely accepted, and a membrane receptor for vitamin D was recently identified as 1,25D3-membrane-associated, rapid response steroid binding (MARRS), also known as ERp57/GRp58.¹⁶

Vitamin D-binding Protein and Chronic Kidney Disease

VDBP is a serum alpha 2 glycoprotein composed of a single polypeptide chain with a molecular mass of 51–58kDa and is structurally related to serum albumin. It is also known as Gc globulin, is synthesised in the liver and is present in plasma at levels of 20–55mg/100ml. VDBP has been detected on the surface of several cell types, including yolk sac endodermal cells and some T lymphocytes. In B cells, VDBP participates in the linkage of surface immunoglobulins. The protein is 458 residues in length¹⁷ and forms three domains, the first of which contains the vitamin D-binding site. The three domains share limited sequence homology with each other and with similar repeats in human serum albumin. Naturally occurring VDBP carries one trisaccharide composed of N-acetylgalactosamine with dibranched galactose and sialic acid termini at the 420 threonine residue; this peculiarity will be discussed in the following paragraph dealing with GcMAF. VDBP is a multifunctional protein that, in addition to vitamin D, binds actin and acts as an actin scavenger. The affinity for actin monomers is high and the actin-binding site has been reported to reside within domain III, between residues 350 and 403.¹⁸ The structure of the complex of VDBP and actin¹⁹ confirms that domain III forms an actin-binding contact between subdomains one and three of actin. These characteristics, although not directly related to vitamin D metabolism, are of paramount importance in CKD since

actin is the most abundant protein in eukaryotic cells and is a major cellular protein released during cell necrosis, which may cause fatal formation of actin-containing thrombi in the circulation if the actin scavenging capacity of VDBP is exceeded.²⁰ Thus, recent studies demonstrated that the determination of serum level of VDBP is useful as a prognostic indicator in patients with acute hepatic failure, acetaminophen overdose, multiple trauma or multiple organ dysfunction syndrome, or sepsis. Other studies suggest an association between VDBP and resistance or susceptibility to chronic obstructive pulmonary disease (COPD), thyroid diseases, diabetes, multiple sclerosis and sarcoidosis.²¹

As far as the role of VDBP in CKD is concerned, an old study demonstrated that patients on peritoneal dialysis and nephrotic patients had lower levels of VDBP than normal subjects and had a significantly higher percentage of free vitamin D, indicating that the loss of VDBP correlated with a rise in the per cent free vitamin D.²² However, more recent studies focused on the association between VDBP polymorphisms and CKD. Similar to the gene coding for VDR, the gene coding for VDBP is polymorphic and a recent study demonstrated that there is an altered VDBP allele frequency in haemodialysis patients compared with the general population.²³ In addition, this study demonstrated that vitamin D intake differed depending on the VDBP polymorphism, and was greatest for end-stage renal disease (ESRD) patients with a VDBP 2-2 phenotype. The authors concluded that vitamin D treatment deserves more careful monitoring among VDBP 2-2 patients with ESRD. These conclusions are supported by a recent meta-analysis of available studies examining the association between common single nucleotide polymorphisms of the vitamin D axis components and serum vitamin D concentrations.²⁴ In fact, plasma levels of VDBP are known to vary according to VDBP gene polymorphisms²⁵ and there is evidence suggesting that the VDBP phenotype influences serum vitamin D concentrations in a non-linear fashion.²⁶ However, while the evidence linking VDBP polymorphisms with serum vitamin D is strong, the underlying mechanism of action remains unclear and further complications may arise following the consideration that, apart from providing transport around the circulatory system, VDBP polymorphisms influence the facilitated transport of the VDBP-bound vitamin D into the cell, or back from the renal tubule.²⁴

