New Vitamin D analogs and changing therapeutic paradigms

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Vitamin D compounds have been used successfully to treat secondary hyperparathyroidism for almost three decades. Side effects of increased levels of serum calcium and phosphate and potential complications have increasingly been recognized as problematic, and this has become an even more difficult clinical challenge with the desire to capitalize on some of the pleiotropic effects of vitamin D. Nonclassical nuclear vitamin D receptor (VDR) effects on the cardiovascular system, kidneys, and immune system, with the prospect of improved patient survival, have moved to center stage. Selective vitamin D compounds with minimal effects on mineral metabolism and with maximal cardiovascular and renal benefits are now needed. New vitamin D compounds already in clinical use, which have an improved side-effect profile and differential nonclassical effects compared with calcitriol, are limited to the three licensed pharmaceuticals—paricalcitol, 22-oxacalcitriol, and doxercalciferol. Other compounds are under early development and it is anticipated that these novel therapeutic concepts will result in new vitamin D therapies that will help to reduce the high mortality rate patients with kidney disease experience.

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Our understanding of the biology and clinical consequences of disordered vitamin D metabolism in the context of chronic kidney disease (CKD) has progressed over recent years. Advances are being made on several fronts including clinical epidemiology, therapeutic trials, laboratory science and the development of 'new' and possibly enhanced compounds. Conspicuously absent, however, have been good quality prospective intervention studies with patient-level outcomes.

This review will focus on the therapy of CKD patients with vitamin D compounds in the light of an emerging paradigm shift that takes in to account 'nonclassical' as well as 'classical' vitamin D effects.

For the purpose of the following discussion, 'old' will refer to the active compound, calcitriol ($1\alpha,25(OH)_2D_3$), its prodrug alfacalcidol (1α-$(OH)D_3$), or calcifediol ($25(OH)D_3$). 'New' will include other vitamin D compounds currently in use or in a pipeline, and which may possess enhanced qualities that are clinically useful to patients. The terms 'native vitamin D', 'vitamin D', and 'cholecalciferol' will be regarded as synonymous, unless stated otherwise, and will refer to cholecalciferol (vitamin D_3) or ergocalciferol (vitamin D_2). 'Active vitamin D' refers to compounds that directly activate the nuclear vitamin D receptor (VDR).

The group of 'new' vitamin D compounds already in clinical use remains quite small, comprising paricalcitol (19-nor-$1\alpha,25(OH)_2D_2$), 22-oxacalcitriol, or maxacalcitol (22-oxa-$1\alpha,25(OH)_2D_3$) and doxercalciferol (1α-$(OH)D_2$) (Figure 1). None is new in the strict sense—most of them have been in clinical use for a decade or more. Paricalcitol and 22-oxacalcitriol bind directly to the VDR and are, therefore, active compounds, whereas the third, doxercalciferol, is analogous to alfacalcidol, a prodrug for 1, 25-dihydroxyvitamin D_2 that needs an enzymatic 25-hydroxylation activation step in the liver.

VITAMIN D AND MINERAL METABOLISM IN CKD

Of the myriad metabolic components of moderate and advanced CKD, failure of renal bioactivation of 25-hydroxycholecalciferol is a prominent and extremely well-studied feature. Relative failure of this metabolic step, and consequent sabotage of the endocrine vitamin D hormonal system, is demonstrable early in the progression of CKD, and in advanced CKD, severe deficiency of circulating calcitriol with

