Cholesterol in Vascular and Valvular Calcification

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In the early 1990s, the late Jeff Hoeg had the foresight to ask whether vascular calcification relates to the duration and severity of exposure to cholesterol. In a group of homozygous hyperlipidemic patients for whom detailed records of cholesterol levels were available over a long period of time, he and his colleagues found that coronary calcification scores by ultrafast computed tomographic scanning correlated significantly with the cholesterol year product. Studies that lack long-term cholesterol history may miss a correlation between lipids and calcification, because current treatments so dramatically change cholesterol levels as to make them unrepresentative of long-term exposure.

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In the present issue of Circulation, Pohle et al\textsuperscript{2} successfully overcame these difficulties by studying the rate of change in calcification as a function of change in lipid levels, excluding patients in whom treatment was altered during the study period. These investigators found that both coronary calcification and aortic valve calcification progress more rapidly in subjects with levels of LDL $>130$ mg/dL. This finding dovetails with those of Callister et al,\textsuperscript{3} who discovered that patients who successfully lowered their cholesterol levels with lipid-lowering agents significantly reduced the progression of coronary calcification. In a large population of young subjects, the risk factor that correlated most significantly with coronary calcification was LDL cholesterol.\textsuperscript{4} This relation was stronger than the correlation with age, smoking, fasting insulin, blood pressure, or male sex.\textsuperscript{4} In another study of asymptomatic outpatients, LDL cholesterol was the only risk factor correlating with progression of coronary calcification by electron-beam computed tomography over an 18-month period.\textsuperscript{5}

The correlation of LDL cholesterol exposure to valvular and vascular calcification raises the possibility of a mechanistic role, which is also supported by in vitro studies. Two groups showed that lipid elements in atherosclerotic plaque may serve as the nidus for mineralization, as they do in bone.\textsuperscript{6,7} In addition, in vitro studies have shown that the artery wall contains cells capable of osteoblastic differentiation and mineralization.\textsuperscript{8–10} Further studies have shown that osteogenesis in these cells is induced by oxidized lipids.\textsuperscript{11} Although the source of these cells has not been established, the presence of heterogeneous subpopulations in the media has long been proposed by Julie and Gordon Campbell and colleagues, and the possibility that calcifying valvular or vascular cells derive from circulating precursor cells is supported by their recent demonstration that marrow stromal-derived cells are present in atherosclerotic neointima.\textsuperscript{12}

Heart valve calcification has received less research attention than artery wall calcification. Textbooks have distinguished calcific and atherosclerotic aortic stenosis as 2 separate diseases. The calcific form has been attributed to degeneration or “wear-and-tear,” a factor that was thought to contribute to a variety of diseases until adequate research identified their true causes. Ongoing research now suggests that it is time to replace the old view on aortic stenosis.

Calcific aortic stenosis may simply represent a later stage or one end of the spectrum of atherosclerotic disease, as calcified plaque does in the artery wall. As with vascular calcification, calcification in cardiac valves is now known to have many features of bone formation. The degree and location of valvular calcification closely corresponds with mRNA expression of osteopontin, a protein regulating biomineralization.\textsuperscript{13} Feldman et al\textsuperscript{14} described ossification in human aortic valve specimens. In a more systematic examination of $\approx350$ human aortic valves removed for replacement surgery, Mohler et al\textsuperscript{15} found that most were calcified and that $\approx15\%$ contained fully-formed, lamellar or endochondral bone tissue with hematopoietic marrow and evidence of remodeling. Specimens containing bone tissue also showed expression of the potent osteogenic factors bone morphogenetic protein (BMP)-2 and BMP-4.

In vitro studies of cardiac valve cells have also revealed similarities with in vitro vascular calcification. Mohler’s group also harvested cells from the aortic valves of humans and dogs. They identified and characterized a subpopulation of mesenchymal cells from the interstitial cell population that were able to produce nodules containing hydroxyapatite-mineralized matrix in vitro. These nodules were essentially identical to the calcifying vascular cells that were previously isolated from the aortic media,\textsuperscript{7} and they were induced to mineralize by the same factors: TGF-\(\beta\), 25-hydroxycholesterol, and BMP-2.

