Mechanisms of Aortic Valve Calcification

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The aortic valve is a supple membrane that opens and closes ~100,000 times a day. To remain pliable, the aortic valve must undergo continuous repair. Accumulation of calcium and sclerotic tissue in a valve may lead to decreased pliability and ultimately to stenosis. In the early 1900s, eminent pathologists, such as Monckeberg,[1] described aortic valve calcific disease as a passive process that is associated with rheumatic fever or aging. Over the past several decades, the etiology of aortic valve stenosis has changed considerably. The decline in the prevalence of rheumatic heart disease and increased longevity in industrialized countries has resulted in a pattern shift from rheumatic aortic stenosis to degenerative calcification as the most common cause of aortic valve stenosis.[2] The bicuspid aortic valve, a congenital anomaly with male predominance that occurs in ~1% of the population, is the second most common etiology associated with aortic valve stenosis.[3,4]

There are many terms used to describe calcification of valves as opposed to calcification of the skeleton, including ectopic calcification, dystrophic calcification, and metastatic calcification. Ectopic calcification (defined in this review as accumulation of calcium in nonskeletal sites) occurs when calcium and inorganic phosphate ions that had been soluble in extracellular fluid crystallize to form hydroxyapatite, the same crystal that comprises much of bone. The nidus for crystallization is a matrix vesicle that is derived from the plasma membrane of dying cells, suggesting a senile pathologic mechanism.[5,6] However, a relative minority of elderly patients accumulate calcium in the aortic valve cusps, suggesting pathologic influences other than age play a role. The pathologic mechanisms leading to the development of aortic valve stenosis were traditionally believed to be due to passive accumulation of hydroxyapatite mineral in the setting of sclerosis, but more recently have been understood to involve active processes similar to those that occur in atherosclerotic arteries, including inflammation and lipid infiltration.[7] The data concerning the pathologic processes of ectopic aortic valve calcification are reviewed and a working hypothesis is generated to indicate that calcific aortic valve stenosis involves an atherosclerotic process (Figure 1). This review also includes a description of risk factors involved in progression of aortic valve calcification and novel therapeutic strategies for prevention of disease progression. Rheologic processes are also important in aortic valve disease, but are mentioned only briefly in this review.

From the Cardiovascular Division, Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania. This work was supported in part by grant KO8 HL03974-01 from the National Institutes of Health, Bethesda, Maryland. Manuscript received June 23, 2004; revised manuscript received and accepted August 2, 2004.

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ENDOTHELIAL DISEASE

The normal aortic valve leaflet is composed of a single layer of endothelial cells that envelopes a spongiosa layer made up of loosely organized connective tissue on the aortic side and a ventricularis layer that contains elastin on the ventricular side. Similar to atherosclerotic disease in arteries, an early event in valve disease appears to be endothelial injury (Figure 1). The endothelial cell layer of calcified aortic valves appears damaged on electron microscopy (Figure 2), especially at sites of prominent lesions that frequently develop in the spongiosa layer on the aortic side of the valve.[8–10] Development of an asymmetric lesion indicates that systemic pressure and abnormal blood rheology (as seen with congenital bicuspid aortic valves) predisposes to sclerosis and calcium accumulation.[11] This developmental pattern of a valve lesion in an area of abnormal blood rheology is similar to that reported for atherosclerotic arteries, where lesions frequently occur at branch points.

ATHEROSCLEROTIC RISK FACTORS AND AORTIC VALVE CALCIFICATION

Traditional atherosclerotic risk factors, such as total cholesterol, increased low-density lipoprotein cholesterol, increased lipoprotein(a), increased triglycerides, decreased high-density lipoprotein cholesterol, male gender, cigarette smoking, hypertension, and diabetes are reported to increase the incidence of aortic stenosis and likely contribute to endothelial dysfunction and leaflet damage.[7,12–18] Although age and hypertension are associated with aortic valve calcification in most of these studies, dyslipidemia and diabetes mellitus are weakly[15] or not associated[18,19] with aortic valve calcification. Two limitations of these population-based studies were that a full lipid profile was not reported in some, and there were relatively few patients who had diabetes. However, the overall evidence indicated by the presence of atherosclerotic risk factors may partly explain why some patients who have congenitally abnormal valves develop aortic stenosis and require valve replacement sooner than others without risk factors. Metabolic bone diseases, which are characterized by increased bone remodeling rates and include Paget's disease, secondary hyperparathyroidism, and renal disease, and increased serum creatinine and calcium are also linked to progression of valve calcification but include only a relative minority of patients who have aortic stenosis (Table 1).[20–22]