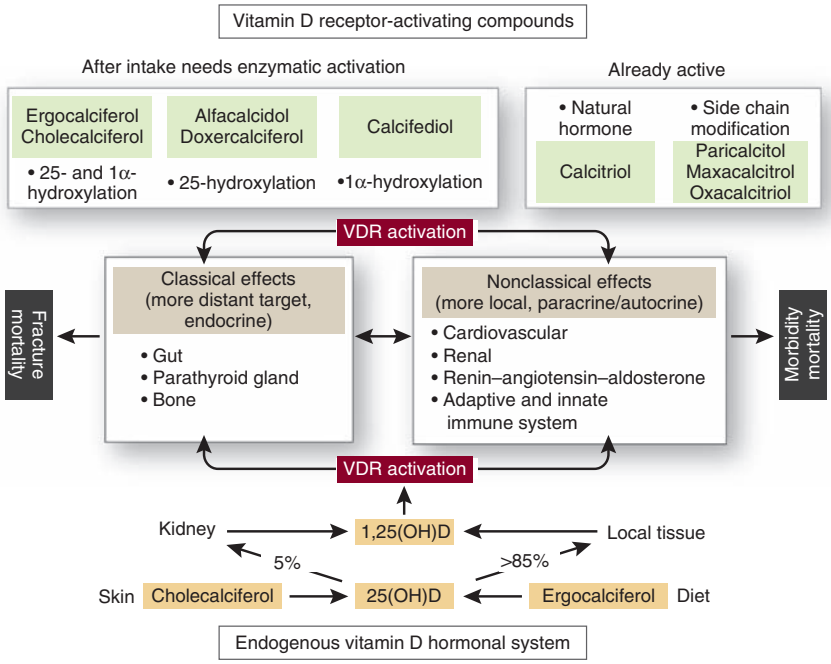

Figure 1 | Vitamin D receptor (VDR) activation: there is an endogenous vitamin D hormonal system with renal (endocrine) and local tissue (paracrine and autocrine) synthesis of calcitriol. The synthesis of calcitriol relies on vitamin D (25(OH)D) sufficiency. The pharmacological treatment with VDR-activating compounds in clinical setup can be divided into two main groups: the prodrugs needing hydroxylation after intake and the already active compounds. Data suggest that active compounds with side chain modification may have an improved side-effect profile. 1,25(OH)D, 1,25-dihydroxyvitamin D.

failure of the vitamin D endocrine system is the norm.[1] As is the case with, for example, thyroid or adrenal insufficiency, physiological replacement of the deficient active hormone is associated with readily apparent and generally advantageous responses. Despite this apparently simple paradigm, a recent meta-analysis painted a surprisingly negative picture of the utility of active vitamin D compounds, particularly in patient-level outcomes and control of biochemical parameters,[2] although this analysis has been the subject of some criticism and the view of most nephrologists, supported by two recent Cochrane reviews, is that treatment of hyperparathyroidism with active vitamin D compounds is logical and generally effective.[3–5]

It is important to remind ourselves that over the last few decades, product development has focussed mainly on the classical endocrine actions of active vitamin D compounds mediated through the principle target tissues, intestine, bone, and parathyroid gland. The three new compounds already in use have all been developed and marketed on the basis of enhanced efficacy and/or reduced toxicity compared with calcitriol. Here, the picture is a very mixed one. Two of these compounds, namely 22-oxacalcitriol[6] and paricalcitol,[7] showed considerable promise during early testing in experimental laboratory systems. In the case of 22-oxacalcitriol, dramatically reduced calcemia with maintenance of potent downregulation of the parathyroid gland was seen *in vitro* and in animal models. In the case of paricalcitol, less striking, but still significant, advantages were observed, particularly with regard to a reduction of the tendency to elevate plasma phosphate during treatment of rodents. On this background, it was both surprising and disappointing to note the complete failure of 22-oxacalcitriol to outperform calcitriol in comparative studies conducted in Japan—both appeared equally effective and equally toxic.[8,9] In the case of paricalcitol, comparison with calcitriol yielded some advantage, as judged by predefined end points relating to efficacy and toxicity, the results pointing to more rapid parathyroid hormone reduction and fewer calcemic episodes in paricalcitrol-treated patients.[10] However, no prospective, placebo-controlled, and blinded study involving 22-oxacalcitriol, paricalcitol, or doxacalciferol has yet demonstrated additional patient-level benefits when compared with calcitriol. Nor, for that matter, have any studies been published showing that either calcitriol or alfacalcidol has an advantage over the other, with respect to biochemical or clinical end points. Further uncertainty arises, because no distinction between low-dose physiological replacement of deficient calcitriol and higher-dose pharmacological use of these compounds in an attempt to treat established hyperparathyroidism has been made in these studies. Finally, it is important to recognize that the published work on the use of active vitamin D compounds in CKD at all levels has been based on populations with a high prevalence of vitamin D insufficiency or deficiency.

Vitamin D sufficiency in non-CKD populations is important for the normal functioning of the vitamin D endocrine system, involving terminal activation of vitamin D in the kidney by the key enzyme, 25-hydroxyvitamin D

1α-hydroxylase (CYP27B1). It is estimated that ~5% of serum prohormone 25(OH)D is used in the kidney for the endocrine vitamin D system and >85% of serum 25(OH)D is used by local target tissues for autocrine/paracrine activation to calcitriol (Figure 1). In addition, the importance of serum prohormone vitamin D sufficiency for patients with CKD, with loss of kidney activation, has only emerged recently, with the observation of a very wide tissue distribution of VDR together with CYP27B1 in the same or neighboring cells, and many nonclassical vitamin D effects are attributed to this paracrine/autocrine vitamin D system, which is still functional in CKD patients. Vitamin D sufficiency, quantified by serum 25-hydroxyvitamin D, is therefore likely to be advantageous to our CKD population and should be one of the first therapeutic interventions when renal function deteriorates.

