Uremic Vascular Calcification

Neal X. Chen and Sharon M. Moe

Recent evidence suggests that uremic vascular calcification is an active, cell-mediated process resembling osteogenesis in bone rather than passive precipitation. We identified increased expression of bone-associated proteins (osteopontin, bone sialoprotein, alkaline phosphatase, type I collagen) and the bone-specific transcription factor core-binding factor α_1 (Cbfα_1) in histologic sections of inferior epigastric arteries obtained from patients with stage V chronic kidney disease or calcific uremic arteriolopathy. In in vitro experiments, the addition of uremic serum to cultured vascular smooth muscle cells up-regulated osteopontin and Cbfα_1 expression and accelerated mineralization. This implies that the uremic mileau may lead to dedifferentiation of vascular smooth muscle cells, with subsequent mineralization. However, a lack of inhibitors of calcification may also be important. Dialysis patients with low levels of serum fetuin A, a circulating inhibitor of mineralization, have increased coronary artery calcification, and fetuin A can inhibit mineralization of vascular smooth muscle cells in vitro. Further understanding of the pathophysiology of uremic vascular calcification is needed to design effective therapeutic strategies to intervene with this devastating condition in patients with stage V chronic kidney disease.

Key words: vascular calcification, dialysis, core-binding factor α_1

Cardiovascular disease is the leading cause of death in patients undergoing dialysis.[1] In 1974, Lindner and colleagues reported accelerated atherosclerosis in maintenance hemodialysis patients,[2] suggesting that the dialysis process itself is responsible for the increased cardiovascular disease observed in chronic kidney disease (CKD) patients. Over the years, others have argued that the high cardiovascular mortality is a result of the underlying causes of renal failure, especially diabetes and hypertension.[3,4] However, the traditional Framingham risk factors do not completely account for this increasing mortality,[5] leading to the notion that there are dialysis-specific cardiovascular risk factors. Many dialysis-specific risk factors have been identified, although, at this time, demonstration that alteration of these risk factors reduces cardiovascular disease in CKD is lacking. In addition, there is now increasing evidence that disorders of mineral metabolism may play an important role in cardiovascular disease in CKD.

Pathologically, there are two patterns of vascular calcification. Typical atherogenesis is found in large vessel disease and coronary arteries and is associated with lipid-laden macrophages and intimal hyperplasia. Calcification is evident later in the disease course in grade IV and V lesions by Stary's classification.[6] Intravascular ultrasonography has demonstrated earlier circumferential calcification, but another form of calcification located only in the medial layer of the artery, Mönckeberg's calcification, has been observed for some time, especially in the distal vessels of diabetics and patients with CKD.[7] It is now clear that medial calcification is very common in multiple arteries in patients with CKD. In addition, another form of arterial calcification, calciphylaxis, is seen in CKD patients and more recently has been called "calcific uremic arteriolopathy" (CUA) to more accurately describe this rapidly progressive form of medial calcification.[8] Histologically, the calcification of the medial layer of CUA cannot be differentiated from the medial calcification of distal arteries of the leg described by Mönckeberg. Thus, calcification can

From the Department of Medicine (N.X.C., S.M.M.), Indiana University School of Medicine, and Department of Medicine (S.M.M.) Roudebush Veterans Affairs Medical Center, Indianapolis, IN.

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Address correspondence to: Dr. Neal X. Chen, Indiana University School of Medicine, 1001 West 10th Street, OPW 526, Indianapolis, IN 46202; e-mail: xuechen@iupui.edu.

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occur in both intimal lesions (atherosclerotic disease) and in the medial layer of all sizes of arteries. Although it was initially felt that the calcification represented deposition of calcium and phosphorus owing to serum supersaturation (which is obviously increased in CKD) and thus a passive deposition, recent evidence strongly supports the contention that this is an active, regulated process that appears to be accelerated in patients with CKD.

In 1979, Ibels and colleagues demonstrated that both the renal and the internal iliac arteries of patients undergoing renal transplantation had increased atherogenic or intimal disease and increased calcification compared with transplant donors.[9] In addition, the medial layer was thicker and more calcified in the uremic patients compared with the donors.[9] A more recent study evaluated coronary arteries obtained at autopsy in dialysis patients compared with age-matched, nondialysis patients who had died from a cardiac event.[10] This study found a similar magnitude of atherosclerotic disease (plaque area), but the lesions were more heavily calcified in dialysis patients. In addition, morphometry of the arteries demonstrated increased medial thickening.[10] Thus, there is histologic evidence for increased arterial calcification in patients on dialysis compared with nondialysis patients.

