Role of vitamin D in vascular calcification: bad guy or good guy?

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Induction of vascular calcification by hypervitaminosis D

The role of vitamin D and its derivatives in vascular calcification is complex. It has long been known that in humans, hypervitaminosis D may be associated with extensive arterial calcium phosphate deposits, mostly in the form of apatite crystals. In experimental animals, the administration of pharmacological doses of vitamin D sterols can lead to widespread arterial calcification, especially in association with favourable conditions such as atherosclerosis, diabetes and chronic kidney disease (CKD) [1–5].

The mechanisms by which high doses of vitamin D or its derivatives induce vascular calcification include an increase in serum calcium and phosphate, the formation of fetuin-A mineral complexes in association with a decrease in free serum levels of fetuin-A [6] and the local induction of osteochondrogenic programmes with transformation of vascular smooth muscle cells (VSMCs) into osteoblast-like cells [7].

In adult patients with CKD, both before [8] and after the initiation of dialysis therapy [9], the severity and progression of vascular calcification have been found by two groups to correlate with circulating 25-hydroxyvitamin D [25(OH)D] levels. However, another group failed to identify an independent association of arterial calcification with serum 25(OH)D and 1,25-dihydroxyvitamin D concentrations although both of them were negatively correlated with aortic pulse wave velocity and positively with brachial artery distensibility and flow-mediated dilatation [10]. Our group also did not find an association between serum 25(OH)D levels and aortic calcification or stiffness in patients with different stages of CKD [11].

The long-term administration of vitamin D sterols to children and young adults with CKD was found to induce vascular calcification [12, 13]. The prevalence of calcinosis was higher in the children treated with calcitriol than in those treated with vitamin D2 or vitamin D3 [13]. Differences between studies may be explained by different doses, types of vitamin D sterols used and treatment duration. Of note, different types of active vitamin D derivatives, when given in high amounts to animals with CKD, are not endowed with the same calcification-inducing capacity. Thus, paricalcitol has been shown to be less pro-calcifying in uraemic rats than calcitriol or doxercalciferol [3, 14]. Whether this also holds true for human patients with CKD remains a matter of debate. No prospective trials are available in such patients comparing the effects of calcitriol with those of the newer active vitamin D derivatives.

Physiological versus pharmacological effects of vitamin D sterols

The effects of vitamin D overload on the vessel wall need to be distinguished from the role of vitamin D under physiological conditions. It is well known that vitamin D exerts pleiotropic actions in multiple organs and tissues, ranging from regulation of the immune system to that of mineral metabolism [15]. It is generally assumed that most of the vitamin’s action occurs through the binding of its active metabolite, 1,25(diOH)D (calcitriol) to the vitamin D receptor (VDR), although effects mediated by other metabolites such as 25(OH)D and 24,25-dihydroxyvitamin D are also possible. In addition to the endocrine effects of VDR activation by circulating calcitriol, local 1,25(diOH)D production can also activate VDRs expressed in many tissues via an autocrine mechanism, including endothelial cells and VSMCs [16–19]. Calcitriol can increase the expression of the VDR and decrease the proliferation of VSMCs [20, 21]. At high doses, it also can promote VSMC migration, transition into an osteoblast-like phenotype and calcification, together with up-regulation of proteins regulating mineralization and calcium transport [7, 22, 23]. Thus, it is likely that autocrine, paracrine and endocrine functions of vitamin D can influence vascular structure, function and remodelling [15]. However, Wang et al. recently challenged the concept of direct VDR activation in the blood.
vessel wall, based on results obtained in an original mouse model. They claim that VDRs are not expressed in arterial endothelial or smooth muscle cells, arguing that previous positive results were an artefact owing to technical problems [24]. This issue needs to be clarified by future studies.