In this context, it is important to emphasize that both, the local and endocrine vitamin D systems, are working together. This has been nicely illustrated with monocytes from CKD patients. These monocytes demonstrated a 50% lower 25(OH)D uptake compared with cells from patients with normal renal function. This reduced cellular access of 25(OH)D to monocyte CYP27A1, with the consequence of impaired paracrine/autocrine function, was corrected and normalized after these CKD patients were treated with calcitriol.[11]

VITAMIN D, CARDIOVASCULAR DISEASE, AND MORTALITY

One of the refreshing, but also very challenging, changes in nephrology over the past decade has been the emphasis placed on patient-level outcomes rather than surrogates. In this respect, nephrology remains somewhat 'behind the curve' compared with a number of other specialties, but the emphasis is now very strong to the extent that the credibility and acceptability of new therapies rests increasingly heavily on these hard-to-demonstrate end points. Conversely, it is because these end points are so hard to demonstrate that the day of the surrogate has not yet passed and nor has that of a number of different types of retrospective analysis. The latter, in particular, have powerfully influenced our thinking as to which clinical end points are relevant to active vitamin D therapies. Perhaps surprisingly, cardiovascular, rather than bone and mineral metabolism outcomes, have come to the fore. This thinking has been catapulted forward by studies showing effects of *VDR* gene polymorphisms on mortality risk in hemodialysis patients[12] followed by a series of very large historical cohort studies, the first of which demonstrated that dialysis patients treated with paricalcitol lived longer than did those treated with calcitriol.[13] This was quickly followed by other equally large studies appearing to show a substantial survival advantage among dialysis patients treated with injectable active vitamin D compounds when compared with those not in receipt of active vitamin D therapy.[14] Doxercalciferol may also confer a greater survival benefit than calcitriol, although similar to that of paricalcitol.[15] These early studies generally used US patients treated with intravenous vitamin D compounds, but more recently, a similar large historical cohort study was conducted in South America in which enhanced survival was again seen in active vitamin D-treated patients, this time in using exclusively oral agents.[16] Most of the previous studies observed a survival advantage for any dosage compared with no treatment with vitamin D compound. When the association of incremental vitamin D dosages and survival were analyzed, an apparent inverse dose–response effect were observed, with paradoxically reduced survival at higher administered vitamin D dosages.[16,17] This seemingly counter-intuitive observation may be due to confounding by medical indication or consequence of vitamin D compound-mediated altered soft tissue minerals. When the data were analyzed using parathyroid hormone-adjusted vitamin D dosage, a dose–response association between vitamin D compound and survival was observed.[18]

Collectively, these observations are of great importance, and, to date, no large retrospective study of this type has found an increased mortality with VDR activation therapy. Even so, confounding by intention, whereby unrecognized bias between comparative groups is introduced, remains a danger in studies of this type; however, even if they do not conclusively establish the benefits suggested, they certainly mount a persuasive case that makes it incumbent on the renal community to generate appropriately powered and designed prospective studies capable of providing definitive answers.

The focus on survival, largely mediated by the effects of cardiovascular disease, has led to an enormous research effort probing the mechanisms by which active vitamin D compounds might affect the pathogenesis of arterial function, vascular inflammation and calcification, cardiac function, and blood pressure control. The relevant tissues all express the nuclear VDR, which is bound by the three active VDR ligands under discussion, as well as by the products of the prodrugs, alfacalcidol and doxacalciferol, after 25-hydroxylation. Experimental models using knockout mice missing either the VDR[19,20] or the CYP27B1 enzyme with resulting calcitriol deficiency,[21] manifest a phenotype of hypertension, left ventricular hypertrophy, and upregulation of the renin–angiotensin–aldosterone system. In the case of the CYP27B1 knockout, this phenotype can be rescued by physiological replacement with calcitriol, although not by correction of serum calcium and phosphorus by dietary manipulation in the absence of calcitriol. Similar results were observed in a salt-sensitive rat model, in which paricalcitol therapy suppressed left ventricular hypertrophy and normalized cardiac function.[22]

Experimental mouse or rat models have also been used to investigate the impact of vitamin D compounds on vascular calcification and loss of arterial elasticity, processes often occurring in patients with CKD and particularly in those on dialysis. In these CKD models, treatment with paricalcitol produced less arterial calcification than calcitriol, although more than a calcimimetic agent in 5/6 nephrectomized rats with secondary hyperparathyroidism.[23]