INFLAMMATION

A manifestation of endothelial dysfunction is decreased availability of nitric oxide and prostacyclin, 2

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doi:10.1016/j.amjcard.2004.08.013

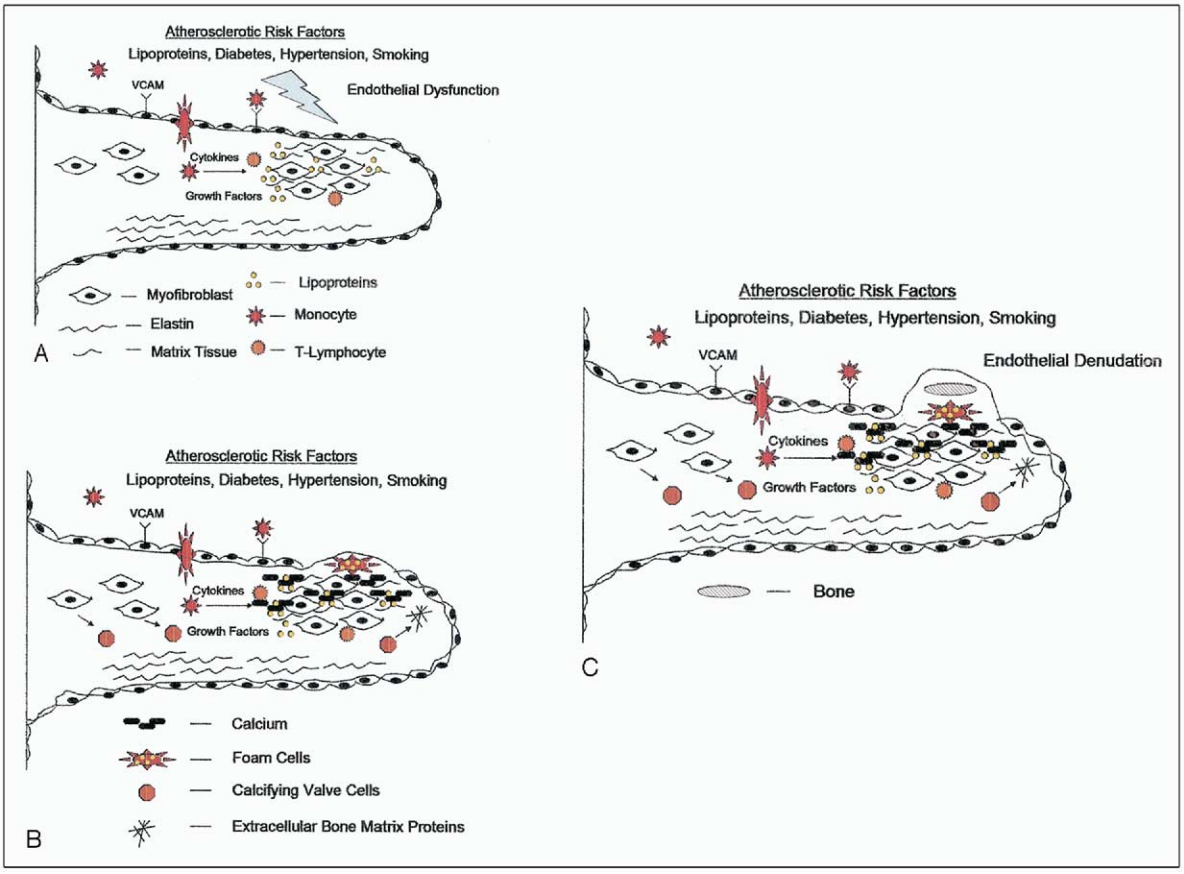

FIGURE 1. Atherosclerotic processes of aortic valve calcification in *(A)* early, *(B)* mature, and *(C)* aortic valve lesions. Rheologic influences on valve calcification are not included. Cardiovascular risk factors and abnormal blood flow rheology result in endothelial dysfunction that in turn results in a cascade of events, such as recruitment of monocytes by expression of adhesion molecules. Inflammatory cells in conjunction with modified lipoproteins induce generation of cytokines and growth factors that in turn induce myofibroblast cell proliferation and synthesis of extracellular matrix primarily on the aortic side of the valve. Ectopic calcification ensues and is facilitated by apoptosis of cells and matrix vesicles. In mature lesions, MMPs and proinflammatory cytokines interact with macrophages to generate a lesion that is similar to the atherosclerotic plaque in arteries. Components of the rennin-angiotensin system, such as angiotensin II likely stimulate the calcification process through a myriad of mechanisms. A subpopulation of myofibroblasts is hypothesized to transdifferentiate into an osteoblastic-like cell, produce alkaline phosphatase, and secrete extracellular bone matrix proteins such as osteopontin and BMP-2 that advance calcification. In severe lesions, lamellar bone, presumably from endochondral calcification, may appear and show evidence of remodeling. VCAM = vascular cell adhesion molecule.

molecules believed to modulate inflammation of vessels.[23] Inflammation is a prominent feature of aortic valve calcification and may develop due to endothelial dysfunction fueled by atherosclerotic risk factors.