It is not always easy to distinguish physiological from pharmacological actions. On the one hand, low doses of both calcitriol and paricalcitol have been shown to be protective against aortic calcification in uraemic mice with low-density lipoprotein (LDL)-receptor deletion, possibly via stimulation of skeletal osteoblast surfaces and bone formation rates [4]. In contrast, high doses of these active vitamin D sterols induced vascular mineralization, possibly in association with enhanced bone resorption. On the other hand, the observation by another group is noteworthy; the group found that non-hypercalcaemic doses of calcitriol-induced diffuse aortic calcification involving the intima and media layer only in uraemic rats but not in non-uraemic rats. These data would indicate a permissive effect of the uraemic state for cardiovascular damage, which could be induced even by relatively low doses of calcitriol [7, 25].

This problem may be solved by the possibility of a U-curve relationship between vitamin D sterols and vascular calcification, as postulated by Zittermann [26] (Figure 1). In keeping with this, Shroff et al. [27] reported a U-shaped bimodal distribution across serum 1,25(dihydroxy)D levels in paediatric dialysis patients. Both calcification scores and carotid intima-media thickness were significantly greater in the patients with either low or high calcitriol levels than in those with normal levels. In contrast, serum 25(OH)D levels did not correlate with any vascular measure. Of interest, low 1,25(dihydroxy)D levels were associated with higher high-sensitivity C-reactive protein (CRP) concentrations. Calcification was most frequently observed in patients with the lowest calcitriol and the highest CRP levels. In another recent study in children with CKD, serum fetuin-A levels were inversely correlated with serum CRP but positively with the cumulative intake of 25(OH)D and calcitriol [28].

**Interaction of vitamin D sterols with other players involved in the regulation of mineral metabolism**

Vitamin D sterols interact with other major factors playing a role in vascular calcification such as calcium, phosphate, parathyroid hormone (PTH), fibroblast growth factor 23 (FGF23), klotho and inflammation. Calcitriol increases serum calcium and phosphate, whereas it reduces serum PTH. Calcitriol is a strong inducer of FGF23 and klotho. In contrast, FGF23 suppresses calcitriol synthesis by reducing renal expression of 1α-25OH vitamin D hydroxylase and increasing 24,25OH vitamin D hydroxylase. Genetic inactivation of either FGF23 or klotho leads to increased serum calcium, phosphate and calcitriol levels in these mutant mice; such abnormal mineral ion and vitamin D homeostasis in FGF23- and klotho-knockout mice are associated with widespread soft tissue and vascular calcifications [15]. More importantly, genetically reducing active vitamin D synthesis in either FGF23- or klotho-knockout mice can completely eliminate soft tissue and vascular calcifications in FGF23/1α-25(OH)ase and klotho/1α-25(OH)ase double-knockout mice [15]. However, it should be mentioned that the loss of vitamin D-related processes, either from FGF23 or klotho mice, resulted in a change from severe hyperphosphataemia to hypophosphataemia. Moreover, when feeding FGF23-knockout mice on a phosphate-poor diet, the development of vascular calcification is prevented [29]. For these reasons, it is extremely difficult to dissociate the *in vivo* effects of vitamin D excess from that of phosphate excess [15] and possible direct effects of excessive concentrations of FGF23 [30] and klotho [31].

**Vitamin D, inflammation and vascular calcification**

Inflammation is another important mechanism in the pathogenesis of vascular calcification. Associations of circulating levels of pro-inflammatory factors such as tumour necrosis factor-alpha (TNF-α), interleukin-1β and interleukin-6 with arterial calcification have been observed in general population and in patients with CKD [32, 33]. Circulating cytokines have also been shown to be inversely related to serum fetuin-A, an inhibitor of extraskeletal calcification [34]. LDL receptor-deficient mice with Type 2 diabetes, which become obese upon a high-fat diet, develop aortic calcification in association with an increase in serum TNF-α and an up-regulation of the Msx2-Wnt signalling pathway [2]. The latter favours the elaboration of osteogenic and chondrogenic programmes in the vessel wall. By dosing with TNF-α neutralizing antibody infliximab, it was possible to decrease the activity of this pathway and thereby reduce aortic calcium accumulation. Furthermore, vascular overexpression of a TNF-α transgene in mice induced aortic calcification,
together with an up-regulation of the inflammation marker haptoglobin and the osteo chondrogenic transcription factors Msx2, Wnt3a and Wnt7a [2].

