Invited Comment

What’s new in vitamin D for the nephrologist?

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Introduction

In this review we discuss some of the clinical implications of progress in our understanding of the action of vitamin D and its derivatives on calcium, phosphate homeostasis and skeletal function in uraemia.

The parathyroid glands synthesize and secrete parathyroid hormone (PTH) in response to low calcium, low 1,25-dihydroxyvitamin D$_3$ (calcitriol), and high phosphate concentrations. The interplay between these elements is complex, operating through several feedback mechanisms. Both PTH and calcitriol regulate circulating calcium and phosphate concentrations through their action on target organs, namely the kidney, bone, and intestine. PTH and calcitriol regulate one another’s production, and additionally are both regulated separately by extracellular calcium and phosphate, as schematically illustrated in Figure 1. The impairment of phosphate excretion and of calcitriol synthesis that accompanies renal insufficiency results in increased parathyroid stimulation from each of the principal modulators, namely decreased calcium, increased phosphate, and decreased calcitriol. This scenario is further complicated by the blunted target organ responsiveness to both PTH and calcitriol in uraemia.

The vitamin D receptor

Calcitriol mediates its genomic effects through the vitamin D receptor (VDR), which is a member of the steroid/thyroid superfamily of nuclear receptors. Calcitriol binding to VDR results in changes in transcription rates for those genes that contain vitamin D responsive elements (VDREs). There are many other facets to this process, the details of which are beyond the scope of this review. Interested readers are directed to other reviews of this topic [1,2]. Uraemia diminishes the tissue response to calcitriol and this is likely to be due, at least in part, to abnormal regulation and function of the VDR [3]. Down-regulation of VDR content has been demonstrated in the parathyroids of uraemic animals [4]. Uraemic toxins have been shown to decrease the ability of the VDR to bind the VDRE as demonstrated for the osteocalcin gene [5] and it is possible that these effects could be ameliorated by early institution of vitamin D therapies. Experiments in the uraemic rat have shown that administration of either calcitriol or the analogue 22-oxacalcitriol (OCT) can prevent the decrease in VDR content of the parathyroids [6].

Recently, homozygous VDR knockout mice have been generated [7,8]. These animals demonstrate phenotypic characteristics similar to that of clinical hypocalcaemic vitamin D-resistant rickets (HVDRR). When these mice are placed on a ‘rescue diet’, in which mineral ion homeostasis is ‘normalized’, they do not develop hyperparathyroidism or the bone abnormalities observed in untreated animals [9], suggesting that the major consequence of VDR ablation is the impairment of intestinal calcium absorption and mobilization of skeletal calcium.

Studies of vitamin D receptor gene polymorphisms have suggested an influence on bone mineral density.
For example, the presence or absence of a cleavage site for the restriction enzyme BsmI in the 7th intron of the VDR gene confers a polymorphism that has been linked to bone mineral density in renal patients [10]. The presence or absence of the restriction site defines a ‘b’ or ‘B’ allele respectively. A relationship between serum intact PTH (iPTH) concentrations and genotypes was found in renal failure patients, with lower iPTH concentrations associated with a BB genotype [11]. However, studies that have tried to evaluate a physiological basis to explain the effect of VDR polymorphisms have so far been unsuccessful [12,13] and it is not yet clear whether these polymorphisms are of use in predicting an individual’s responsiveness to calcitriol therapies.

Rapid actions of calcitriol that could not be adequately explained by an effect on gene transcription have been demonstrated. This has led to the postulation of a second vitamin D receptor, located in the plasma membrane, that is able to transduce a signal from calcitriol independent of the genomic pathway (reviewed in [14]). This prompts speculation that the tissue specific selective actions of vitamin D metabolites could be the result of different effects on both the classical genomic (VDR) and non-genomic receptors.

**Calcitriol and the parathyroids**

**PTH synthesis and secretion**

Calcitriol suppresses PTH directly by powerfully reducing PTH gene transcription [15], as well as by several other processes (Figure 2). This repression of PTH transcription is mediated by a negative vitamin D response element (VDRE) in the gene promoter [16]. The effects of calcium on the parathyroids are mediated through a specific calcium sensing receptor (CaR) [17] which, in the rat, may be upregulated by calcitriol [18], although this view is contradicted by others [19]. In contrast to calcitriol, both calcium and phosphate regulate PTH production by post-transcriptional effects on pre-proPTH mRNA [20].

