Pathologic calcification of cardiovascular structures, or vascular calcification, is associated with a number of diseases including ESRD and cardiovascular disease. Calcium phosphate deposition, in the form of bioapatite, is the hallmark of vascular calcification and can occur in the blood vessels, myocardium, and cardiac valves. In blood vessels, calcified deposits are found in distinct layers of the blood vessel and are related to underlying pathology. Intimal calcification occurs in atherosclerotic lesions (1,2), whereas medial calcification (also known as Monckeberg’s medial sclerosis) is associated with vascular stiffness and arteriosclerosis observed with age, diabetes, and ESRD (3,4). Intimal calcification may occur independently of medial calcification and vice versa. In patients with ESRD, a mixture of intimal and medial calcification has been observed in affected vessels (5,6).

Clinical Consequences of Vascular Calcification

Vascular calcification can lead to devastating organ dysfunction depending on its extent and the organ affected. In the heart, calcification of cardiac valve leaflets is recognized as a major mode of failure of native as well as bioprosthetic valves (7,8). In dialysis patients, vascular medial calcification is responsible for calcific uremic arteriolopathy, a necrotizing skin condition associated with extremely high mortality rates (9). Finally, a genetic deficiency in pyrophosphate levels causes idiopathic infantile arterial calcification, a disease characterized by arterial calcification, fibrosis, and stenosis that leads to premature death in affected neonates (10).

In contrast, calcification of blood vessels commonly seen with aging, ESRD, diabetes, and atherosclerosis has historically been considered a benign finding. However, the introduction of new techniques to measure vascular calcification noninvasively, such as electron beam computed tomography, have revolutionized our current thinking about the risks of vascular calcification. In coronary arteries, calcification is positively correlated with atherosclerotic plaque burden (11,12), increased risk of myocardial infarction (13–15), and plaque instability (2,16). Although some of these findings may relate to the correlation of coronary calcification with extent of underlying atherosclerotic disease, it is also possible that vascular calcification itself may contribute to initiation or progression of cardiovascular disease (CVD). This possibility seems particularly plausible in the case of coronary calcification associated with ESRD (see below). Finally, vascular calcification, especially that found in the media of large arteries, leads to increased stiffness and therefore decreased compliance of these vessels. The consequent loss of the important cushioning function of these arteries is associated with increased arterial pulse wave velocity and pulse pressure, and leads to impaired arterial distensibility, increased afterload favoring left ventricular hypertrophy, and compromised coronary perfusion (17,18). Indeed, medial arterial calcification is strongly correlated with coronary artery disease and future cardiovascular events in patients with type 1 (19,20) and is a strong prognostic marker of CVD mortality in patients with ESRD (21). Thus, vascular calcification has a profound influence on cardiovascular function and health.

Cardiovascular Calcification and CVD Mortality in ESRD

More than half the deaths in patients with ESRD are due to CVD. In fact, the risk of CVD mortality in adult patients with ESRD is 20 to 30 times higher than that of the general popu-
lution (22). Growing evidence suggests that this increased risk of CVD mortality may be partly explained by the predisposition of this population to vascular calcification. Hyperphosphatemia and elevated Ca × P ion product (Ca × P; prevalent in patients with ESRD) promote vascular calcification, and are significantly linked to all cause and CVD mortality in patients with ESRD (23). In a landmark study, Goodman et al. (24) found that coronary artery calcification occurred in young patients with ESRD decades before this pathology was observed in the normal population. Furthermore, progression of vascular calcification in this group was positively correlated with serum P levels, Ca × P, and daily intake of Ca (24).

Similar findings were observed by Eifinger et al. (25) as well as Oh et al. (26) in young adults with childhood-onset ESRD. In addition, Raggi et al. (27) found that coronary artery calcification was very common and severe in adult hemodialysis patients, and significantly correlated with ischemic CVD. Again, calcification was highly correlated with elevated serum Ca and P levels. Finally, arterial medial calcification, a strong prognostic marker for CVD mortality in patients with ESRD, was also associated with elevated serum Ca, P, Ca × P, and prescribed Ca intake (18,21). Thus derangements in Ca and P balance are now considered major nontraditional risk factors for CVD in ESRD.

