Biology of Vascular Calcification in Renal Disease

Afshin Farzaneh-Far\textsuperscript{a} Catherine M. Shanahan\textsuperscript{b}

\textsuperscript{a}Division of Cardiology, New York Presbyterian Hospital, Cornell University Medical Center, New York, N.Y., USA; \textsuperscript{b}Division of Cardiovascular Medicine, University of Cambridge, Cambridge, UK

Introduction

The very high risk of cardiovascular death in patients with end-stage renal disease (ESRD) has become increasingly apparent over the last several years. Interest from both the cardiovascular and renal communities has been driven in response to the worldwide epidemic of diabetes and hypertension which has led to a huge increase in the number of patients with ESRD complicated by cardiovascular disease. Moreover, it appears that patients with chronic renal disease have inferior clinical outcomes following percutaneous coronary intervention and coronary artery bypass grafting independent of procedural success. Although conventional Framingham risk factors such as hypertension, diabetes mellitus, and dyslipidaemia are commonly associated with chronic renal disease, these risk factors alone do not fully explain the prevalence and adverse sequelae of cardiovascular disease in this population. As a result, interest has arisen in the identification of ‘novel’ factors in renal patients that may explain their increased risk.

One of these factors that have recently become the subject of intense investigation is vascular calcification. Indeed, the presence of calcium apatite crystals (similar to bone) in the arterial wall has been well described by pathologists. Although increased vascular calcification on plain X-rays was noted decades ago in the very early days...
of dialysis, it is only recently that its molecular and cellular mechanisms have begun to be understood. Extensive valve calcification is also seen in ESRD patients and may lead to accelerated valvular stenosis. However, this review will focus primarily on arterial vascular calcification. Vascular calcification occurs in both the intima and the media of arteries, and there is evidence that these two sites of calcification may be distinct entities. Intimal calcifications are seen as patchy scattered deposits only occurring within atherosclerotic plaques. In contrast, medial calcifications may occur independently of intimal calcification and atherosclerosis and are seen as continuous linear deposits associated with elastin fibers.

Recent refinements and increasing use of cardiac computed tomography (CT) techniques has allowed more precise quantitative assessments of calcification of the coronary arteries. Indeed, CT-measured coronary calcification scores are far higher in dialysis patients than in the general population. In one series, coronary calcification measured by computed tomography was present in 88% of patients with ESRD (between the ages of 20 and 30 years) compared to 5% of age-matched controls [1]. It is unclear from these studies how much of the detected coronary calcification is within the intima as compared to the media. However, one small postmortem pathological study in dialysis patients demonstrated greater intimal calcification of coronary plaques in these patients despite similar plaque areas and volumes to controls [2].

In general, greater amounts of CT-measured coronary calcification correlate well with overall plaque burden. However, for a given volume of plaque the effect of increased intimal calcification on plaque stability is unclear. In patients with acute coronary syndromes, intravascular ultrasound shows less calcification in the ‘culprit’ lesion than in stable plaques in other vessels [3, 4]. More recently, Ehara et al. [5] have shown that spotty calcification as seen by intravascular ultrasound typifies the culprit plaque in patients with acute coronary syndromes, whereas in chronic stable angina patients the culprit plaque tends to show larger and more extensive calcification. Thus, the relationship of intimal calcification to plaque stability is complex and may relate to both the pattern of distribution as well as the total amount of calcification.

A number of studies have suggested adverse hemodynamic consequences of calcification of large elastic arteries – particularly the aorta [6]. These effects appear to relate to increased arterial stiffness resulting in augmentation of aortic pulse wave velocity. Ejection of blood from the left ventricle during systole initiates an arterial pressure wave that travels toward the periphery. At points of impedance mismatch, chiefly at the high-resistance arteries, wave reflection occurs. Faster travelling pressure waves arrive at, and are reflected from, the peripheral circulation earlier. When arteries are relatively compliant and pulse wave velocity is relatively slow, reflected waves return to the central aorta in diastole, augmenting diastolic blood pressure and, therefore, coronary blood flow, which occurs predominantly during diastole. When arteries are stiffer and pulse wave velocity is higher, reflected waves arrive earlier and augment central systolic blood pressure, rather than diastolic blood pressure. This results in increasing left ventricular workload, left ventricular hypertrophy, increased myocardial oxygen demand and subendocardial ischemia. These effects appear to be proportional to the amount of calcification and are associated with increased mortality in ESRD patients.

Mechanisms

Calcium and Phosphate

Some studies have suggested that ESRD patients with higher serum calcium and phosphate concentrations as well as calcium-phosphate products have significantly increased vascular calcification. Moreover, recent work with sevelamer, a noncalcium-based phosphate binder, has shown significantly reduced vascular calcification within the coronary arteries and thoracic aorta after 1 year, as compared with patients on calcium-based phosphate binders [7]. The group treated with calcium-containing phosphate binders had significantly more episodes of hypercalcemia.