VDBP is also implicated in a peculiar kidney pathology that is related to cadmium intoxication – Itai-itai disease – which is a syndrome that includes renal tubular dysfunction, osteomalacia and generalised pain due to multiple bone fractures. A recent paper by a Japanese group demonstrated that excretion of urinary VDBP is increased after long-term cadmium exposure and that the loss of VDBP in urine is linked to renal tubular dysfunction and possibly bone lesions in the inhabitants of cadmium-polluted areas, even though the mechanism of how renal dysfunction relates to the development of bone lesions is unresolved.²⁷ Finally, it should be noted that low affinity for VDBP is responsible for the lower calcaemic activity of some of the vitamin D analogues commonly used in CKD treatment. Calcipotriol (calcipotriene) and 22-oxacalcitriol (OCT) show low VDBP affinity, which has been held responsible for the reduced calcaemic action.²⁸

In addition to the multiple roles quoted above, VDBP is the precursor of the most potent macrophage activator, GcMAF, a protein that is being extensively studied after it was demonstrated to eradicate advanced cancer and HIV infection.

Group-specific component-protein-derived Macrophage Activating Factor and Chronic Kidney Disease – Future Perspectives

Inflammation-associated macrophage activation is the principal macrophage activation process and requires serum VDBP and participation of B and T lymphocytes. VDBP carries a trisaccharide composed of N-acetylgalactosamine with di-branched galactose and sialic acid termini. This oligosaccharide is hydrolysed by the inducible membranous β -galactosidase of inflammation-primed B cells to yield a macrophage proactivating factor. This in turn is hydrolysed by the membranous Neu-1 sialidase of T cells to yield the MAF.²⁹ Naturally produced as part of the immune response, this factor should be simply termed MAF whereas the term GcMAF should be reserved for the factor obtained *in vitro* by stepwise treatment of purified Gc protein (VDBP) with immobilised β -galactosidase and sialidase. The procedure for producing probably the most potent MAF ever discovered was conceived and perfected by Professor Nobuto Yamamoto of the Socrates Institute for Therapeutic Immunology in Philadelphia, PA, the researcher who has provided the most notable scientific contributions to the topic. The interest for administering GcMAF to humans derives from the observation that different chronic pathologies such as cancer, HIV infection and systemic lupus erythematosus show elevated levels of serum alpha-N-acetylgalactosaminidase (nagalase), an enzyme that degrades VDBP resulting in the loss of MAF precursor activity with consequent impaired immune response.^{29–31} Consequently, MAF precursor activity and serum nagalase activity have been used as diagnostic indices for a variety of cancer patients and as prognostic indices during radiation therapy, surgical resection of tumours and GcMAF therapy of tumour-bearing mice.²⁹ However, there are no data on serum nagalase activity in CKD and this lack of data opens an entire field of future research focusing on the intricate relationship between immune system function and CKD. In fact, externally administered GcMAF was quite efficient in restoring a deficient immune system and this property awaits study by researchers in nephrology. It could be argued that elevated serum nagalase will degrade also externally administered GcMAF, but it was demonstrated that serum nagalase has no effect on GcMAF because serum nagalase acts as an endo-nagalase and not as an exoenzyme: i.e. it is unable to deglycosylate a monosaccharide, N-acetylgalactosamine (GalNAc), of GcMAF. Thus, when GcMAF was added to cancer patient serum containing high nagalase activity and incubated for four hours at 37°C, the potency of GcMAF activity did not decrease.³² Consequently, administration of 100ng of GcMAF per human resulted in the maximal level of macrophage activation; the macrophages develop an enormous variation of receptors that recognise abnormality in the malignant cell surface and kill cancerous cells as well as HIV-infected cells. The therapeutic potential of GcMAF has been validated in patients with metastatic breast cancer,²⁹ metastatic colorectal cancer³³ and prostate cancer,³⁴ as well as in HIV-infected patients.³⁰ Studies are under way to assess its efficacy in other types of cancer as well as in pathologies as diverse as psoriasis, chronic fatigue syndrome and systemic lupus erythematosus.

