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Vascular Calcification in Uremic Conditions: New Insights into Pathogenesis

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Chronic kidney disease (CKD) patients have an higher incidence of cardiovascular morbidity and mortality compared with the general population. In the past 10 years, several studies pointed out that vascular calcification is a major cause of cardiovascular disease in the dialysis population. In CKD patients, high levels of serum phosphate and parathyroid hormone play a critical role in the pathogenesis of cardiovascular events. Calcium- and aluminum-free phosphate binders provide a new and effective therapeutic tool in preventing cardiovascular calcifications in CKD in animal models and in hemodialysis patients. Moreover, the pathogenesis of vascular and soft-tissue calcification, which traditionally has been associated with a passive calcium-phosphate deposition, certainly also is related to an active, cell-mediated process. In fact, some bone regulatory proteins seem to be able to induce or inhibit mineral deposition in the vasculature. In particular, bone matrix protein 7, α2-HS glycoprotein, and matrix GLA protein may be regulatory keys in preventing extraskeletal calcification in uremic conditions. This review presents the current understanding of the pathogenesis of vascular calcification in CKD patients, focusing on these 3 proteins and their protective action on extraskeletal calcification.
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Severe cardiovascular calcification is very common in chronic kidney disease (CKD).[1-3] Patients develop extensive medial calcification, which causes increased arterial stiffness and high morbidity and mortality caused by cardiovascular events.[4-6] A large number of risk factors are associated with vascular calcification in dialysis patients (time on dialysis, uremic toxins, history of diabetes, and inflammation), but abnormalities in bone mineral metabolism may play a critical role.[7] In fact, increased serum phosphate, calcium-phosphate product, and parathyroid hormone (PTH) levels contribute to vascular calcification, although their roles are understood incompletely.[8-11]

In addition to these classic alterations in bone and mineral metabolism, an active process has been documented.[12] In the past decade, several studies defined calcification of atherosclerotic lesions as an active process similar to bone formation. Several gene products may induce or inhibit the process of extraskeletal calcification. However, the potential role of protective proteins associated with reduced vascular calcification in CKD needs to be elucidated better.

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Vascular Calcifications in Uremia

Dialysis patients develop atherosclerotic vascular disease earlier than the general population.[3,4] In CKD-induced cardiovascular disease, 2 groups of vascular calcification risk factors need to be considered: classic risk factors are age, sex, family history, smoking, obesity, hypertension, diabetes, and dyslipidemia; uremia-associated risk factors are time on dialysis, uremic toxins, inflammation (advanced-glycation end products, oxidative stress and nitric oxide, asymmetric dimethylarginine, and homocysteine), and increased serum levels of phosphate, calcium-phosphate product, and PTH.

Increases of serum phosphate and calcium-phosphate product levels may worsen cardiovascular events in the uremic population by causing a progressive increase in calcium deposition in the coronary arteries and cardiac valves.[13] Furthermore, calcium-containing phosphate binders can increase calcium load.[5] Recently, an elegant study by Goodman et al[14] on children and young adults on hemodialysis showed a correlation between coronary artery calcification detected by electron-beam computed tomography and years of dialysis, serum phosphorus levels, calcium-phosphate product

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levels, and daily intake of calcium. Moreover, experimental observations in uremic rats[15,16] and in dialysis patients[17] showed that a new calcium-free and aluminum-free phosphate binder (sevelamer HCl) attenuated the progression of cardiovascular calcification better than calcium-based phosphate binders.

In the past decade, several investigators analyzed the pathogenic mechanisms of phosphate-induced vascular calcification, pointing out not only a calcium-phosphate passive deposition in the vessels, but also an active role of genes associated with osteoblastic functions in vascular smooth muscle cells.[8,9] Recently, very enlightening in vitro studies by Jono et al[18] and Giachelli[19] showed that high phosphorus levels in the culture media (2 mmol/L) stimulated calcification in vascular smooth muscle cells. Moreover, additional in vitro studies indicated that high phosphate levels increase vascular calcification by a direct induction of the osteoblast-specific gene Cbfa-1, which regulates the expression of several bone morphogenic proteins (BMPs).[20]

In CKD the expression of these bone morphogenic proteins also was induced by uremic serum independently of phosphate concentrations, in an in vitro model of bovine vascular smooth muscle cells.[21] Importantly, Moe at al[22] showed an increased expression of Cbfa1 and osteopontin in the media and intima of calcified epigastric arteries from uremic patients.