The gold standard diagnostic tool for atherosclerotic calcification is angiography as the plaque protrudes into the lumen, producing a filling defect. In contrast, medial calcification is not readily detectable by angiography as no filling defect is produced. However, both forms of calcification can be detected by imaging techniques such as electron beam computed tomography (EBCT), spiral computed tomography (CT), and ultrasonography. Although there is a distinct appearance to medial compared with intimal calcification on plain radiographs,[11] neither EBCT nor spiral CT can differentiate intimal from medial calcification.

Radiographic imaging studies have clearly demonstrated increased calcification in the arteries of patients with end-stage renal disease. Braun and colleagues demonstrated that coronary artery calcification by EBCT increased with advancing age in patients on dialysis and that the calcification scores were two- to fivefold greater in dialysis patients than in age-matched individuals with normal renal function and angiographically proven coronary artery disease.[12] Goodman and colleagues subsequently demonstrated that advanced calcification can also occur in the coronary arteries of children and young adults and found a relationship between increasing doses of calcium-containing phosphate binders, increased calcium × phosphorus product (Ca × P), and increasing calcification scores.[13] Moe and colleagues demonstrated that spiral CT with retrospective gating (described above) can also detect coronary artery calcification.[14] Several other authors have reported vascular calcification using these various techniques and have determined the risk factors associated with the presence or absence of calcification or the degree of calcification. The only risk factors that are uniform across studies are advancing age and the duration of dialysis. Mineral metabolism abnormalities, including elevated phosphorus, elevated Ca x P, or calcium load from phosphate binders, are frequently identified risk factors. Thus, these studies support the contention that the primary risk factors for vascular calcification of all types are the duration of dialysis and increasing age but that mineral metabolism and other factors may also be important.

What is the pathogenesis of vascular calcification, and do all of these forms of vascular calcification (intimal, medial, CUA) represent completely distinct processes or distinct initiating factors with subsequent calcification by similar mechanisms? And why is this so common in dialysis patients? To examine the pathophysiology of vascular calcification observed in dialysis patients, we examined arteries histologically.[15,16] We found expression of osteopontin at the base of the calcium spicules in skin arterioles with calcification from patients with CUA but no expression of osteopontin in arterioles without calcification in the same section.[15] Electron microscopy of CUA specimens revealed the presence of matrix vesicles identical to those observed in human bone.[15] Hyperphosphatemia and elevated calcium x phosphate product were associated with development of CUA,[15] emphasizing the role of phosphorus in uremic vascular calcification. We then prospectively evaluated inferior epigastric arteries obtained from stage V CKD patients at the time of renal transplantation. The degree of vascular calcification by both spiral CT and histologic stains for calcification was proportional to the expression of the bone matrix proteins osteopontin, bone sialoprotein, alkaline phosphatase, and type I collagen. Furthermore, the presence of positive immunostaining for these bone proteins was found more frequently than was overt calcification, which suggests that the deposition of these proteins precedes calcification.[16] Furthermore, we demonstrated the presence of a multinucleated, tartrate-resistant acid–phosphatase, osteoclast-like cell in an area of medial calcification, supporting the contention that not only bone formation but also perhaps bone resorption can occur in calcified arteries.[17] Thus, our ex vivo findings suggest

that the initial changes that occur in the vessels of dialysis patients are the deposition of these bone matrix proteins, followed by calcification. These results also confirm a cell-mediated process in vascular calcification in stage V CKD patients, similar to findings in the vessels of nondialysis patients with atherosclerotic coronary artery disease[18–20] and in the small distal vessels of patients with medial calcinosis.[7]

To further understand the mechanism by which this osteogenesis occurs in vascular calcification, we incubated bovine vascular smooth muscle cells (BVSMCs) in the presence of normal human pooled serum versus pooled human serum from hemodialysis patients on dialysis for at least 2 years (to eliminate residual renal function).[21] Using these pooled sera in vitro, we demonstrated that uremic serum led to increased and accelerated calcification in BVSMC vitro. Furthermore, the uremic serum up-regulated the expression of osteopontin in BVSMC compared with normal serum. Similar to the exogenous addition of phosphorus in the form of β-glycerophosphate, the uremic serum–induced osteopontin was dependent on both alkaline phosphatase and Na/Pi cotransport. However, in contrast to the effect observed with β-glycerophosphate alone, the induction of uremic serum–induced osteopontin was only partially blocked by inhibition of Na/Pi cotransport (foscarnet), indicating that the mechanism was not completely dependent on Na/Pi cotransport. Of importance, the final media concentration of phosphorus was similar in the BVSMC cultures, with 10% normal and 10% with uremic serum (≈0.5 mM),[21] well below levels known to induce calcification in the work by Jono and colleagues.[22]