Parallel in vitro work appears to be consistent with these clinical observations. When human vascular smooth muscle cells (VSMCs) were exposed to elevated levels of inorganic phosphate, a calcium-phosphate precipitate was formed in association with the extracellular matrix. Elevated levels of extracellular calcium had similar effects. Moreover, when human VSMCs were incubated with solutions containing both elevated calcium and phosphate, the effect on calcification was synergistic [8, 9]. Interestingly this process was completely inhibited by phosphonoformic acid, an antagonist of the sodium-phosphate co-transporter (Pit-1), suggesting that calcification is not just a passive phenomenon, but an active cellular process, dependent on phosphate uptake [9]. There is evidence to suggest that increased intracellular phosphate may stimulate calcification through production of core-binding factor 1 (Cbfa-1) which is a pivotal

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transcriptional regulator of osteogenesis [8, 10]. VSMCs and osteoblasts are both derived from mesenchymal precursors and Cbfa-1 is thought to be the switch that turns on osteogenic differentiation.

**Matrix Vesicles**

Observations during bone development, as well as in human calcified aortas, have shown the presence of small (cell derived) membrane-bound remnants – matrix vesicles – that appear to act as initiation sites for apatite crystallization. We have previously shown that similar membrane-bound remnants (apoptotic bodies) were released by apoptotic VSMCs in vitro and appeared to act as nucleating structures for calcium crystal formation. Recently, we demonstrated that calcium and inorganic phosphate induced vascular smooth muscle cell death and apoptotic body release as well as matrix vesicle release from living cells [9]. Moreover, vesicles released by VSMCs after prolonged exposure to calcium and phosphate contained preformed calcium phosphate apatite and calcified extensively. However, vesicles released in the presence of serum did not contain apatite and calcified minimally [9]. These data emphasize the regulated nature of vascular calcification and the importance of matrix vesicles in this process. It is interesting to speculate that phagocytosis of matrix vesicles or apoptotic bodies may provide another level of control by removing the nidus of apatite crystal formation (fig. 1).

**Calcification Inhibitors**

When calcium and phosphate are mixed at concentrations found in human serum, apatite precipitation occurs and yet widespread calcification does not usually occur in vivo. This suggests the presence of natural inhibitors of calcification.

Matrix Gla protein (MGP) was one of the first inhibitors of vascular calcification identified. It is found in association with areas of vascular calcification and the MGP knockout mouse develops overwhelming medial calcification of the vascular tree [11]. Interestingly, it is also found within VSMC vesicles and pre-treatment with the MGP inhibitor warfarin is able to increase vesicle calcification, probably by reducing post-translational modification of vesicular MGP which is required for its activity [9]. Although high doses of warfarin can cause vascular calcification in rats, such effects on calcification in man have not been described with one possible exception. Warfarin use has been associated with some cases of calciphylaxis, a devastating form of vascular calcification occurring almost exclusively in ESRD resulting in extensive medial calcification of small arterioles, resulting in skin necrosis and often death from sepsis.

Elevated calcium concentrations can increase expression of MGP in VSMCs via a mechanism involving the
calcium sensing receptor, possibly as a defense against calcification [12]. This raises the intriguing possibility that calcimimetic drugs, that are agonists of the calcium-sensing receptor, may be able to inhibit vessel wall calcification by increasing MGP expression.

Fetuin-A is a recently described major inhibitor of calcification found in human serum. It has been shown to be a very potent inhibitor of apatite crystal formation in solutions containing calcium and phosphate. Furthermore, Fetuin-A knockout mice have a phenotype demonstrating extensive tissue calcification. In humans, serum from dialysis patients has lower levels of Fetuin-A and is less efficient at inhibiting apatite crystal formation – an effect that is reversed by addition of Fetuin-A [13]. It will be important to determine whether serum levels of Fetuin-A are inversely correlated to CT measured coronary calcium scores in a larger group of patients. Interestingly, we have shown that Fetuin-A is also found in VSMC vesicles similar to MGP [10].

Pyrophosphate is another recently described inhibitor of vascular calcification. Observations on incubated rat aortic rings have shown that no calcification occurred in normal vessels despite elevated free calcium and phosphate concentrations, but mechanical injury resulted in extensive medial calcification [14]. The inhibitor was identified as pyrophosphate on the basis of the inhibition of calcification in injured aortas by pyrophosphate, and the production of inhibitory levels of pyrophosphate by normal aortas. Moreover, mutations in the cell surface enzyme ecto-nucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) that generates pyrophosphate cause infantile idiopathic arterial calcification, a condition characterized by calcification of the internal elastic lamina of muscular arteries with subsequent occlusive intimal proliferation usually leading to death within the first 6 months [15]. Pyrophosphate may also be present in VSMC-derived vesicles and serve an inhibitory function analogous to MGP and Fetuin-A [10].