[24] T lymphocytes are present in calcified valves,[25–27] even during early disease, and when found with inflammatory cytokines indicate that an injury-repair cycle is operative, which may initiate disease development. Adhesion molecules, such as vascular cell adhesion molecule 1, not normally expressed by the endothelium, are found in diseased valves. Monocytes adhere to adhesion molecules, migrate into the subendothelial space of the valve in response to locally produced chemoattractant molecules, and differentiate into macrophages.[28–30] Similar to arterial atherosclerotic lesions, there are few, if any, neutrophils present in the aortic valve lesion.

Proinflammatory cytokines are believed to be pivotal in mediating valve calcification. Data have indicated that transforming growth factor β1 (TGF-β1) is an important cytokine that contributes to the calcific process because it has "bone-inducing" properties and is a member of the gene superfamily that includes bone morphogenetic proteins.[31,32] Immunohistochemical studies of calcified human aortic valves have found higher levels of TGF-β1 in calcified cusps than in noncalcified cusps.[33] The TGF-β1 identified in calcified valves may bind to TGF-β–latent binding proteins in the extracellular matrix. Activation and release of TGF-β1 from latent binding proteins may occur from protease activity of matrix metalloproteinases (MMPs).[34,35] In cell culture, the addition of TGF-β1 to aortic valve interstitial cells increased cellular migration, cell aggregation, and formation of apoptotic alkaline phosphatase-enriched calcified nodules.[33,36]

LIPIDS

Lipid in calcified aortic valves was reported decades ago, but the pathologic mechanistic relation to calcium was unknown. Recent evidence has indicated that lipid in vascular tissue stimulates calcifica-

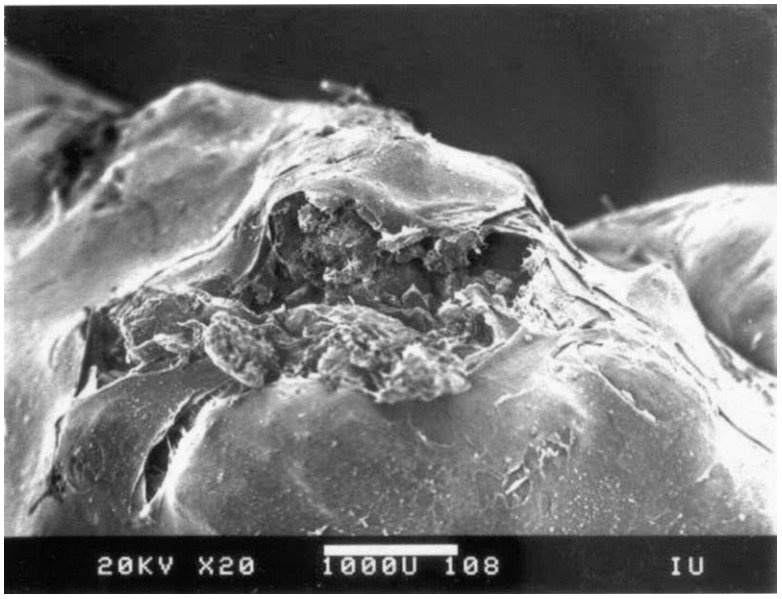

FIGURE 2. Electron micrograph of a severely calcified aortic valve removed at the time of aortic valve replacement shows endothelial denudation and a calcified lesion that predominantly expanded into the aortic side of the valve.

