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Uremic Vasculopathy

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Recent evidence suggests that uremic vascular calcification is an active cell-mediated process resembling osteogenesis in bone rather than passive precipitation. We have 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 (Cbfa1) in histologic sections of inferior epigastric arteries obtained from patients with end-stage renal disease (ESRD) or calcific uremic arteriolopathy. In in vitro experiments, the addition of uremic serum to cultured vascular smooth muscle cells up-regulated osteopontin and Cbfa1 expression and accelerated mineralization. This implies that the uremic milieu may lead to dedifferentiation of vascular smooth muscle cells with subsequent mineralization. Further understanding of the pathophysiology of uremic vascular calcification is needed to design effective therapeutic strategies to intervene with this devastating condition in ESRD patients.
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Cardiovascular disease is the leading cause of death in patients undergoing dialysis.[1] In 1974, Lindner et al[2] reported accelerated atherosclerosis in maintenance hemodialysis patients, implying 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 rate is a result of the underlying causes of renal failure, especially diabetes and hypertension.[3] However, analysis of the recent hemodialysis (HEMO) trial showed that 15% of the cardiovascular disease observed in CKD cannot be explained by the traditional risk factors of diabetes, hypertension, family history, hyperlipidemia, and smoking,[4] 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 alteration in mineral metabolism also may play a role in cardiovascular disease in CKD.

The observation that calcification, or mineralization, can occur in arteries is not new. Work performed by Stary,[5] using monkey models of hypercholesterolemia and human autopsy specimens, noted that atherosclerotic lesions of the intima frequently were calcified, although this occurred very late in the progression of these lesions. Another form of calcification located only in the medial layer of the artery, Mönkebergs calcification, has been observed for some time, especially in the distal vessels of diabetics and patients with CKD.[6] In addition, another form of arterial calcification, calciphylaxis, also has been observed for many years. However, the initial pathologic lesion of calciphylaxis described by Selye et al[7] was calcification in the dermis of the skin, and not in the medial layer of small arteries of the skin, which is the pathologic lesion of calciphylaxis observed in CKD. Thus, Coates et al[8] coined the term *calcific uremic arteriolopathy* (CUA) to describe more accurately this rapidly progressive form of medial calcification. Histologically, the calcification of the medial layer of CUA cannot be differentiated from the medial calcification of distal arteries of the leg described by Mönkeberg. Thus, calcification can occur in both intimal lesions (atherosclerotic disease), and in the medial layer of all sizes of arteries. Because of the variety of locations of calcification in arteries, it initially was felt that the calcification represented deposition of calcium and phosphorus owing to serum supersaturation (which obviously is increased in renal failure), and thus a passive deposition. However, recent evidence supports that this is a complex regulated process that appears to be accelerated in patients with CKD.

Ibels et al[9] in 1979 showed that both renal and internal iliac arteries of patients undergoing a renal transplant had increased atherogenic/intimal disease and increased calcifica-

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tion (detected by chemical methods) compared with transplant donors. 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 calcified more heavily in dialysis patients. In addition, morphometry of the arteries showed increased medial thickening.[10] Although calcification in the medial layer did not appear to be increased, it also was not evaluated specifically (K. Amann, personal communication). Thus, there is histologic evidence for increased arterial calcification in patients on dialysis compared with nondialysis patients.

Clinically, arterial calcification can be detected by a number of techniques including visualization of plain radiographs, tomography, scintigraphy, and computed tomography (CT) scan. Most recently, electron beam CT scan (EBCT) was developed to evaluate coronary artery calcification, with sufficient speeds to avoid motion artifact from the heart. The scanner is linked to the electrocardiogram tracing (gating), and images are obtained only during diastole. Evolution of the spiral CT scanners also has allowed rapid acquisition of images. Two techniques for gating to electrocardiogram tracings exist for spiral CT, including retrospective and prospective. The latter is similar to EBCT, and retrospective is performed by taking images all through the cardiac cycle and then retrospectively using only the images in diastole. Although there is some 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 clearly have shown increased calcification in arteries in patients with end-stage renal disease (ESRD). Braun et al[12] showed that coronary artery calcification by EBCT increased with advancing age in patients on dialysis and that the calcification scores were 2- to 5-fold greater in dialysis patients than age-matched individuals with normal renal function and angiographically proven coronary artery disease.[12] Goodman et al[13] subsequently showed that advanced calcification also can 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. Guerin et al[14] confirmed a potential role for mineral metabolism, showing that vascular calcification by ultrasound progressively increased as the prescribed dose of calcium-containing phosphate binder increased. We subsequently showed that spiral CT with retrospective gating (described earlier) also can detect coronary artery calcification.[15]

Several other investigators 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 were uniform across studies were advancing age and duration of dialysis. Mineral metabolism abnormalities including increased phosphorus levels, increased Ca × P, or increased calcium load from phosphate binders frequently are identified as risk factors. However, the only randomized, controlled, prospective study to evaluate the potential role of mineral metabolism in vascular calcification is the Treat to Goal study.[16] In this study, dialysis patients were randomized to receive calcium-containing phosphate binders or the non–calcium-containing phosphate binder sevelamer (Renagel; Genzyme Therapeutics, Waltham, MA) to control serum phosphorus levels to a target of less than 5.5 mg/dL. The primary end-point was EBCT of the coronary arteries and aorta. The results showed that despite equivalent phosphorus levels in both arms, the calcification score of both the aorta and the coronary arteries was attenuated in the sevelamer-treated group but increased in the calcium binder–treated group. The serum calcium levels and the low-density lipoprotein (LDL) cholesterol levels were lower in the sevelamer group than the calcium-treated arm. In addition, the parathyroid hormone level was more suppressed in the calcium-treated arm. These results lend support to the hypothesis that calcium load is a determinant of coronary artery and aorta calcification, but the results also may be explained by the lower LDL cholesterol levels.