Clearly, these data suggest that vascular calcification is an active process. The cellular formation of a bone-like structure in calcified vessel walls indicates that the uremic environment and increases in serum phosphate levels may be regulatory keys on the pathogenesis of vascular calcification.

Considering serum calcium and phosphate levels, physiochemical crystallization should occur immediately in the absence of active inhibitors. However, serum biologic macromolecules have an inhibitory effect on calcium-phosphate precipitation. Loss of these inhibitory proteins determines mineralization in the arteries.

Bone Morphogenetic Protein 7

Recently, exciting new data from Lund et al,[23] Davies et al,[24] and Li et al[25] suggested that a new player, BMP-7, is involved in the process of vascular calcification of chronic renal failure and in the pathogenesis of renal osteodystrophy.

BMPs are members of the transforming growth factor-β superfamily of cytokines and consist of a group of at least 15 morphogens involved in intracellular messaging.

BMP-7 is a crucial element for the development of kidneys, eyes, and bones.[26] In the skeleton, BMP-7 deficiency produces a patterning defect causing extra digits and ribs to be formed and leads to a deficiency of precursor cell commitment to the osteoblastic differentiation and mineralization program. In the adult, BMP-7 maintains a role in osteoblast function, suggesting a hormonal role in bone metabolism. Interestingly, BMP-7 expression decreases early in the course of renal failure.[27]

This state of BMP-7 deficiency has important consequences in the pathogenesis and treatment of chronic renal insufficiency,[28] but also is very interesting for the pathogenesis and treatment of vascular calcifications. Indeed, BMP-7 maintains vascular smooth muscle cell (VSMC) differentiation and prevents their transformation into an osteoblastic phenotype.[23,29] Thus, the state of BMP-7 deficiency, characteristic of chronic renal failure, could favor vascular calcification, especially in the context of atherosclerotic lesions.

In renal osteodystrophy, BMP-7 affects osteoblast morphology and number, eliminates peritrabecular fibrosis, decreases bone resorption, and increases bone formation in secondary hyperparathyroidism. Moreover, it restores normal rates of bone formation in the adynamic bone disorder, as shown in a renal ablation animal model.[30] In this study, the reversal of adynamic bone disease was shown with a physiologic bone anabolic factor: the results of BMP-7 treatment differs from that of increasing PTH levels because bone resorption is not stimulated and increased bone formation rates thereby truly are anabolic. Therefore, BMP-7 is broadly efficacious in renal osteodystrophy, and importantly increases the skeletal deposition of ingested phosphorus and calcium, improving ion homeostasis in CKD.

Given the known role of BMP-7 in osteoblast biology it may seem paradoxic that it would reduce vascular calcification. However, the effects of individual BMPs are highly dependent on the target cell type, the receptors expressed, and the state of differentiation and maturity.[31] Transdifferentiation of VSMCs in osteoblastic phenotype could be the critical first step in the cause of vascular calcification and it is clear that BMP-7 has a positive influence in maintaining VSMC differentiation.

Suggesting that BMP-7 may be viewed as a fourth renal hormone, along with renin, erythropoietin, and calcitriol, Davies et al[24] showed that BMP-7 is an effective treatment of vascular calcification in the context of a murine model of atherosclerosis and chronic renal failure, a finding that may have important implications for the development in human beings of future therapies for this condition, which currently is without treatment and with strong negative influences on cardiovascular morbidity and mortality.

α2-Heremans-Schmid Glycoprotein

α2-Heremans-Schmid glycoprotein (AHSG), also known as human fetuin, is another important inhibitor of extraskeletal calcification. Serum concentration of AHSG decreases during the cellular immunity phase of inflammation.[32] In vitro, fetuin inhibits the de novo formation and precipitation of calcium phosphate, with no effects on hydroxyapatite once it is formed.[33] AHSG antagonizes the antiproliferative action of transforming growth factor-β1 and blocks the osteogenesis and deposition of calcium-containing matrix in dexamethasone-treated rat bone marrow cells.[34]