TABLE 1 Risk Factors for Aortic Valve Calcification

Dyslipidemia
Hypertension
Diabetes mellitus
Smoking
Congenitally abnormal valve (i.e., bicuspid valve)
Hyperparathyroidism
End-stage renal disease
Paget's disease

tion.[37,38] The mineral component of aortic valves is intimately associated with cholesterol at the ultrastructural level, and cholesterol is believed to play a role in the precipitation of calcium crystals.[39] Products of cholesterol oxidation, such as 25-hydroxy cholesterol, found in coronary atherosclerotic lesions, also accelerate valvular calcification in vitro.[36] Lipid accumulates in macrophages in atherosclerotic lesions and, likely through a similar mechanism in aortic valves, through membrane scavenger receptors that are capable of binding oxidized low-density lipoprotein cholesterol.[40]

Oxidized lipids are found in early lesions of calcified valves (Figure 3) and likely stimulate the atherosclerotic process in valves through a myriad of mechanisms, such as enhancement of inflammation, apoptosis, and expression of MMPs.[27,41,42] Rabbits fed a diet high in cholesterol developed macrophages and bone matrix proteins in the aortic valve, and the effect was inhibited with atorvastatin.[43] Delipidation of bioprosthetic valves by pretreatment with ethanol decreased calcific degeneration in rats[44] and sheep,[45] further supporting the role of lipids in the calcific process.

CALCIFYING VASCULAR CELLS

Recent studies have indicated that cells that reside in the valve may undergo transdifferentiation and participate in the calcific process. Vascular smooth muscle cells are important in fibrous tissue accumulation and calcification of atherosclerotic plaques in arteries. The aortic valve contains cells similar to vascular smooth muscle cells in the interstitial layer, called myofibroblasts, that are primarily believed to be secretory in nature, with protein-staining characteristics similar to vascular smooth muscle cells and fibroblasts. Myofibroblasts presumably secrete collagen and other extracellular matrix proteins to maintain the interstitial integrity of the valve leaflet.

A clonal population of cultured aortic valve interstitial cells or myofibroblasts spontaneously undergo phenotypic transdifferentiation into osteoblast-like cells and form calcific nodules[36] similar to those seen with vascular smooth muscle cells.[46,47] The nodules are composed of nonviable myofibroblasts and hydroxyapatite mineral. Viable cells that exhibit osteoblastic-like properties surround the calcified nodules. Osteoblastic properties include the presence of alkaline phosphatase and secretion of extracellular bone matrix proteins. Although TGF-β1 stimulates nodules in cell culture, further studies are needed to determine the pathologic signals involved in transdifferentiation of myofibroblasts into calcifying vascular or "valve" cells.

MATRIX METALLOPROTEINASES

To maintain integrity and pliability, the aortic valve must undergo physiologic remodeling that entails degradation and reorganization of the interstitial tissue or so-called extracellular matrix. Putatively important regulators of aortic valve remodeling are the MMPs and tissue inhibitors of metalloproteinases. MMPs are endopeptidases with common functional domains and a common mechanism of action due to their ability to degrade extracellular matrix components.[48,49] MMPs have been implicated in a variety of conditions that affect blood vessel structure.[48,50–56]

Several types of cells found in the cardiovascular system secrete MMPs, including endothelial cells and smooth muscle cells. MMP-1, MMP-2, MMP-3, and MMP-9 are present in calcified aortic valves.[57,58] In pathologic processes, inflammatory cells are a source of MMPs and other proteases, such as cathepsins, that degrade vascular matrix. Studies have indicated that activated macrophages secrete cytokines that upregulate MMP gene expression, a process that is stimulated by the presence of oxidized lipoproteins.[51] Reactive oxygen species, which have been implicated in the development of atherosclerotic lesions, can trigger

