Published in final edited form as:

Curr Pharm Des. 2014 ; 20(37): 5829–5833.

Vascular Calcification in Chronic Kidney Disease: Role of Disordered Mineral Metabolism

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Abstract

In patients with chronic kidney disease (CKD), vascular calcification is associated with significant morbidity and mortality. The prevalence of vascular calcification increases as glomerular filtration rate (GFR) declines and calcification occurs years earlier in CKD patients than in the general population. The mechanisms of vascular calcification in CKD patients are complex and not completely understood but likely involve non-traditional risk factors, which may be unique to patients with CKD. These unique risk factors may predispose patients to early and more accelerated calcification. Experimental and clinical studies show that disorders in mineral metabolisms including calcium and phosphorus homeostasis initiate and promote vascular calcification in patients with CKD. It is currently unknown if vascular calcification can be prevented or reversed with therapies aimed at maintaining calcium and phosphorus homeostasis. This review focuses on the potential mechanisms by which disordered mineral metabolism may promote vascular calcification in patients with CKD.

Keywords

vascular calcification; chronic kidney disease; mineral metabolism; phosphorus

Introduction

Cardiovascular disease is the most common cause of death in patients with chronic kidney disease (CKD) and vascular calcification is one of the strongest predictors of cardiovascular risk. Vascular calcification is a process characterized by thickening and loss of elasticity of muscular arteries walls [1]. This thickening and loss of elasticity occurs in two distinct sites, the intimal and medial layers of the vasculatures. Intimal calcification is associated with atherosclerotic plaques and medial calcification is characterized by vascular stiffening and arteriosclerosis [1]. Medial calcification is more common in patients with CKD, but both forms of calcification occur [2]. With decreasing kidney function, the prevalence of vascular calcification increases and calcification occurs years earlier in CKD patients than in the

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Conflict of Interest: Authors declare no conflicts of interest.

general population [3]. Vascular calcification is associated with many adverse clinical outcomes including ischemic cardiac events and all-cause and cardiovascular mortality [4]. Hence, preventing or reversing vascular calcification may result in increased survival in patients with CKD.

Vascular Calcification in CKD is a complex and active pathological process. Promoters and inhibitors of vascular calcification are under the control of cell-mediated processes and under normal conditions, vascular calcification is eluded [2]. Initiation of calcification occurs due to an imbalance of these regulators. Traditional risk factors for vascular calcification include age, male gender, smoking, diabetes, hypertension, dyslipidemia and other atherosclerotic risk factors. While CKD patients have a high prevalence of these traditional risk factors, vascular calcification in this population is also associated with non-traditional risk factors. These unique risk factors to CKD may predispose these patients to earlier, more accelerated calcification (Table 1). Disordered mineral metabolism may be one such risk factor and is emerging as a key regulator of vascular calcification in the CKD population. This review focuses on the potential mechanisms by which disordered mineral metabolism may initiate and/or promote progression of vascular calcification.

Mineral Metabolism and Vascular Calcification

Disturbances in calcium and phosphate metabolism are common in patients with CKD and begin early in the course of CKD. The most important factors that regulate mineral metabolism are calcium, phosphate, parathyroid hormone (PTH) vitamin D and fibroblast growth factor-23 (FGF23). FGF23 increases early in the course of CKD resulting in inhibition of 1- α hydroxylase and a decrease in 1,25-dihydroxyvitamin D (calcitriol) synthesis. The decrease in calcitriol results in decreased intestinal calcium absorption and decreased total calcium leading to an increase in PTH. This increase in PTH levels is termed secondary hyperparathyroidism. The increase in PTH levels stimulates calcium and phosphorus release from bone and increases phosphate excretion in the kidney. Hyperphosphatemia is a late finding in CKD as FGF23 and PTH result in decreased tubular reabsorption of phosphate and the fractional excretion of phosphate can reach as high as 90%. Thus, serum phosphate is maintained within normal limits until the glomerular filtration rate (GFR) falls to less than approximately 35 mL/min [5]. These alterations in mineral metabolism are associated with increased morbidity and mortality in patients with CKD. Elevated phosphate, calcium, PTH, and FGF23 levels are all associated with vascular calcification in patients with CKD and may directly promote calcification [3, 6-11]. There are many factors hypothesized to play a role in vascular calcification but only the role of disordered mineral metabolism in vascular calcification will be discussed in this review.