**Mechanisms of Vascular Calcification**

Vascular calcification is currently considered an actively regulated process that may arise by several different, nonmutually exclusive mechanisms (28). As shown in Figure 1, four different mechanisms for initiating vascular calcification have been proposed. First, human and mouse genetic findings have determined that blood vessels normally express inhibitors of mineralization, such as pyrophosphate and matrix gla protein, respectively, and that lack of these molecules (“loss of inhibition”) leads to spontaneous vascular calcification and increased mortality (10,29). Likewise, fetuin/a2-HS-glycoprotein is a major inhibitor of apatite found in the circulation, and decreased fetuin levels have recently been correlated with elevated CVD mortality in hemodialysis patients (30). Second, the presence of bone proteins such as osteopontin (31), osteocalcin (32), and BMP2 (33), matrix vesicles (34), and outright bone and cartilage formation in calcified vascular lesions (1,35) has suggested that osteogenic mechanisms may also play a role in vascular calcification. Indeed, cells derived from the vascular media undergo bone- and cartilage-like phenotypic change and calcification in vitro under various conditions, and is discussed in further detail below (36–40). Third, bone turnover leading to release of circulating nucleational complexes has been proposed to explain the link between vascular calcification and osteoporosis in postmenopausal women (41–43). Fourth, cell death can provide phospholipid-rich membranous debris and apoptotic bodies that may serve to nucleate apatite, especially in diseases where necrosis and apoptosis are prevalent, such as atherosclerosis (34,44,45).

Finally, via thermodynamic mechanisms (sometimes referred to as “passive” mechanisms), elevated Ca, P, and Ca × P promote apatite nucleation and crystal growth and would be expected to exacerbate vascular calcification initiated by any of the other mechanisms described above. Furthermore, new evidence suggests that Ca and P may additionally have direct effects on vascular cells that predispose to mineralization, as described below.

**Roles of Ca and P in Vascular Smooth Muscle Cell Mineralization**

As indicated above, elevated serum P, Ca, and increased Ca burden are correlated with vascular calcification and cardiovascular mortality in patients with ESRD. To determine whether elevated Ca or P directly affect cell-mediated regulation of vascular calcification, we and others have made use of vascular smooth muscle cell culture systems. Increasing inorganic P in the culture media to those seen in hyperphosphatemia (>2.4 mM) leads to deposition of apatite into the extracellular matrix surroundings the cells (37,46–48). Concomitant with mineralization, the cells undergo a phenotypic change characterized by loss of smooth muscle specific gene expression and upregulation of genes commonly associated with bone differentiation including osteocalcin, osteopontin and Runx2. Similar phenotypic changes have also been observed in vivo in human as well as animal models of vascular calcification (46,49,50). Elevated P-induced phenotypic transition and mineralization were shown to be dependent on a sodium-dependent phosphate cotransporter, Pit-1, on the basis of their ability to be inhibited by phosphonoformic acid (37) and Pit-1 specific small interfering RNA (Giacchelli and Li, unpublished data). These data confirm the importance of inorganic P as a signaling molecule with the ability to initiate both phenotypic change and mineralization in vascular smooth muscle cells.

Likewise, elevating Ca levels in the culture media to levels considered hypercalcemic (>2.6 mM) leads to enhanced mineralization and phenotypic transition of vascular smooth muscle cells (51). Elevated calcium-induced mineralization was also dependent on the function of a sodium-dependent phosphate cotransporter. Although elevated Ca did not appear to increase P uptake acutely, prolonged exposure of smooth muscle cell cultures to elevated Ca induced Pit-1 mRNA levels, suggesting that elevated Ca regulates P sensitivity of vascular smooth muscle cells. These findings were recently confirmed and extended by Proudfoot et al. (52). In those studies, elevated Ca (1.8 to 5.0 mM) stimulated human smooth muscle cell calcification in vitro. Furthermore, elevated Ca levels stimulated release of mineralization-competent matrix vesicles from human smooth muscle cells, and diltiazem and BAPTA, both inhibitors of intracellular Ca influx, blocked smooth muscle cell mineralization. A rise in intracellular Ca was also associated with altered alkaline phosphatase, decreased matrix GlA protein (MGP), and increased fetuin levels. Together with our own, these findings confirm that elevated Ca has pro-mineralizing effects beyond simply raising the Ca × P, and regulates multiple systems in smooth muscle cells that promote susceptibility to matrix mineralization. A diagram summarizing the possible roles of elevated Ca and P on vascular smooth muscle calcification is shown in Figure 2.
Can Vascular Calcification Be Controlled?