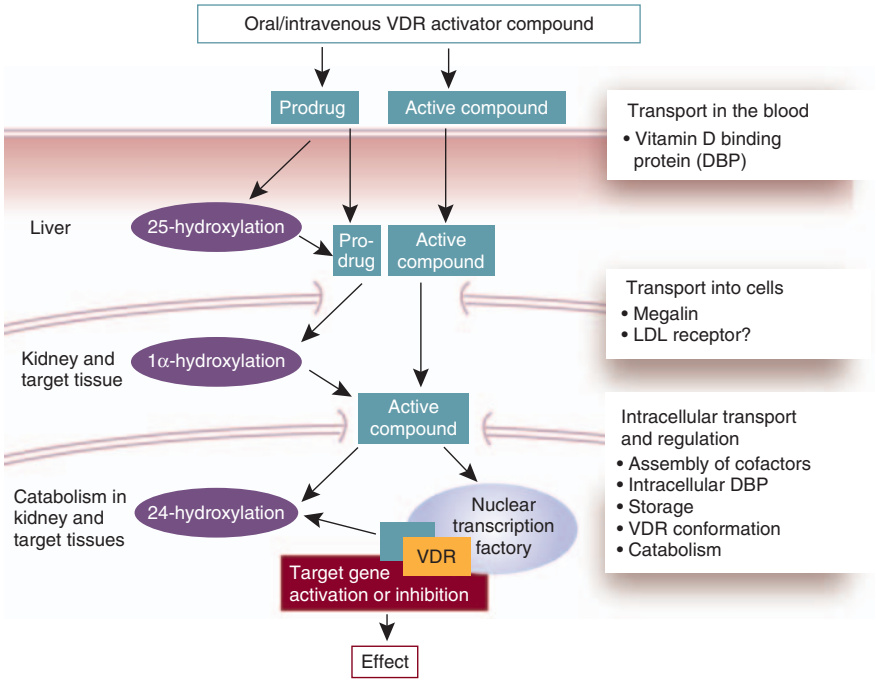

Figure 2 | Transfer of vitamin D compounds to the vitamin D receptor (VDR). The diagram shows different levels where compounds can vary in their transport, storage, or effect. Prodrug or active compound is transferred to site-of-action bound to vitamin D binding protein; a cell surface receptor (in the kidney, megalin) enables the uptake into the cell where the compound binds to intracellular binding protein. Prodrugs can be either activated in the liver (25-hydroxylation) or target tissues (25-hydroxylation, 1-hydroxylation). Active compound binds to the VDR and recruit nuclear proteins to activate the target gene. Catabolism occurs in the target tissue. LDL, low-density lipoprotein.

Mechanistically, there may be direct effects on cells of the artery wall by modulation of the cellular responses and transformation triggered by uremia, or indirect effects from modulation of intestinal mineral absorption and mineral buffering or release from the bone.[24] Observational studies support this beneficial role of vitamin D, and a double-blind randomized study using paricalcitol is now testing the hypothesis that active vitamin D treatment leads to the regression of left ventricular cardiac hypertrophy (PRIMO study, http://www.clinicaltrials.gov, NCT00497146 and NCT00616902).

VITAMIN D AND RENAL PROTECTION

Improvement of proteinuria and renal inflammation is considered an important determinant of cardiovascular and renal outcome. Animal studies with vitamin D compounds have found improvement of proteinuria and inhibition of renal inflammation, similar to that seen with inhibition of the renin-angiotensin-aldosterone system with angiotensin-converting enzyme or angiotensin II receptor blockade and consistent with evidence that renin expression is suppressed by VDR activation. Some preliminary clinical studies using paricalcitol support these observations, and a prospective double-blind, randomized, placebo-controlled study investigating above hypothesis, the effect of paricalcitol in addition to renin-angiotensin-aldosterone system blockade on diabetic proteinuria, is due to be published shortly (VITAL study, NCT00421733).

ARE ACTIVE VITAMIN D COMPOUNDS SAFE?

The most obvious side effects of VDR activators in clinical use are increased levels of serum calcium and phosphate and oversuppression of parathyroid hormone. Biologically, the endocrine role of VDR activation is primarily to regulate calcium balance, and CKD is associated with hypocalcemia. Of concern in CKD is that such changes are meeting a malfunctioning biological system, already unable to keep calcium in solution. The concerns about altered minerals are excess soft tissue and vascular calcification and their link to adverse outcome. However, this effect is mirroring the endocrine effect of VDR activation, is likely linked to other factors, and is dose dependent.

CAN TISSUE SELECTIVITY BE ACHIEVED?