Phosphorus

Serum phosphorus has emerged as a key regulator of vascular calcification in patients with CKD. Elevated serum phosphate levels, even within the normal laboratory range, have been associated with vascular calcification and stiffness in patients with and without CKD [3, 6, 11, 12]. Phosphorus appears to have a direct role in vascular calcification through its effect on vascular smooth muscle cells (VSMC). Exposure of VSMC to high levels of inorganic

phosphate induces calcification in the extracellular matrix surrounding the VSMC [13]. Phosphate also directly induces phenotypic changes in VSMCs causing them to transform from a contractile phenotype into an osteochondrogenic phenotype [12]. When VSMC are exposed to elevated phosphate *in vitro*, there is increased transcription of proteins involved in matrix mineralization and bone formation. These proteins include osteocalcin and Cbfa-1/Runx2 [14-15]. This phosphate induced VSMC phenotypic change is dependent on the activity of sodium-dependent phosphate cotransporters (Na/Pi), specifically the type III Na/Pi cotransporter Pit-1, which has been characterized in VSMC [2]. These cotransporters appear to play key roles in controlling vascular calcification [2]. It has been shown *in vitro* that treatment of VSMCs with an inhibitor of Pit-1 results in decreased phosphate uptake and calcification [15-17]. Whether these cotransporters contribute to vascular calcification in human disease remains unknown.

Apoptosis of VSMC is a key regulator of VSMC calcification [18]. Studies have found that apoptosis in VSMC occurs before the onset of calcification [18]. Matrix vesicles, produced by budding from chondrocytes and osteoblasts, appear to play a role in apoptosis induced vascular calcification [19]. The matrix vesicles originated from VSMC may be fragments of apoptotic cells. These matrix vesicles/apoptotic bodies possess the capacity to concentrate and crystallize calcium as they have all of the essential proteins for calcification [18]. Studies have shown that in terminally differentiated chondrocytes phosphate induces apoptosis [20]. The mechanism behind how phosphorus results in apoptosis is uncertain, but may be due to interruptions in normal mitochondrial energy metabolism [21]. Apoptosis and calcification occur in a dose and time dependent manner when human aortic smooth muscle cells are exposed to high phosphate [22]. In human aortic smooth muscle cells, HMG CoA reductase inhibitors stop phosphate induced calcification by preventing apoptosis [22]. In human trials of HMG CoA reductase inhibitors, a decrease in vascular calcification is seen and this decrease may be due to inhibition of phosphate induced apoptosis [23,24]. Further studies are needed to elucidate the role of phosphate induced apoptosis in vascular calcification in humans.

It has been postulated that a delicate balance between mineral deposition and resorption exists in the vasculature and disturbances result in calcification of the vessel walls [25]. VSMC with an osteoblast-like phenotype are found in the calcified vessel wall. Interestingly, the calcified wall also contains cells, such as monocytes and macrophages that are able to differentiate into osteoclast-like phenotypes [12, 26]. These osteoclast-like cells are important regulators in vascular homeostasis and extracellular mineralization [26]. Accordingly, initiation and progression of calcification occurs if an imbalance favoring the osteoblast-like phenotype ensues [25]. For example, *in vitro*, the differentiation of monocytes and macrophages into osteoclast-like cells decreases after exposure to elevated phosphorus concentrations to levels seen in advanced kidney disease. The decrease in differentiation is due to down regulation of RANK-ligand induced signaling [27]. Hence, elevated phosphorus may result in vascular calcification through reductions in osteoclast activity. It is unclear if activating osteoclast-like cells in the vessel wall prevents or reverses calcification.