In this issue of the Journal, Aoshima et al. [35] further examined the role of vitamin D sterols in inflammation-dependent vascular calcification (ref. to be inserted by Publisher). They used an in vitro system of human VSMCs maintained in culture for 9 days. First of all, the authors confirmed the observation that TNF-α enhances the vascular calcification process induced by a high phosphate concentration in the incubation medium. TNF-α actually increased the deposition of calcium phosphate in the VSMC cultures by nearly 100%. When adding the most active natural vitamin D sterol calcitriol or the synthetic active vitamin D derivative maxacalcitol to the incubation medium, the phosphate- and TNF-α-induced stimulation of VSMC mineralization was drastically reduced. In contrast, the two sterols failed to reduce VSMC mineralization induced by exposure to high phosphate alone.

One of the mechanisms of the vitamin D sterol effects was downregulation of the expression of Cbfa1/Runx2 and osteocalcin, that is genes that are involved in the osteo chondrogenic process, with transformation of VSMCs to osteoblast-like cells. This action involved binding to the VDR and its activation; however, there was no increase in VDR expression.

The authors demonstrated the involvement of yet another remarkable mechanism of action of active vitamin D sterols. Both calcitriol and maxacalcitol reduced the expression of matrixmetalloproteinase-2 (MMP-2) in their VSMC culture system, which was greatly increased in response to high phosphate and TNF-α concentrations, both at the messenger RNA and the protein level. MMP-2, which is a major elastase, degrades elastin in the vessel wall and elsewhere. It has been shown to be activated by the uraemic state [36], and elastin degradation by elastases activated in inflammatory states has been shown to be a major contributor to vascular calcification [37]. Of interest, in the study by Aoshima et al., the inhibitory effect of maxacalcitol on MMP-2 expression was significantly more pronounced than that of calcitriol.

It must be pointed out that the authors examined the effects of pharmacological, not physiological, concentrations of calcitriol and comparable concentrations of maxacalcitol. This could mean that even high doses of vitamin D sterols might be beneficial in inflammation-linked vascular calcification and in the concomitant presence of a high phosphate environment. However, the studies were performed in a non-uraemic environment. Therefore, any extrapolation from these in vitro findings to the uraemic state in vivo must be done with extreme caution. That being said, the findings are in agreement with observations of beneficial effects of various active vitamin D derivatives made in some models of uraemic animals [3, 4, 14] although not in other experimental models or with other vitamin D derivatives [3, 7, 25], as mentioned above.

Figure 2 illustrates in a schematic way how low doses of vitamin D or its derivatives, i.e. doses in the physiological range, might exert protective actions against vascular calcification in the condition of CKD whereas high pharmacological doses might promote the vascular mineralization process. The existence of such apparently opposite actions is supported by the central and right part of the above mentioned U-shaped curve of the vitamin D–calcification relationship.

Remaining problems and perspective

There are no prospective randomized intervention trials in CKD patients comparing the effect of native vitamin D or active vitamin D derivatives on vascular calcification with that of placebo. Although the results obtained in experimental studies done in vitro and in vivo are encouraging, it remains to be seen whether in patients with CKD, the increasingly prescribed correction of vitamin D insufficiency or deficiency with pharmacological doses of vitamin D3 or vitamin D2 have positive or negative effects with respect to arterial calcification. The same question needs to be answered with respect to the pharmacological doses of active vitamin D sterols administered for the treatment of secondary hyperparathyroidism. The negative results of the PRIMO study [38] that failed to show a beneficial effect of long-term paricalcitol administration on cardiac structure and function in chronic haemodialysis patients with left ventricular hypertrophy confirms the need for intervention studies with firm outcomes.

Conflict of interest statement. None declared.

(See related article by Aoshima et al. Vitamin D receptor activators inhibit vascular smooth muscle cell mineralization induced by phosphate and TNF-α. Nephrol Dial Transplant 2012; 27: 1800–1806.)

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