The increased understanding of VDR-mediated biological effects, with the changing therapeutic paradigms, raises the question, how tissue selectivity is achieved and how this could be used therapeutically? The VDR is also expressed in tissues not directly involved in calcium homeostasis, providing means for the nonclassical effects of vitamin D. An important question is if and how tissue or cell selectivity is achieved by the endogenous vitamin D system and how the effect of active VDR ligands could differ from one another and, perhaps, between tissues as well.[25,26] The key vitamin D-activating enzyme, CYP27B1, is expressed and differentially regulated in VDR-expressing target tissues, complementing the endocrine system and enabling local calcitriol synthesis,

depending on tissue-specific requirements (Figure 2). Activation of the catabolic enzyme 24-hydroxylase (CYP24A1) in target tissue provides another point for regulation and may protect cells from excessive VDR activation. CYP27B1, CYP24A1, the vitamin D binding protein in the blood, intracellular vitamin D binding protein, vitamin D membrane binding protein affinity for the vitamin D compound, and alteration of the nuclear transcription factory are other mechanisms that could enable tissue selectivity of vitamin D compound.[27] For example, differential tissue effects observed with 19-nor compounds, such as paricalcitol, resulting in less calcemia, is likely caused by differential nuclear VDR coactivator and corepressor recruitment with altered, tissue-specific target gene activation and inhibition.

OTHER VITAMIN D COMPOUNDS: WHAT'S IN THE PIPELINE?

The pharmaceutical industry has a large number of vitamin D compounds and analogs 'on the shelf'. Some of these have been examined as potentially enhanced treatments for mineral disturbances in patients with CKD, although much greater interest has focused on the possibility that these agents may exert antiproliferative effects useful in oncology and dermatological conditions without the calcemic toxicity associated with calcitriol. There is considerable scientific plausibility to this approach, which has certainly borne fruit in the case of calcipotriol, widely used in the treatment of psoriasis. The hope that one or more of these vitamin D compounds would prove effective in the treatment of malignancy has, however, not yet been realized.

Activation of the catabolic enzyme, CYP24A1, in target tissue is an additional regulator of VDR activation and its potential consequences. Although this enzyme is potentially a treatment-limiting factor for all the vitamin D compounds in clinical use, it is also a potential therapeutic target. New compounds under development with dual action, namely activation of the VDR and simultaneous inhibition of CYP24A1, are in phase II clinical investigation.

NEW COMPOUNDS FOR NATIVE VITAMIN D DEFICIENCY

Vitamin D insufficiency is highly prevalent amongst the general healthy population in temperate climates, and yet more so in patient groups manifesting chronic disease.[1,28,29] Those with CKD are no exception, with prevalence rates of vitamin D insufficiency in the order of 75% and of vitamin D deficiency in the order of 30%. There is a convergence of epidemiological data, basic cell biology, and physiological plausibility that points to a wide range of 'nonclassical' effects of vitamin D compounds that can only be properly subserved by provision of one of the precursors rather than of the active hormonal form itself.[30,31] This is possible because most cells that express the VDR and are vitamin D-responsive also express their own differentially regulated cellular CYP27A1 machinery, in effect allowing calcitriol to be 'locally produced for local need'. It is on this background that recent reports of new compounds designed to perform the same role as native vitamin D are arousing interest. These compounds, some of which require bioactivation by one or more hydroxylations, may differ from native vitamin D in a number of ways, including altered pharmacokinetics and capacity to upregulate or downregulate catabolic and other involved enzymes. Whether these differences are in any way tissue specific remains to be seen; if they are, there is considerable potential for therapeutic enhancement.

CONCLUSIONS AND RECOMMENDATIONS

Although the appearance of new active vitamin D compounds is limited to three licensed pharmaceuticals, all of which have been in routine clinical use for several years, many others lurk in the wings. Despite the paucity of new available compounds available for clinical testing, both clinical and laboratory investigation of the vitamin D compounds continues apace. Importantly, with the changing therapeutic paradigms that have emerged over recent years, vitamin D compounds now have a real prospect of improving patient outcome.

However, we believe that the endemic vitamin D insufficiency/deficiency in CKD populations has been largely ignored. It is critical that future studies of active vitamin D compounds, as well as calcimimetics, should be designed to take into account the high prevalence of native vitamin D insufficiency by first correcting this or including a native vitamin D treatment arm as a positive control.

Whether or not the newer compounds genuinely enhance patient outcomes continues to be a matter of intense debate—the arrival of additional new compounds, both active and precursors, will further stimulate that debate and perhaps result in the substantial advances that have been lacking in recent years.

DISCLOSURE

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