Hyperphosphataemia predicts a poor outcome to vitamin D therapy and new data have shown a direct role for phosphate on PTH secretion [21–23]. In intact rat parathyroids incubated in culture, high phosphate (3–4 mmol/l) concentrations were able to increase PTH secretion 3–4-fold above basal levels in a normal calcium environment and high phosphate concentrations also blunted the suppressive actions of calcitriol on PTH secretion in this assay [23]. These data suggest that there is likely to be a mechanism whereby parathyroid cells ‘sense’ and respond to extracellular phosphate, presumably through a cell membrane system that can mediate these signals. A possible candidate is the sodium-dependent phosphate co-transporter identified in rat parathyroid, termed rat PiT-1, which is regulated by calcitriol and shows close homology with the mouse and human PiT-1 type III sodium-phosphate cotransporters [24].

**Parathyroid cell proliferation**

Although normal parathyroid tissue has a constitutively low basal cell proliferation rate [25], a common feature of chronic renal failure is irreversible parathyroid hyperplasia [26]. The same factors that augment PTH secretion also modulate parathyroid growth, namely low calcium, low calcitriol and high phosphate [21,27–29]. Calcitriol is a potent inhibitor of proliferation and promoter of differentiation in many cell types including parathyroid cells [30] and, in a uraemic animal model, calcitriol administration was shown to inhibit parathyroid proliferation [31]. These issues are of great clinical importance in that the dynamics of parathyroid cell turnover are such that hyperplasia can develop quite quickly, whereas apoptosis is exceedingly slow—effectively a one-way ticket.

**Calcitriol and the kidney**

PTH stimulated renal 1α-hydroxylation of 25-hydroxyvitamin D₃ to the active hormone is checked by a feedback inhibition, whereby calcitriol promotes its own degradation directly by suppressing 1α-hydroxylase and stimulating 24-hydroxylase activity, and indirectly by elevating ECF calcium. The 1α-hydroxylase gene has now been characterized and studies of its promoter region have identified positive regulatory regions for PTH and calcitonin and a negative regulatory region for calcitriol [32], confirming earlier functional studies. Similar studies identified two VDREs in the upstream region of the 24-hydroxylase gene [33]. These data further corroborate at a molecular level the well-documented physiological role of calcitriol on these enzymes.

Another potentially important regulatory site has
been recently identified. Megalin, a multifunctional clearance receptor located on the luminal surface of proximal convoluted tubule (but not other tubular cells), is responsible for the delivery to the 1α-hydroxylase in the proximal tubule cells of the vitamin D binding protein/25(OH)D₃ complex which is filtered through the glomerulus [34]. These findings are of great interest since they contradict the previous assumption that the substrate for renal 1α-hydroxylase was free 25(OH)D₃, that diffused from ECF across the baso-lateral membrane into the proximal tubular cell, as well as raising the possibility that decreased GFR of any cause could reduce substrate delivery to the renal 1α-hydroxylase.

The role of calcitriol on calcium and phosphate regulation in the kidney has often been contradictory although most evidence points to conservation. Calcitriol upregulates the mRNA and protein expression of the renal type II sodium-dependent phosphate transporter (NaPi-2) in the rat, increasing phosphate uptake in vitamin D deficient rats [35]. Furthermore, the human NaPi-3 gene, was shown to contain a calcitriol-sensitive VDRE in its promoter region [35], pointing to a possible mechanism of calcitriol-mediated phosphate reabsorption in the kidney. However, in another twist to this puzzle a recently identified phosphate-regulating gene with homology to neutral endo-

Calcitriol and the intestine

Pharmacokinetic issues may be important—the maturation cycle of small-bowel epithelial cells is in the order of 70 h, thereby setting a finite limit on the duration of effect of a pulsed dose of a vitamin D analogue. Such considerations would not apply in, for example, parathyroid or bone cells. The promotion of intestinal calcium transport by calcitriol may be partly non-genomic—a near instantaneous response has been observed in some studies—transcalcitachia [36] and the postulated membrane receptor for calcitriol may be involved in this process. These issues are reviewed in detail elsewhere [37].