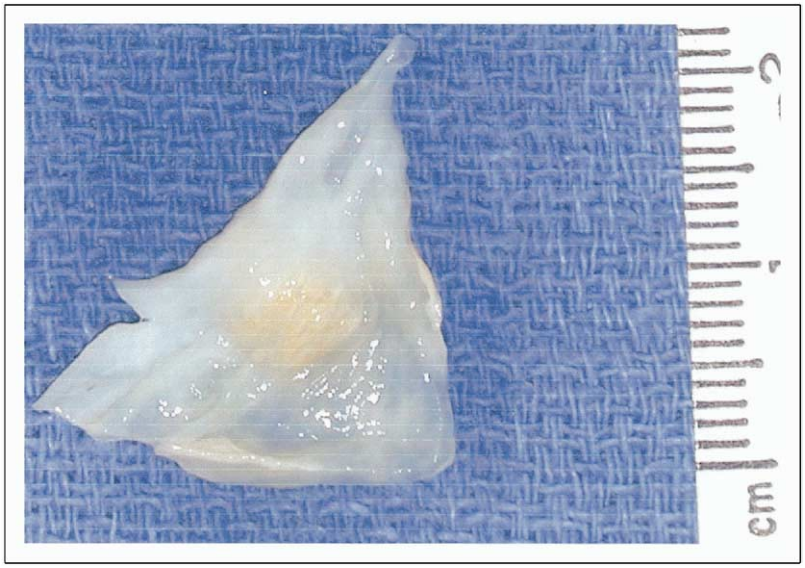

FIGURE 3. Photomicrograph shows an aortic valve that was removed because of severe regurgitation, with lipid accumulation and no gross calcification in the body of the leaflet. Patients who have this type of valve are susceptible to valve calcification as they age, and the presence of lipids even before large amounts of calcification occur implicates lipid accumulation as an early event in lesion development.

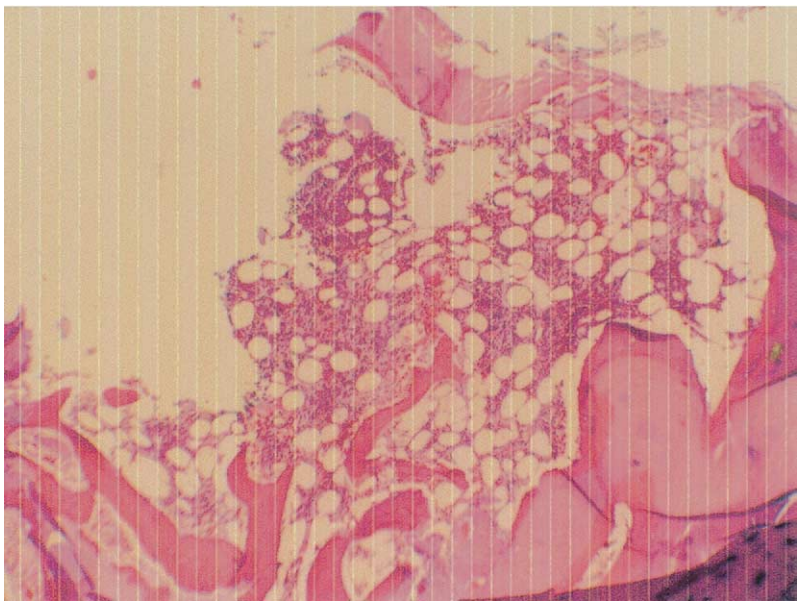

FIGURE 4. Photomicrograph of a heavily calcified aortic valve shows lamellar bone with hematopoietic elements, also known as osseous metaplasia.

activation of latent MMP zymogens that are stored in the extracellular matrix, thus providing an arsenal for matrix degradation.[59] More specifically, T lymphocytes have been found to trigger MMP-2 secretion from endothelial monolayers in vitro, a process that likely occurs in calcified valves.[60] The release of MMP-2 depends on the presence of vascular cell adhesion molecule-1 on endothelial cells, a molecule that is expressed on calcified aortic vales.[30]