Several recent studies suggest that vascular calcification may be slowed, and potentially even reversed, in humans as well as experimental animal models. In light of the findings that elevated serum P and Ca are strongly correlated with vascular calcification and CVD mortality in ESRD, an emphasis has been placed on the use of non–Ca-containing P binders, such as sevelamer, to treat hyperphosphatemia in these patients.

Figure 1. Schematic illustrating four, non–mutually exclusive theories for vascular calcification: (1) loss of inhibition as a result of deficiency of constitutively expressed tissue-derived and circulating mineralization inhibitors leads to default apatite deposition, (2) induction of bone formation resulting from altered differentiation of vascular smooth muscle or stem cells (3), circulating nucleational complexes released from actively remodeling bone, and (4) cell death leading to release of apoptotic bodies and/or necrotic debris that may serve to nucleate apatite at sites of injury. Figure reprinted from (28) with permission from Elsevier.

Figure 2. Proposed model for the effects of elevated Ca and P on vascular smooth muscle cell (SMC) matrix mineralization. Elevated Ca and P are proposed to stimulate vascular matrix mineralization in two ways. First, both Ca and P increase the activity of Pit-1: elevated P stimulates P uptake via Pit-1, and elevated Ca induces expression of Pit-1 mRNA. Both mechanisms are proposed to enhance P uptake into SMC as well as matrix vesicles. Elevated intracellular P than leads to SMC phenotypic modulation, which includes upregulation of osteogenic genes (Runx2, osteocalcin, and alkaline phosphatase), and generation of a mineralization-competent extracellular matrix. In addition, increased Pit-1 in matrix vesicles promotes P loading of matrix vesicles, promoting nucleation of mineral within the extracellular matrix. Second, elevated Ca and/or P lead to increased Ca × P ion product, thereby promoting growth of apatite crystals in the matrix via thermodynamic mechanisms.

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(53). Indeed, when sevelamer was compared to commonly used Ca-based P binders in a large hemodialysis patient group it was found that patients receiving sevelamer had unchanged median coronary artery and aorta calcification scores after 1 yr as opposed to Ca-treated patients whose arterial calcification scores increased 28% over baseline (12). Significantly, although both treatments controlled P levels equivalently, treatment with Ca containing binders led to an increased frequency of hypercalcemic episodes and greater suppression of serum parathyroid hormone (PTH) levels in hemodialysis patients (54,55). Similar effects of sevelamer were also noted in a rat uremia model, where renal calcification was greatly reduced compared to Ca carbonate treatment (56). Thus, decreasing Ca and P burdens appear to be beneficial in blocking vascular calcification.

Antihypertensive agents have also been implicated in control of vascular calcification. In a clinical study, the Ca channel blocker nifedipine slowed progression of coronary calcification in hypertensive patients compared to diuretics (57). Moreover, Moreau’s group has developed a novel animal model of isolated systolic hypertension that is caused by arterial calcification (58,59). In this model, rats are treated with warfarin and vitamin K and this leads to calcification of the aortic wall and isolated systolic hypertension. Treatment with an endothelin (ET-1) receptor antagonist or angiotensin II blocker increases in pulse pressure as well as calcification of the vessels. Remarkably, treatment of rats with ET-1 antagonist after calcification was established caused regression of vascular calcification and normalization of pulse pressure. These data support the idea that calcification of compliance vessels leads to hypertensive effects, especially increased pulse pressure as a result of increased stiffness, and identify ET-1 antagonists as major regulators of vascular calcification. Furthermore, these data suggest that regression of vascular calcification can occur and is actively regulated. Indeed, evidence for regression of coronary calcification in humans has also been obtained. In a study of 102 asymptomatic patients with coronary calcification currently being treated with standard medications, 15% of patients showed evidence of regression on the basis of electron beam computed tomography calcium scores measured at baseline and approximately 6 mo later (60).

Conclusions

Vascular calcification is highly correlated with CVD morbidity and mortality, especially in high-risk populations such patients with ESRD or diabetes. Four non–mutually exclusive mechanisms for vascular calcification are emerging, including (1) loss of inhibition, (2) induction of bone formation, (3) circulating nucleational complexes, and (4) cell death. Disruption in Ca and P metabolism effect all of these mechanisms by elevating the $Ca \times P$, and also by direct effects on smooth muscle cells that promote bonelike differentiation. The challenge remains to understand which mechanisms are active and/or predominate under various disease states, and to develop effective therapeutic strategies that may prevent and potentially reverse vascular calcification.

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References