Fetuin-deficient mice develop extensive ectopic calcifica-

Table 1 MGP, Fetuin, and BMP-7

	MGP	Fetuin	BMP-7
Molecular weight	10 kd	63 kd	46.75 kd
Serum levels	60-130 U/L	0.5-1.0 g/L	0.1-0.5 ng/mL
Synthesis	VSMCs, chondrocytes	Hepatocytes	Osteoblasts
Phenotype of knock-out mice	Aortic media calcification, cartilage calcification, lethal aortic rupture	Diffuse metastatic soft-tissue and intra-arterial calcification, impaired survival	Hypoplastic-dyplastic kidneys, bone mineralization deficiency, developmental defects of eyes
Properties	Vitamin K–dependent γ-carboxylation necessary for activation	Negative acute phase reactant, transforming growth factor-β antagonist, inhibitor of insulin-receptor tyrosine kinase activity	Transforming growth factor-β superfamily member, regulator of osteoblast differentiation, VSMC differentiation

tions in myocardium, kidney, lung, tongue, and skin.[35] In addition, hemodialysis patients with lower serum fetuin levels have increased mortality owing to cardiovascular events.[36] This observation by Ketteler et al[36] suggests that AHSG is involved in preventing the accelerated extraskeletal calcification observed in CKD.

Price et al[37,38] have investigated the mechanisms by which proteins inhibit vascular calcification. They showed a high molecular weight complex of a calcium-phosphate mineral phase and the 2 inhibitory proteins AHSG and matrix GLA protein (MGP) in the serum of rats treated with the bone-active bisphosphonate etidronate.[39] Furthermore, in a recent in vivo study, Price et al[39] found that vitamin D–induced vascular calcification is associated with a 70% reduction of serum fetuin level, probably caused by the clearance of the fetuin–mineral complex from serum.

The potential role of fetuin in the pathogenesis of extraskeletal calcification in CKD patients still is understood poorly. Clearly, AHSG binds calcium and hydroxyapatite because fetuin knock-out mice have soft-tissue calcifications, and serum AHSG levels are reduced in both uremic and inflammatory conditions. Therefore, loss of serum and local fetuin could promote vascular calcification directly in uremic patients.

MGP

MGP is a member of the vitamin K–dependent protein family with unique structural and physical properties. During the first 2 months of life, MGP-deficient mice develop diffuse arterial calcification, osteoporosis, and pathologic fractures.[40] For its properties as an extracellular matrix protein with a high affinity for calcium and phosphate, MGP plays an important role in the prevention of vascular calcification and in the pathogenesis of osteoporosis,[41] although its effects in CKD patients still are unclear.

Binding BMP-2, MGP elicits an inhibitory mechanism on mineralization.[42] A recent study by Wajih et al[43] showed an uptake mechanism for serum fetuin by cultured human VSMCs. Fetuin uptake and secretion by these cells could represent an additional protective mechanism against arterial calcification.

The localization of MGP and other bone matrix proteins such as osteopontin has been investigated by Canfield et al[44] in calcified atherosclerotic arteries and in normal vessel walls. Although MGP was not detected in normal blood vessels, its expression was enhanced at loci of arterial calcification such as atherosclerotic and calciphylactic lesions. Therefore, the MGP localization in calcified arteries suggests an etiopathogenic role for this inhibitory protein on the development of vascular calcification.[44]

An association between polymorphisms of the MGP gene and myocardial infarction in low-risk individuals has been described.[45] Potentially, the definition of polymorphisms of the MGP gene represent a critical step in understanding pathogenic mechanisms of vascular calcification in CKD and dialysis patients. Recently, Jono et al[46] reported an association between serum MGP levels and coronary artery calcification detected by electron-beam computed tomography in 115 patients with suspected coronary artery disease and normal renal function. Patients with coronary artery calcification had lower serum MGP levels compared with those with no calcium in the coronary tree, suggesting the potential role of MGP in the prevention of vascular calcification.[46]

Conclusions

Vascular calcification is a complex phenomenon in which basic mechanisms have been identified. Lack of inhibitory proteins (BMP-7, fetuin, MGP) is a first possible principle of calcium-phosphate deposition in soft tissues (Table 1). In addition, an organized calcification process similar to bone formation has been identified clearly in calcified atherosclerotic lesions. In the setting of CKD, different stimuli such as hyperphosphatemia, hyperparathyroidism, diabetes, dyslipidemia, hypertension, and iatrogenic vitamin D administration have a common target in increased calcium load. In particular, the alteration of bone mineral metabolism is a typical risk factor for a higher incidence of vascular calcification and cardiovascular events in CKD patients. Therefore, it becomes mandatory to prevent vascular calcification by decreasing serum phosphate and calcium-phosphate product levels, and re-

ducing the active process through regulation of specific genes. In fact, modulation of the production and activity of BMP-7, AHSG, and MGP could offer a novel approach to the treatment of several vascular diseases.

The advent of these new pieces of information has opened important pathways for better understanding the puzzle of the pathogenesis of increased risk for extraskeletal calcification and cardiovascular events in CKD patients.

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