Little is known so far about GcMAF intracellular signalling and GcMAF–vitamin D interactions at the cellular/molecular level. There is indirect evidence suggesting interaction of GcMAF with a C-type lectin receptor on the macrophage surface,³⁵ and a preliminary report approved for public release by the US Army Medical Research and Materiel Command states that GcMAF blocked the phosphorylation of a band with the approximate molecular weight of 75kDa in prostate

cancer cell lines.³⁶ We recently demonstrated that the F and b alleles of the polymorphic VDR gene were associated with the highest response (in terms of cyclic adenosine monophosphate (cAMP) formation and proliferation) to 100pg of GcMAF per millilitre in human peripheral blood mononuclear cells (PBMC). In subjects harbouring FF and bb genotypes, GcMAF maintained cell viability for about 98 hours after drawing whereas unstimulated cells were no longer viable after 48 hours, as if, in those subjects, GcMAF had rescued monocytes from apoptosis.³⁷ It is worth noting that the VDR alleles termed 'F' and 'b' are also associated with the highest sensitivity to vitamin D and its analogues; an interconnection of vitamin D and GcMAF signalling pathways can thus be hypothesised. However, we do not know as yet whether GcMAF exerts its effects through direct interaction with the VDR or whether VDR polymorphisms influence the response to GcMAF in an indirect manner. As a matter of fact, VDR polymorphisms indirectly influence a variety of conditions, from cancer³⁸ to acquired immunodeficiency syndrome (AIDS)³⁹ and CKD.¹⁵ Further studies will elucidate whether VDR polymorphisms are also associated with the known polymorphisms of VDBP, the precursor of GcMAF,⁴⁰ or with polymorphisms of the gene coding for the GcMAF receptor. Whatever the case, these results may prove instrumental in identifying those subjects who could benefit the most from GcMAF treatment, in particular considering that such treatment is currently being proposed in different settings (see www.gcmf.eu/info/immunemedicine.com/available-therapies/gcmf/; or www.gcmf.nl).

Vitamin D Axis and Angiogenesis in Chronic Kidney Disease

In addition to the effects on the immune system, the components of the vitamin D axis are involved in the control of angiogenesis, a process frequently deregulated in CKD.⁴¹ In fact, in experimental models of diabetic nephropathy, therapeutic effects of angiogenesis inhibitors have been reported,⁴¹ and vitamin D and GcMAF are known to have antiangiogenic properties. Vitamin D has antiproliferative effects on

endothelial cells by promoting G0–G1 cell cycle arrest and by inducing apoptosis.⁴² At the molecular level, vitamin D inhibits angiogenesis by controlling the expression of the gene coding for vascular endothelial growth factor (VEGF), possibly through the formation of cAMP, a second messenger that inhibits angiogenesis.⁴³ Thus, in several cell types, vitamin D exerts its effects by increasing intracellular cAMP levels;⁴⁴ the intracellular effects activated by the cAMP cascade on the VDR-dependent transactivation pathway have been demonstrated.⁴⁵ It is also well established that vitamin D rapidly downregulates gene transcription via a cAMP-dependent signalling pathway.⁴⁶ Also, GcMAF is a potent inhibitor of angiogenesis and this property was associated with its anticancer effects.^{47,48} We recently observed that GcMAF inhibited angiogenesis on an assay completely different from those used in previous studies, reinforcing the hypothesis that GcMAF could be useful in the control of angiogenesis in CKD; thus, we demonstrated that its effect is independent of the tissue of origin and of the stimulus used to induce the angiogenic response.³⁷ Therefore, GcMAF-induced inhibition of angiogenesis could be crucial in determining its effects in conditions where angiogenesis plays a major role in the progression of the disease as it happens in CKD.

Conclusion

All the components of the vitamin D axis participate in the pathogenesis of CKD and this knowledge can be successfully exploited to devise targeted therapeutic strategies. Some of the components of the axis have been studied in greater detail so far, and supplementation of vitamin D is a common feature of CKD treatment. The research on other components, such as VDBP and GcMAF, is still in its infancy and it is thought that new exciting applications deriving from basic research will see the light in the next few years. Thanks to its effects on the immune system and angiogenesis, GcMAF appears to be a promising candidate and it is quite likely that all those interested in CKD will become familiar with this protein of multifaceted properties in the near future. ■

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