A unique feature of cardiac valve tissue, the lack of conventional smooth muscle cells, provides a clue about the origin of calcifying valvular cells. Although some \(\alpha\)-smooth muscle actin has been observed in diseased valves, in general, only endothelial, fibroblastic, and interstitial cells are considered normal constituents. This would support the concept that calcifying valvular cells are not dedifferentiated or redifferentiated smooth muscle cells. Whatever the origin, whether embryonic remnant, blood-borne precursor, or local mesen-
chymal tissue, Mohler’s mesenchymal cells in valves may be the same cells as calcifying vascular cells in the artery wall.

As with artery wall calcification, lipids may also contribute to valvular calcification. Cholesterol concentration is significantly higher in patients with calcific aortic stenosis than in control subjects. In bioprosthetic valves, eliminating lipids from the biological matrix by ethanol extraction prevents in vivo calcification. Bioprosthetic valves raise an interesting question. They are considered free of viable cells after glutaraldehyde fixation. Nevertheless, they undergo mineralization, suggesting that valvular calcification may be cell-independent and passive. However, it is possible that the cell-produced matrix and/or its fixative-modified proteins have the proper physical characteristics to serve as crystallization foci. Thus, the regulated aspect of mineralization, cellular production of a mineral-competent matrix, may have already been completed. Another possibility is that cells migrate into or deposit on the bioprosthetic valves. Recent studies from a variety of laboratories indicate that circulating blood contains immature mesenchymal cells capable of differentiation into endothelial, smooth muscle, and other lineages, presumably from the marrow stromal cell population, much the same way as hematopoietic stem cells are present in peripheral blood and are capable of incorporating themselves into tissue. If cells can deposit themselves onto devitalized bioprosthetic valve tissue and deposit appropriate matrix, then cellular-regulated mineralization may take place.

One consequence of the fact that vascular calcification resembles osteogenesis deep within the artery wall is that infusions capable of dissolving the calcium mineral that forms within matrix in vascular tissue should be equally capable of dissolving the calcium mineral that forms within skeletal bone. Newly forming bone in the skeleton is located immediately adjacent to the sub endothelial space of the Haversian canals and marrow, only a few cell thicknesses from the blood lumen. Because atherosclerotic calcification is usually much deeper, skeletal calcification may be more likely to be removed by intravenous chelators than atherosclerotic calcification.

Some evidence raises concerns that warfarin has a role in cardiovascular calcification. The small protein matrix GLA protein (MGP) is thought to serve as an inhibitor of soft-tissue calcification, possibly through inhibitory interaction with BMP-2. GLA proteins, which include coagulation cascade factors, are unusual in having post-translational carboxylation of certain glutamic acid residues, a modification thought to be important for function. The carboxylase responsible for this modification depends on vitamin K. Because warfarin interferes with vitamin K–dependent enzymes, it is theoretically possible that warfarin treatment or dietary vitamin K deficiency may increase the risk of vascular calcification by reducing the mineral-inhibitory activity of MGP.

It has been suggested that calcification does not increase the risk of plaque rupture because it does not weaken the plaque’s resistance to hoop stresses. If anything, calcification should increase resistance to circumferential hoop stresses. However, instability is expected to be induced by calcium deposits through dramatically increased solid shear stresses along the sharp interface where the pulsating, compliant soft tissue meets mineral. This interface is the known site of plaque rupture during balloon angioplasty.

With aging, soft tissues harden and hard tissues soften. One explanation may be that both processes are stages of last resort in chronic inflammatory responses to various stimuli, including oxidized lipids or autoimmune phenomena. Chronic inflammation may have evolved from mechanisms protecting against microorganisms and even macroorganisms such as helminths. The last resort of immune defense in soft tissue is to wall off the invader with a bone barrier. The amorphous calcium mineral may simply be the first stage in osteogenesis. In hard tissue infection, the anchorage-dependent microorganisms thrive in mineral matrix, and the defense of last resort is to eliminate that anchorage by dissolving the mineral matrix. This process is known as inflammatory osteolysis.

It is optimistic to conclude, on the basis of this study’s correlation, that cholesterol lowering may prevent or reverse valvular calcification. Drawing treatment conclusions from correlations may oblige us to send coronary patients to plastic surgeons for the correction of ear lobe creases. However, the findings of Pohle et al² warrant aggressive investigation of a causative relation between cholesterol and cardiovascular calcification.

References


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