Calcium

Numerous clinical and epidemiologic studies have shown that elevated circulating calcium levels are associated with vascular calcification [3,10]. Even though calcium and phosphorus have distinct effects on VSMCs they are synergistic in promoting vascular calcification [28]. Experimental studies have found that vascular calcification requires an increased uptake of both calcium and phosphate by the VSMC [15]. Calcium entry into the VSMC is regulated by the calcium-sensing receptor (CaSR) which is highly sensitive to small changes in extracellular calcium levels [29]. The CaSR is down-regulated in the setting of high extracellular calcium. Inhibition of CaSR function increases VSMC calcification whereas increased sensitivity of the CaSR to calcium reduces calcification [30]. Drugs called calcimimetics increase the sensitivity of the CaSR to calcium and are currently being used for the treatment of secondary hyperparathyroidism in patients with CKD (discussed below).

Calcium also appears to play a central role in inducing apoptosis and matrix vesicle release resulting in promotion of vascular calcification. Exposure of VSMC to high calcium concentrations *in vitro*, results in apoptotic cell death leading to further release of calcium and apoptosis [31]. As discussed earlier, these apoptotic bodies/matrix vesicles initiate calcification through increases in promoters and decreases in inhibitors of calcification [18]. For example, exposure to high calcium levels results in depletion in the endogenous calcification inhibitors, Matrix Gla protein and Fetuin-A [31]. The expression of both of these calcification inhibitors are reduced in patients with CKD.

Patients with CKD have accelerated calcification compared to the general population. The cause of this accelerated, progressive calcification is unclear but may be due to calcium-phosphate nanocrystals. It has been reported that calcium-phosphate nanocrystals undergo lysosomal degradation by VSMCs leading to high intracellular calcium levels and ultimately cell death [28]. The resulting apoptosis further promotes calcification. Studies have found that the osteochondrocytic differentiation of VSMCs is induced by calcium-phosphate nanocrystals, not phosphorus alone, suggesting that it is the synergistic effect of calcium with phosphate that results in phenotypic changes in the VSMC [31]. Additionally, calcium-phosphate nanocrystals increase the expression of BMP-2, a promoter of calcification that promotes VSMC differentiation into osteoblast-like cells [31]. From a clinical perspective, serum calcium levels are independently associated with death in patients on dialysis, but the greatest risk of death in dialysis patients occurs when high calcium and high phosphate coincide [10]. Patients with CKD are believed to have increased total body calcium levels despite usually normal serum levels [32]. Further calcium balance studies in CKD patients are needed in order to better understand calcium homeostasis. The current recommendations by the National Kidney Foundation, Kidney Disease Improving Global Outcomes (KDIGO) are to limit the total calcium intake from phosphate binders and diet in dialysis patients with hypercalcemia and/or arterial calcification [33].

Parathyroid Hormone and Vitamin D

Secondary hyperparathyroidism is a common finding in CKD patients. Secondary hyperparathyroidism is characterized by increased parathyroid hormone (PTH) levels and

parathyroid gland hyperplasia [34]. Data regarding the role of elevated PTH levels in vascular calcification is conflicting. Physiologically, the activation of the PTH receptor by PTH decreases arterial blood pressure which could result in protection against vascular calcification [35]. However, continuously high PTH levels (as seen in secondary hyperparathyroidism) are associated with higher arterial blood pressures not lower ones. PTH directly stimulates the reninangiotensin-aldosterone system and the sympathetic nervous system resulting in increases in arterial blood pressure [36, 37]. The resultant hypertension may induce endothelial dysfunction and promote vascular calcification. Interestingly, significant decreases in both coronary and carotid artery calcifications have been reported in CKD patients following parathyroidectomy [38]. A study examining the use of a calcimimetic for secondary hyperparathyroidism in patients on dialysis found a reduction in aortic valve calcification after 52 weeks of treatment [39]. However, other studies of calcimimetics have not found such promising results [40]. Hence, the beneficial effect of lower PTH levels on vascular calcification has not been definitely established.