Uremic serum, compared with control human serum, also up-regulated the expression of the osteoblast transcription factor core-binding factor α_1 (Cbfα_1) in BVSMCs in a time-dependent, non–phosphorus-mediated mechanism.[23] Confirming a role for Cbfα_1, we found ex vivo evidence of the expression of Cbfα_1 in vascular smooth muscle cells (VSMCs) adjacent to both medial and intimal calcification in inferior epigastric arteries obtained at the time of kidney transplantation.[23] Previous studies have demonstrated that exogenous phosphate added to human VSMC culture up-regulated Cbfα_1 expression,[24] a transcription factor critical for osteoblast differentiation and the expression of the bone matrix proteins osteopontin, osteocalcin, and type I collagen.[25] Cbfα_1 knockout mice fail to form mineralized bone, proving that Cbfα_1 is critical for the initial differentiation of osteoblasts,[26] thus supporting the contention that VSMCs dedifferentiate to osteoblast-like cells. In addition, arteries from the matrix Gla protein knockout mice lose smooth muscle markers and gain expression of Cbfα_1 as they progressively mineralize their arteries.[27] Furthermore, expression of Cbfα_1 has also been observed in calcification of the atherosclerotic plaques from patients without CKD.[28] Taken together, these results support the contention that Cbfα_1 may be a key regulatory factor in vascular calcification observed in dialysis patients.

Vascular calcification, although very prevalent in dialysis patients, is not uniform. Depending on the series, an average of 17% of dialysis patients have no vascular calcification and continue not to have calcification on follow-up.[29] Although younger age is partially responsible for the protection against calcification, the data also support the presence of naturally occurring inhibitors of calcification.

One potential inhibitor of vascular calcification is fetuin A (α_2- Heremans-Schmid glycoprotein), a circulating inhibitor. Fetuin A is abundant in the plasma and is mainly produced by liver in adults.[30] Fetuin A inhibits the de novo formation and precipitation of the apatite precursor mineral basic calcium phosphate but does not dissolve it once the basic calcium phosphate is formed.[31] Therefore, fetuin A can prevent undesirable calcification in the circulation without inhibiting bone mineralization. Fetuin A knockout mice have extraskeletal calcification in the presence of hypercalcemia or when crossbred on a mouse strain with a predisposition to calcification.[32] Fetuin A is inversely correlated with the acute phase response. A study by Ketteler and colleagues demonstrated that fetuin concentrations in the serum of dialysis patients were inversely related to C-reactive protein.[33] Furthermore, low fetuin A levels were associated with increased cardiovascular mortality. Serum from dialysis patients with calcific uremic arteriolopathy had impaired ex vivo capacity to inhibit hydroxyapatite precipitation, which could be normalized by the addition of purified fetuin A.[32]

We also recently demonstrated that the serum levels of fetuin A in individual dialysis patients were inversely correlated to coronary artery calcification by spiral CT scan.[34] In addition, we showed the presence of fetuin A expression by immunostaining of the inferior epigastric artery positively correlated with the presence of calcification. In vitro, fetuin A can inhibit both control and uremic serum–induced calcification in BVSMCs in a dose-dependent manner.[34] Thus, fetuin A may be an important protector and there may be a relative deficiency of fetuin A in most dialysis patients given the high serum concentration of calcium and phosphorus. The precise role of fetuin A and other inhibitors in the vascular calcification in CKD patients remains to

be determined, but, clearly, there are multiple mechanisms to regulate extraskeletal calcification. We are only beginning to understand this complex process.

In conclusion, we hypothesize that vascular calcification in dialysis patients may be a three-step process. First, VSMCs are stimulated by uremic toxins,[21,35] including phosphorus,[22] oxidized low-density lipoprotein,[36] and possibly aging or hypertension (leading to increased shear stress) to transform into osteoblast-like cells. Whether expression of Cbfα_1 is critical for the dedifferentiation or is simply a marker of dedifferentiation remains unknown. In step 2, these osteoblast-like cells in arteries lay down a bone matrix of type I collagen and noncollagenous proteins. The final step may be mineralization of this matrix through a process ''guided'' by the matrix proteins and osteoblast-like cells. This latter step is likely to be accelerated in the presence of elevated supersaturation of calcium x phosphorus product in the serum as well as excessive positive calcium balance owing to excessive calcium-containing phosphate binders.[11,13,37,38] These promineralizing forces are balanced by mineralization inhibitors such as circulating fetuin A[33,39] and locally produced matrix gla protein.[7,40,41] Therefore, deficiency of mineralization inhibitors, which may be a problem in the chronic inflammatory state of uremia,[32] could alter this balance toward calcification. Should our hypothesis prove true, gaining control of key regulatory factors in the pathogenesis of early stages of vascular calcification and augmentation of naturally occurring inhibitors offer the potential hope of developing specific therapeutic agents to arrest this process.

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