Role of Molecular Regulation in Vascular Calcification

Farhad Parhami, Kristina Boström, Karol Watson, and Linda L. Demer

Departments of Medicine and Physiology, UCLA School of Medicine, Los Angeles, California

Calcium deposits account for most of the dry weight of atherosclerotic lesions. Previously considered uncommon, vascular calcification is now known to be present in 80% of significant lesions and in at least 90% of patients with coronary artery disease. Previously considered a passive process, it is increasingly recognized as an active, regulated process. Previously considered benign, it is now becoming recognized as a major risk factor for cardiovascular events, and a major contributor to systolic hypertension, heart failure, plaque rupture and stenosis. To confirm the similarity of vascular calcification with embryonic osteogenesis, we demonstrated the expression of bone morphogenetic protein in calcified human lesions, and we developed an in vitro model of vascular calcification that provides a useful experimental system for elucidating the molecular regulation of this process, which we have shown to include alkaline phosphatase induction and expression of bone matrix proteins and differentiation factors. Understanding the regulatory mechanisms of vascular calcification will allow future therapeutic approaches to prevent and possibly reverse this disease and its clinical consequences. J Atheroscler Thromb, 1996; 3: 90-94.

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Background

Calcification in the coronary arteries has been widely regarded as an uncommon, end-stage, insignificant, passive, degenerative process of aging—a notion that has paralyzed research in this area for decades. Interestingly, these same terms were once used to describe atherosclerosis. Three features of vascular calcification should have been clues that it is an active, regulated process similar to bone formation. (A) It occurs in the absence of hypercalcemia, unlike metastatic calcification. (B) The crystals are in the form of bone mineral apatite (1-3), rather than amorphous calcium phosphate, and (C) the deposits often have the architecture of mature bone tissue, including vascularized marrow with adipocytes, cartilage and evidence of remodeling, leading the early pathologists to label the process ossification rather than calcification (4-7). The origins of bone-forming cells, marrow stromal cells, adipocytes and osteoclast-like cells in the artery wall remain unknown. Possibilities include that they are derived from (A) blood-borne cells that migrate into the artery wall, (B) pericytes accompanying angiogenesis from the vasa vasorum in the adventitial layer, (C) differentiation of immature, pluripotent—perhaps embryonic remnant—cells in the artery wall, and/or (D) dedifferentiation of existing artery wall cells. The association of vascular calcification with cell death and degeneration should no longer imply lack of regulation now that it is recognized that apoptosis and other regulated mechanisms of necrosis are normal elements of embryonic osteogenesis (8).

Clinical Significance

Electron beam computed tomography (EBCT) and intravascular ultrasound imaging of the coronary arteries have revealed that vascular calcification is not limited to the most advanced lesions but begins as early as the second and third decades of life (9) before coronary narrowing. The relatively early onset of calcification, preceding impingement of lumen diameter, led to high
sensitivity but low specificity in identifying coronary stenoses. Thus, although EBCT may accurately identify atherosclerosis in the coronary artery wall, it may not correlate with stenosis severity so that, ironically, the impression has developed that EBCT is "inaccurate" for diagnosing "coronary artery disease". Raising the threshold criteria for positivity may improve the correlation with stenosis, but reduce the sensitivity for atherosclerosis. Most importantly, coronary calcification detected by EBCT has now been shown to be a major predictor of cardiac events (10).

Vascular calcification, not limited to coronary arteries, is associated with increased risk of myocardial infarction and cardiovascular mortality (11-14). The odds ratios of 1.5 for infarction and 1.6 for cardiovascular mortality remain significant even when adjusted for age, duration of diabetes, hypertension, smoking, total cholesterol, HDL cholesterol, fasting glucose, triglycerides and baseline cardiovascular disease (15). The basis for this relationship is not established, but it may relate to effects on calcification of the aorta, plaque rupture, and arrest of compensatory enlargement.

Calcification of the aorta, leading to loss of compliance, is an independent cardiovascular disease risk factor (16). It is the major factor in increased aortic rigidity and its adverse cardiovascular consequences (17-20) including ischemia (21), systolic hypertension (22, 23), left ventricular hypertrophy (24), heart failure (25-28) and stroke (29).

Coronary calcification is the single most important feature for predicting plaque dissection and its location (30-31). The same mechanical predisposition to breakage may apply in spontaneous plaque rupture and in balloon valvuloplasty, which would explain the increased cardiovascular risk in individuals with coronary calcification and the increased risk of complications in balloon valvuloplasty of calcified valves (32). Since calcification is now recognized to occur early and even precede stenosis, this concept is not inconsistent with evidence that most plaque ruptures leading to infarction represented mild stenoses. Narrowing of the atherosclerotic vessel lumen is counteracted initially by compensatory enlargement of the arterial circumference through remodeling (33). Eventually, for unknown reasons, this process fails, and stenosis occurs. Calcification may be one of the responsible factors (34).

**Similarities between Vascular and Bone Calcification**

Calcium deposits are found in intimal and/or medial layers of the artery wall, primarily, but not always in or near atherosclerotic plaques. Medial calcification not associated with atherosclerosis is known as Monckeberg's. Both types contain bone mineral, matrix vesicles and the bone regulatory protein osteopontin (35-39). When it takes on the architecture of fully-formed bone, which appears to require angiogenesis, ossification has many features of trabecular bone including trabeculae, marrow, adipocytes, resorption pits, osteocytes, bone lining cells, and matrix vesicles. The time course resembles that of endochondral ossification, a type of embryonic bone formation. Ossification frequently occurs at the base of lesions on the luminal side of the fragmented internal elastic lamina.