Patients with CKD have a very high prevalence of vitamin D deficiency and low circulating levels of the active form of vitamin D (1,25-dihydroxyvitamin D or calcitriol) [34]. The decrease in vitamin D levels is multifactorial but in early CKD the fall in calcitriol levels is likely due to FGF23. Several studies have found an association between vitamin D deficiency and increased cardiovascular mortality and morbidity including increased vascular calcification and stiffness [41,42]. The role of vitamin D in vascular calcification is unclear. Calcitriol is a potent inhibitor of the renin-angiotensin system and inhibition of the renin-angiotensin system is anti-atherosclerotic [43]. Vitamin D also has anti-inflammatory properties and deficiencies in vitamin D are associated with increases in inflammatory factors such as TNF- α and IL-6 [44]. Inflammation plays a key role in vascular endothelial dysfunction and atherosclerosis, thus vitamin D may be protective against vascular calcification. Additionally, VSMC have vitamin D receptors as well as functional 1- α hydroxylase and relax in the presence of calcitriol [45]. However, calcitriol also promotes reabsorption of calcium and phosphorus from the gut potentially promoting calcification.

Studies examining the role of vitamin D supplementation on vascular calcification have found conflicting results. Some studies have found that vitamin D promotes calcification by upregulating calcification promoters Runx2, osterix and osteocalcin and by increasing calcium uptake into VSMCs [46,47]. However, other studies have found that vitamin D has protective effects against calcification by increasing calcification inhibitory proteins such as osteopontin [48]. The reason for the differing results may be due to the doses of vitamin D receptor activators (VDRAs) used in the studies. Studies showing increased calcification used supraphysiological doses while studies using therapeutic doses did not find increased calcification. Further studies are needed to determine whether vitamin D supplementation is protective against calcification in patients with CKD.

Fibroblast Growth Factor-23 and Klotho

Fibroblast Growth factor 23 (FGF23) is a circulatory peptide secreted by osteocytes and plays a key role in the control of serum phosphate, vitamin D metabolism and secondary hyperparathyroidism. FGF23 inhibits phosphate reabsorption in the proximal tubules and

inhibits 1- α hydroxylase activity resulting in decreased calcitriol and decreased phosphate reabsorption in the intestine [49]. FGF23 requires a co-receptor, Klotho, a single-pass transmembrane protein for activity [49] as FGF23 is presumed to only be active in tissues that also contain Klotho (distal tubule of kidney, parathyroid glands, brain). FGF23 levels increase early in the course of kidney disease in order to keep serum phosphorus within the normal range.

Several studies have linked FGF23 to death and cardiovascular events in patients with CKD [12, 50-52]. Studies have also linked FGF23 to vascular calcification but the results are conflicting. In moderately uremic mice fed high phosphate diets, serum FGF23 levels, not serum phosphate levels, were associated with extensive arterial medial calcification [53]. FGF23 has been independently linked to coronary artery, aortic and peripheral vascular calcification in patients on dialysis [54-56]. However, a recent study found that FGF23 was not associated with vascular calcification in CKD patients, not on dialysis, but serum phosphate was [57]. Furthermore this study found no expression of FGF23 or Klotho in human VSMCs. This data suggests that phosphate and FGF23 have different roles in the pathogenesis of cardiovascular disease. Hence, it is uncertain if elevated FGF23 levels directly cause vascular calcification or if they inhibit calcification as patients with inactivating mutations of FGF23 have an increase not a decrease in vascular calcification. Further studies are required to determine whether FGF-23 is a marker or a potential mechanism for vascular calcification.

Klotho may also play a role in vascular calcification. CKD appears to be a state of Klotho deficiency as secreted Klotho is decreased in the blood and urine in patients with CKD [58,59]. The decrease in urine Klotho starts early in CKD and continues as kidney function declines [58,59]. Klotho deficient mice and FGF23 null mice exhibit a similar phenotype, characterized by an accelerated aging process with shortened life span, atherosclerosis and soft tissue calcification including vascular calcification [60]. In an experimental model of CKD, mice with overexpression of klotho had better renal function, higher urinary phosphate excretion and decreased ectopic calcification than wild-type mice with CKD suggesting that Klotho may inhibit vascular calcification [58]. Recent data suggests that Klotho may have a direct effect on vascular calcification. VSMC from animals without Klotho have increased expression of the Na/Pi cotransporter and the osteogenic transcription factor Runx2. This finding suggests that decreases in Klotho promote calcification [58]. If Klotho is added to VSMC in vitro, it decreases the activity of the Na/Pi cotransporter thereby decreasing phosphate uptake and preventing the change of VSMC to an osteochondrogenic phenotype [58]. It is unclear how Klotho mediates its effects on VSMC and is unknown if VSMC express Klotho. Further studies are needed to confirm the role of Klotho in vascular calcification.