Calcitriol, calcitriol derivatives and bone

Both PTH and calcitriol act synergistically to regulate bone turnover through the osteoblast. In healthy bone the remodelling cycle is ‘plastic’ and responds, amongst other things, to calcium and phosphate perturbations, increasing formation or resorption according to physiological demands. Uraemia may affect the ability of bone to respond to such perturbations, partly because of blunted responses to PTH [38]. This blunting is partially corrected by provision of calcitriol and/or restriction of phosphate [39].

Maintenance of normal bone turnover in uraemia is an important goal. New vitamin D analogues and calcimimetics may allow us to achieve profound PTH suppression in a large number of patients, thereby removing a major stimulator of osteoblasts, potentially resulting in adynamic bone disorders. A useful property for a calcitriol surrogate in these circumstances would be to maintain osteoblast function by substituting for the anabolic effect of PTH, while potently suppressing the parathyroid. Few studies have examined this issue specifically, although in one report the analogue 22-oxacalcitriol (OCT) was shown to suppress PTH in the uraemic dog without increasing the risk of adynamic bone disease [40]. We have found that there are differences in the ability of calcitriol analogues to stimulate production of the cytokine interleukin-6 (IL-6) in human osteoblast-like cells (Table 1). IL-6 is a paracrine factor that mediates osteoblast/osteoclast signalling, and may be important in the recruitment of osteoclast progenitors. Maximum stimulation of IL-6 production was achieved by calcitriol and 19-nor-1,25-dihydroxyvitamin D₃ (paricalcitol) at fairly high concentrations—10⁻⁷–10⁻⁹ mol/l, whereas OCT and 1,25-dihydroxydihydrotachysterol₂ were maximally active at much lower and therapeutically achievable concentrations—10⁻¹¹–10⁻¹₃ mol/l [41].

**Clinical developments**

The drawbacks of calcitriol therapy (unwanted hypercalcaemia, hyperphosphataemia, inadequate parathyroid suppression, or parathyroid oversuppression) were tackled first by novel, highly unphysiological regimens of calcitriol administration and more recently with structurally modified calcitriol analogues (Table 2).

**Current therapies**

The principal therapies still used are calcitriol and its pro-drug, alfacalcidol. They appear to be equally effective, regardless of dosing regimes. Fischer and Harris [42] found that in haemodialysis patients with MG63 cells were incubated with the compound of interest for 24 h. Data taken from Ref. 41.
Table 2. New analogues of calcitriol used in renal medicine

<table>
<thead>
<tr>
<th>Full name</th>
<th>Generic designation</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>22-oxa-lalpha,25-dihydroxyvitamin D$_3$</td>
<td>OCT</td>
<td>Replacement of carbon-22 with oxygen atom in side-chain</td>
</tr>
<tr>
<td>19-nor-1-alpha,25-dihydroxyvitamin D$_3$</td>
<td>Paricalcitol</td>
<td>Lacks carbon-19 methylene group from A-ring</td>
</tr>
<tr>
<td>1-alpha-hydroxyvitamin D$_2$</td>
<td>Hectorol</td>
<td>Pro-drug—converted to 1,25- and 1,24(S)-dihydroxyvitamin D$_2$</td>
</tr>
<tr>
<td>26,27-hexafluoro-1-alpha,25-dihydroxyvitamin D$_3$</td>
<td>Falecaltriol</td>
<td>Fluorinated side-chain—interferes with side-chain catabolism</td>
</tr>
</tbody>
</table>

2'-HPT there was no difference between intermittent oral or i.v. calcitriol regimens. Similar prospective trials in haemodialysis patients also report that the route of calcitriol administration confers no clinical benefit [43,44]. In another randomized trial examining the efficacy of pulse or daily oral calcitriol administration in CAPD patients, both modalities were equally effective at suppressing PTH [45] despite the higher peak concentrations achieved with the pulse regimen. The evidence from these and other controlled studies support the view that there is probably little or no difference between i.v. and oral administration, although it must be borne in mind that with i.v. injection patient compliance is ensured [46].

New analogues

Many vitamin D analogues have been developed outside the context of renal disease. It was shown in the early 1980s that calcitriol has effects on cell proliferation and differentiation and many analogues have since been found to have much more potent anti-proliferative properties. Many of these new analogues are less calcemic. For example, the anti-psoriasis drug calcipotriol [47] is ‘non-calcemic’ because of its rapid catabolism once it penetrates the dermis. Other analogues have been shown to reduce circulating PTH concentrations with only modest effects on serum calcium and no hyperphosphataemia [48], and some of these may find a use in the treatment of hyperparathyroid disorders.