It is important to clarify that bone cells do not directly secrete mineral crystals; the crystals deposit extracellularly within the specialized matrix secreted by bone cells. Similarly, in the artery wall, the crystals are not found intracellularly, but in the matrix. Whether the cells producing the matrix that calcifies are classified as phenotypically modulated smooth muscle cells, fibroblasts, or as osteoblast-like cells is a distinction of questionable value since the main feature used to distinguish between these is the matrix they produce. The distinction is further blurred by the ability of these mesenchymal cells to shift their profiles of actin isoforms.

Evidence for ultrastructural similarity to bone had been reported by two groups (2, 40). To assess whether the calcium deposits in the artery wall form by a process similar to embryonic bone formation, Boström, in our laboratory, examined calcified human atherosclerotic lesions for expression of bone morphogenetic protein (BMP) by in situ hybridization (41). She found evidence for mRNA for BMP-2 in association with calcium deposits. Independent investigators subsequently showed evidence for the entire complement of bone matrix proteins in atherosclerotic lesions, including collagen I, osteocalcin, osteopontin, matrix gla protein, alkaline phosphatase and matrix vesicles. In bone, this matrix calcifies approximately 10 days after its secretion, at about 10 microns from the osteoblast. Some of these matrix proteins may give osteoid its capacity for mineralization despite low ionic concentrations of calcium and phosphate. Many of these factors are also found in calcified valves (42). Atherosclerotic matrix and bone matrix are also similar in their content of growth factors and cytokines, including transforming growth factor-β, bone morphogenetic protein, platelet derived growth factor, fibroblast growth factor, nitric oxide, interleukins (particularly 1,6, and 10), insulin-like growth factor, monocyte colony stimulating factor, monocyte chemotactic protein-1, tumor necrosis factor, and others. Thus, the matrix in atherosclerotic lesions is nearly identical to osteoid, and this may explain its capacity for mineralization.

**In Vitro Model of Vascular Calcification**

We observed that aortic smooth muscle cell cultures maintained for 2-3 weeks produce cellular aggregates or nodules similar to those produced by bone cell cultures (41), confirming related work of Björkerrud and Schor (43,
We went on to show that these nodules also form calcium deposits made of hydroxyapatite crystals. By subcloning, we were able to demonstrate that in vitro calcification was a feature of a subpopulation of cells from the aorta, which we call calcifying vascular cells (CVC). In recognition of the diversity of cells in the aortic media, we prefer to call the parent cultures aortic medial cells rather than smooth muscle cells. Of the approximately 100 clones obtained, approximately 20-30% produce nodules within 3 weeks. Shioi et al. made a significant advance in this in vitro model by demonstrating that the calcification produced by aortic medial cells could be accelerated by the addition of beta glycerocephosphate which provides substrate for the ectoenzyme alkaline phosphatase, yielding phosphate ions for crystal formation (45). This work opens the way for more detailed biochemical investigations.

Calcifying vascular cells resemble microvascular pericytes in growth kinetics, morphology and expression of actin isoforms and a surface ganglioside (46). This is especially important since pericytes are postulated to be immature mesenchymal progenitor cells (47) that undergo in vitro calcification (44) and have osteoblastic potential (48). Calcifying vascular cells are negative for markers of endothelial cells and leukocytes. CVC also have many features of osteoblasts, including expression of alkaline phosphatase, collagen I, osteopontin, osteonectin, and osteocalcin (46). Thus, CVC may be mesenchymal intimal cells partially committed to osteoblastic differentiation which further differentiate in culture.

Factors that Modulate Vascular Calcification

The close colocalization of atherosclerotic lesions and calcium deposits suggest that one induces the other or that a common factor induces both (49). Since cholesterol in plaque would be expected to undergo oxidation, Watson, in this laboratory, treated CVC with an oxidative derivative of cholesterol present in atherosclerotic plaque, 25-hydroxycholesterol, and found acceleration of in vitro calcification (46). She also found that it was accelerated by another factor present in atherosclerotic plaque, transforming growth factor-beta. In contrast, she observed that platelet-derived growth factor (PDGF) has a less positive effect, and interferon-gamma has no effect compared with vehicle alone. These results suggest that factors in atherosclerotic lesions, such as TGF-β1 and oxysterols may induce osteoblastic differentiation of artery wall cells.

Genetic Factors

In collaboration with Lusis, Qiao and colleagues, we have found evidence for genetic predisposition to vascular calcification (50, 51). A series of inbred mouse strains examined for vascular calcification by serial sectioning and H&E staining of the aortic root revealed significant variation among strains, and calcification was increased in the mice fed a high fat diet. The homozygous apoE knockout mouse had particularly high levels of lesion calcification. Cartilage-specific type 2 collagen, also showed genetic patterns.

Understanding the regulatory mechanisms of vascular calcification will allow future therapeutic approaches to prevent and possibly reverse this disease and its clinical consequences.

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Regulation of Vascular Calcification


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