Treatment of Vascular Calcification

Since vascular calcification is one of the strong predictors of cardiovascular mortality and morbidity in CKD patients, prevention and treatment of vascular calcification is crucial. Current treatment strategies focus on treating modifiable traditional risk factors such as dyslipidemia and hypertension as well as abnormalities of mineral metabolism.

Unfortunately, to date, no treatments have been proven to prevent or completely reverse vascular calcification in patients with CKD. In clinical studies, it is observed that established calcification has a tendency to progress and cannot be completely arrested or reversed except in a few casuistic reports after parathyroidectomy of patients with severe secondary hyperparathyroidism [38]. Treatment should ideally focus on prevention of vascular calcification but even in studies of patients with early stage CKD it has been found that nearly half of these patients already have calcification [61]. Thus, we need to focus on preventing progression of or possibly reversing vascular calcification.

It is currently recommended to treat hyperphosphatemia in patients with CKD. However, there is insufficient evidence that any specific phosphate binder significantly impacts patient-level outcomes. There is some evidence suggesting that sevelamer compared with calcium based phosphate binders attenuates the progression of vascular calcification in patients with CKD [62-64]. However, other randomized controlled trials found similar rates of progression of arterial calcification in patients receiving sevelamer and calcium-based binders [65,66]. Kidney Disease Improving Global Outcomes (KDIGO) recommends that therapy should be individualized to each patient and recommends limiting the dose of calcium-based binders in patients with persistent or recurrent hypercalcemia, adynamic bone disease, arterial calcifications and/or if serum PTH levels are consistently low [33]. Interestingly, studies examining the use of phosphate binders in CKD have found no effect on FGF23 levels [61]. Hence, it remains unknown if modifying FGF23 levels will reduce calcification. Randomized controlled trials are desperately needed in order to guide treatment and hopefully improve outcomes.

Calcimimetics may have a role in reducing vascular calcification progression. Calcimimetics are currently used for the treatment of secondary hyperparathyroidism in patients with CKD. Evidence indicates that functional CaSR are also expressed in VSMC [29]. It has been shown experimentally that calcimimetics reduce the accumulation of calcium and phosphorus in the aorta [67] and delay the development of both vascular calcification and atherosclerosis in uremic mice [68]. Human data regarding calcimimetics is conflicting. The ADVANCE study found that the combination of a calcimimetic (cinacalcet) with low dose active vitamin D attenuated the progression of vascular and cardiac-valve calcification in patients on dialysis [39]. However, another recent study found that cinacalcet did not significantly reduce the risk of death or major cardiovascular events in patients with moderate-to-severe secondary hyperparathyroidism who were undergoing dialysis [40]. The role of vitamin D receptor activators (VDRAs) remains unclear as some studies have found VDRAs promote calcification and others that they inhibit it [46-48]. Randomized trials are needed in order to guide therapy.

Conclusion

Vascular calcification is thought to be one of the most important risk factors for the increased cardiovascular morbidity and mortality in patients with CKD. Vascular calcification is an active and complex process and disordered mineral metabolism is thought to be a key regulatory of vascular calcification in patients with CKD. Several measures have been taken for prevention and control of vascular calcification in the CKD population which

mostly target the control of calcium and phosphate homeostasis. However, to date, no treatment strategy has been proven to prevent or completely reverse calcification. The question remains as to whether strategies that target disordered mineral metabolism prevent or reverse vascular calcification.

Acknowledgments

Financial Support: National Institute of Diabetes and Digestive and Kidney Disease Grant K23 DK087859

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Table 1

Nontraditional Risk Factors for Vascular Calcification in Patients with Chronic Kidney Disease

Kidney Function Decline
Dialysis Vintage
Disordered Mineral Metabolism
Elevated serum phosphate levels
Elevated serum calcium levels
Elevated parathyroid hormone levels
Changes in vitamin D metabolism
Elevated FGF23 levels
Inflammation and Oxidative Stress
Osteogenesis Factors (Cbfa1)