Figure 3 shows where it might be possible for an analogue to differ from calcitriol and therefore potentially vary the response. This may in turn lead to either reduced or enhanced expression of proteins involved in calcium and phosphate homeostasis and/or alter cellular functions.

22-oxacalcitriol (OCT)

OCT was developed in the late 1980s and was shown to suppress PTH secretion and synthesis in vitro and in vivo [49] while eliciting much less calcemia than calcitriol. This encouraging selectivity of OCT may be the result of differences in action at different target tissues. OCT, in contrast to the sustained action of calcitriol, stimulated only transient intestinal calcium absorption in a rat model [50]. More recent evidence also suggests that the lack of effect of OCT on intestinal Ca-BP9k in the intestine may help to explain the lower calcemic activity of this agent [51]. However, despite these encouraging data in animal models, clinical studies report that effective PTH suppression is still accompanied by hypercalcemia in many patients [52]. Careful comparisons with calcitriol and/or alfacalcidol are awaited.

1-alpha-hydroxy vitamin D$_2$

It had been assumed that the metabolism of vitamin D$_2$ and vitamin D$_3$ with, respectively, the ergosterol and cholesterol side-chain structure, led to the formation of similar 1,25-dihydroxyvitamin D metabolites, but recent studies in man have suggested that this may not be the case [53,54]. For example, alfacalcidol (1z-OH-D$_3$) is 25-hydroxylated to form calcitriol, whereas 1z-OH-D$_3$ is metabolized to both 1,25-dihydroxyvitamin D$_2$ and 1,24(S)-dihydroxyvitamin D$_2$. The latter metabolite is a potent antiproliferative agent with minimal calcemic properties [53,55]. Initial studies in haemodialysis patients have shown the effectiveness of 1z-OH-D$_2$ in suppressing secondary hyperparathyroidism without causing hypercalcemia and without recourse to adjusting the dose of calcium-based phosphate binders [56]. Early results from a large multicentre trial have also demonstrated the efficacy of this vitamin D metabolite in reducing severe hyperparathyroidism [57] but, as with OCT, comparisons with calcitriol or alfacalcidol are still awaited.

Paricalcitol

Paricalcitol (19-nor-1,25-dihydroxyvitamin D$_3$) has also shown encouraging results as a calcitriol alternative. This compound was found to suppress parathyroid gland secretion and growth in the uremic rat model [58,59]. Of interest was an observed lack of intestinal VDR upregulation that was seen in the calcitriol-treated group [59]. This lack of effect in the intestine may account for the reduced calcemic and phosphatemic action of this analogue in that model. In recently published clinical studies, paricalcitol was found to have good PTH suppression with very few and only transient increases in serum calcium in haemodialysis patients [60,61]. However, preliminary reports of comparisons with calcitriol suggest that there is probably little difference between paricalcitol and calcitriol in haemodialysis patients.
Fig. 3. Areas where an analogue may differ from calcitriol in its effect and thereby modify target organ response.

**Falecalcitriol**

This fluorinated calcitriol analogue has been compared with alfacalcidol [62] and in a crossover study demonstrated better PTH suppression, but also more hypercalcaemia than alfacalcidol.

**Summary**

Our current understanding of the actions of calcitriol on mineral ion homeostasis have been much improved by recent insights at the molecular level. The correction of calcitriol lack in renal disease is undoubtedly beneficial in the prevention of both parathyroid hyperplasia and maintenance of bone integrity, and with respect to hyperparathyroidism it is clear that prevention is essential—cure of established hyperplasia often requires surgery. There is no convincing superiority of any particular regimen or dose schedule of the currently available medications. Although some new analogues appear to have better therapeutic potential in experimental settings, this has not been carried over to the clinical arena where the new metabolites have yet to be shown to have a useful edge over calcitriol and alfacalcidol. Clarification of the reason for the disparate results between animal experimental work (good PTH suppression without calcaemia and phosphataemia) and the clinical studies (significant calcaemic effects reminiscent of alfacalcidol and calcitriol) is important, as is refinement of the dosing regimens for the newer analogues.

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**References**

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