Stimulators of Vascular Calcification in Uremic Serum

Several studies have suggested the presence of other, as yet unidentified, pro-calcific factors in the serum of patients with ESRD. For example, bovine VSMCs incubated in the presence of uremic serum upregulated the osteoblast transcription factor Cbfa1, compared with cells incubated in control serum [16]. Moreover, uremic serum increased and accelerated calcification in bovine VSMCs in vitro, compared with control serum [10]. In addition, the effects of uremic serum on Cbfa1 expression were only partially inhibited by blocking the sodium-phosphate co-transporter (Pit-1) [10]. This suggests that other factors in uremic serum (in addition to hyperphosphatemia) stimulate the development of vascular calcification. Although the identity of these factors remains unclear, one interesting candidate is bone morphogenic protein-2 (BMP-2). BMP-2 is a potent osteogenic differentiation factor in mesenchymal stem cells and the expression of BMP-2 has been detected in human calcified arteries. In a preliminary study the concentrations of BMP-2 in pooled uremic serum was twice that found in normal human serum suggesting a possible role in the calcification process [17]. However, further work is required to demonstrate a causative link between serum BMP-2 levels and uremic vascular calcification.

Serum lipids are another possible stimulator of vascular calcification. Uremic patients often have elevated levels of low-density lipoprotein cholesterol (LDL). Furthermore, uremic stress results in increased concentrations of oxidized LDL, a highly reactive and atherogenic species. We have shown that acetylated LDL stimulates calcification by enhancing osteogenic differentiation of VSMCs in vitro [18]. This effect was inhibited by blockade of the scavenger receptor SRA1. Moreover, acetylated LDL did not increase apoptosis despite an increase in the number of apoptotic bodies implying a defect in apoptotic body phagocytosis. These observations are consistent with the possibility that acetylated LDL competes with apoptotic bodies for SRA1 binding. In contrast, epidemiological studies on the association of hyperlipidemia with uremic vascular calcification have been inconsistent to date. Nevertheless, slowing of atherosclerosis progression by statins has been shown in non-uremic patients along with reduction in the rate of progression of CT measured coronary artery calcification. Interestingly, the non-calcium-containing phosphate binder, sevelamer, is also a bile acid sequestrant with LDL-lowering properties. Thus, it is possible that the slowing of progression of coronary artery calcification with sevelamer was at least partly related to its lipid-lowering properties [7]. Further randomized trials looking at the effects of statins and other lipid-lowering drugs on uremic vascular calcification are therefore eagerly awaited.

Conclusions

Over the last few years, a large number of cell biological and clinical studies have provided important insights into the mechanisms of vascular calcification. Significant
ly, they have shown that vascular calcification is a regulated, cell-mediated process, not just a passive precipitation of calcium and phosphate onto the extracellular matrix. This suggests that it may be possible to regulate the vascular calcification seen in renal disease that could have an impact on the extraordinarily high cardiovascular mortality and morbidity in these patients. A number of stimuli in uremic patients including hypercalcemia, hyperphosphatemia and vascular injury cause vesicle (or apoptotic body) release and osteo-/chondrocytic conversion of VSMCs. Reduced levels of calcification inhibitors including MGP and Fetuin-A allow the calcification process to proceed with the matrix vesicles/apoptotic bodies acting as niduses for apatite crystal formation. Phagocytosis of matrix vesicles/apoptotic bodies may be reduced at least partly by elevated levels of LDL cholesterol that compete for uptake by VSMC scavenger receptors. Factors that affect any one of these processes are likely to effect vascular calcification. In ESRD, the balance of pro-calcific factors vs. calcification-protecting mechanisms is heavily tipped towards the pro-calcific factors. However, the complexity of the calcification process means that no single factor is responsible for the excessive vascular calcification seen in this patient group.

Recent advances in the understanding of the biology of vascular calcification have raised the possibility of novel therapies aimed at directly reducing arterial calcification. The phosphate binder, sevelamer, has been shown to reduce coronary artery calcification [7]. However, it remains to be seen how much of this effect is due to its lipid-lowering properties and whether it leads to improved cardiac outcomes. Other possible therapies for the future include calcimimetics, vitamin D analogues and bisphosphonates. The complex biomechanical interaction of calcium crystals within the atherosclerotic plaque means that reducing plaque calcification may not have a linear relationship with plaque stability. Therefore, it is imperative that any anticalcification therapy be subject to randomized trials with hard cardiovascular endpoints rather than surrogate markers such as CT measured coronary artery calcium scores.

In the meantime it is important to remember that a large component of cardiovascular risk in ESRD patients is explained by conventional risk factors such as hypertension, diabetes mellitus, and dyslipidaemia. Therefore, aggressive treatment of these risk factors with diet, exercise, weight reduction, and drug treatment should continue to be pursued.

References