Finally, another form of vascular calcification, calciphylaxis, or calcific uremic arteriolopathy (CMA), is observed nearly exclusively in ESRD patients. In this disease there is medial calcification of the small arterioles of the dermis, leading to arteriole occlusion and skin necrosis. Risk factors identified in case control studies included female gender, white race, obesity, and increased serum phosphorus levels, but not increased serum parathyroid hormone or serum calcium levels.[17] Thus, these studies support that primary risk factors for vascular calcification of all types are duration of dialysis and increasing age, but that mineral metabolism, possibly lower LDL cholesterol, and other factors also may be important.

To date, there are no studies using EBCT showing adverse cardiac outcomes with coronary artery calcification in ESRD patients, although the degree of calcification in a cross-sectional study correlated with a history of cardiac events.[18] We have shown in a small prospective study that the calcification score by spiral CT was greater in patients who died or were hospitalized during a 15-month follow-up period compared with those who were never hospitalized.[19] Recently, London et al[11] evaluated a large cohort of dialysis patients with plain radiography of the pelvis and thigh and differentiated between intimal calcification and medial calcification. There was an increased mortality risk in patients with vascular calcification in an intimal pattern compared with a medial pattern, but in turn the mortality in patients with medial calcification still was greater than in patients with no calcification. Thus, there is increasing evidence that vascular calcification is indeed associated with morbidity and mortality, regardless of the imaging technique and regardless of the location.

What is the pathogenesis of vascular calcification and do all these forms of vascular calcification (intimal, medial, CUA) represent completely distinct processes, or distinct initiating factors with subsequent calcification by similar mech-

anisms? And why is this so common in dialysis patients? To examine the pathophysiology of vascular calcification observed in dialysis patients, we examined arteries histologically.[20,21] We have 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.[20] In addition, other bone proteins such as bone sialoprotein and alkaline phosphatase also were associated with calcification (Moe, unpublished observation). We then prospectively evaluated inferior epigastric arteries from patients with ESRD undergoing renal transplantation.[21] Many of these arteries showed calcification in the form of medial calcinosis in association with the expression of the bone matrix proteins osteopontin, alkaline phosphatase, type I collagen, and bone sialoprotein. 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. Furthermore, in vessels without histologic evidence of calcification but positive immunostaining for these bone matrix proteins, there was a deposition of purple-stain material by MacNeal's stain in the same location in the medial layer of the arteries.[21] In bone biopsy examinations using a MacNeal's stain, this purple staining correlated with deposition of osteoid, or unmineralized bone, composed of collagen and noncollagenous proteins. Finally, in one artery with calcification, a tartrate-resistant acid phosphatase–positive multinucleated cell (similar features to an osteoclast) was observed, suggesting active bone remodeling (Moe, personal observation). 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 ESRD patients, similar to findings in vessels of nondialysis patients with both atherosclerotic coronary artery disease[22-24] and small distal vessels in medial calcinosis.[6]

To further understand the mechanism by which this bone-like process 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).[25] By using these pooled sera in vitro, we showed that uremic serum led to increased and accelerated calcification in BVSMCs in vitro. Furthermore, the uremic serum up-regulated the expression of osteopontin in BVSMCs 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 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 those with 10% uremic serum (~0.5 mmol/L),[25] well below levels known to induce calcification in the work by Jono et al.[26]

We also have shown that uremic serum, compared with control human serum, induced the expression of Cbfa1 in BVSMCs in a time-dependent, non–phosphorus-mediated mechanism.[27] We also have found ex vivo evidence of the expression of Cbfa1 in VSMCs adjacent to both medial and intimal calcification in inferior epigastric arteries obtained at the time of kidney transplant.[27] Previous studies have shown that exogenous phosphate added to human VSMC culture up-regulated Cbfa1 expression,[28] a transcription factor critical for osteoblast differentiation and the expression of the bone matrix proteins osteopontin, osteocalcin, and type I collagen.[29] Cbfa1 knock-out mice fail to form mineralized bone, proving that Cbfa1 is critical for the initial differentiation of osteoblasts,[30] thus supporting that VSMCs dedifferentiate to osteoblast-like cells. In addition, arteries from the matrix gla protein knock-out mice lose smooth muscle markers and gain expression of Cbfa1 as they progressively mineralize their arteries.[31] Furthermore, expression of Cbfa1 also has been observed in calcification of atherosclerotic plaques from patients without CKD.[32] Taken together, these results support that Cbfa1 may be a key regulatory factor in vascular calcification observed in dialysis patients.

In conclusion, we hypothesize that vascular calcification in dialysis patients may be a 3-step process. First, VSMCs are stimulated by uremic toxins,[25,27] including phosphorus,[26] oxidized LDL,[33] and possibly aging, or hypertension (leading to increased shear stress), to transform into osteoblast-like cells. Whether expression of Cbfa1 is critical for the dedifferentiation or is simply a marker of dedifferentiation remains unknown. In step 2, these bone-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 increased supersaturation of calcium $\times$ phosphorus product in the serum as well as excessive positive calcium balance owing to excessive calcium-containing phosphate binders.[11,13,14,16] These promineralizing forces are balanced by mineralization inhibitors such as circulating fetuin-A[34,35] and locally produced matrix-gla protein.[6,36,37] Therefore, deficiency of mineralization inhibitors, which may be a problem in the chronic inflammatory state of uremia,[38] may be the final straw. 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, offers the potential hope of developing specific therapeutic agents to arrest this process. In the interim, continued efforts to optimize dialysis and normalize mineral homeostasis are of paramount importance.

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