An extracellular matrix glycoprotein, tenascin-C, is often coexpressed with MMPs in a variety of tissues. There is compelling evidence to indicate that tenascin-C actively participates in normal physiologic mineralization through cytokine activation, calcium binding, and alkaline phosphatase activity.[61] Studies have indicated that tenascin-C upregulates expression of MMPs and, conversely, that degradation of type 1 collagen by MMPs promotes tenasin-C expression at the transcriptional level.[62] Jian et al[58] found that human calcified aortic stenotic cusps have prominent deposition of tenascin-C, MMP-2, alkaline phosphatase activity, and MMP-gelatinolytic activity. In that study, significantly less tenascin-C was noted, and MMP-2 and alkaline phosphatase were not detected in grossly noncalcified valve cusps. Thus, the natural homeostatic mechanisms of valve repair that involve MMPs appear to be dysfunctional and may result in accumulation of sclerotic tissue and, indirectly, of calcification.

BONE

Lamellar bone is also found in severely calcified aortic valves and is preceded by ectopic calcification.[24] Hematopoietic elements similar to bone marrow have been seen in areas of lamellar bone (Figure 4). Bone growth and fracture healing are not passive but involve an orchestrated expression of extracellular bone proteins (osteoid components) that provide a scaffold for the calcific process.[63] Similarly, calcified aortic valves contain extracellular bone matrix protein, such as osteopontin, osteonectin, matrix γ-carboxylated glutamate protein, and bone morphogenetic proteins (BMPs) that are believed to play a role in the calcific process.[24,64–66] Bone (heterotopic ossification) with osteoblasts and osteoclasts is estimated to be present in 13% of severely calcified aortic valves and represents an active process of abnormal tissue repair.[24] Cartilage and chondrocytes in calcified valves indicate that endochondral ossification, similar to that seen in bone fracture healing, is operative.[24] Myofibroblasts that reside in the valve leaflet most likely undergo transdifferentiation into osteoblastic-like cells according to data from cell culture models[36] and a rabbit study[43] and indicate the presence of cells with osteoblastic phenotype. Human studies have also suggested osteoblast-like cells in calcified aortic valves.[24,67]

The sequence of events that leads to bone formation in the skeleton includes blood vessel invasion

(neovascularization) with mineralization of the extracellular matrix, apoptosis of hypertrophic conduit sites, and extracellular matrix degradation. Different angiogenic factors are expressed in the bone growth plate, including members of the TGF-β family, vascular endothelial growth factor, fibroblast growth factor, insulin growth factor 1, and platelet-derived growth factor A.[68,69] Current evidence has indicated that vascular endothelial growth factor and fibroblast growth factor are essential coordinators of fracture healing and likely contribute to bone formation in aortic valves. Normal aortic valves have only minor vascularization in the region of the valve near the aortic wall, whereas neovascularization may occur throughout the leaflet in calcified aortic valves, especially those with bone.[24,69]

GENETIC INFLUENCES

Recent genetic studies of 2 extracellular molecules, matrix γ-carboxylated glutamate protein and osteoprotegerin, in mice have indicated that extracellular matrix calcification may be inhibited directly or indirectly by these proteins.[70,71] Although not proved, downregulation of inhibitors of calcification is a plausible scenario that may contribute to accumulation of calcium in aortic valve cusps. If this is the case, then administration of these molecules may attenuate valve calcification.

Different antagonists to BMPs, including noggin, chordin, follistatin, and gremlin, were identified in controlling BMP gene expression and regulate bone formation.[72] These antagonists theoretically may play a role in ossification of aortic valves if not present in adequate numbers or inhibited by other proteins. Smad proteins are intracellular mediators of signaling that are initiated by the TGF-β superfamily of ligands, which includes the BMPs. The inhibitory Smads (6 and 7) are transcriptionally induced in cultured cells treated with the TGF-β superfamily of ligands and downregulate BMP signaling in in vitro assays. These inhibitory Smads interfere with BMP signaling.

Gene manipulation in mice has started to reveal specific developmental and physiologic functions of the signal-transducing Smads. Galvin et al[73] explored the role of an inhibitory Smad in vivo by targeting a mutation of Madh-6 (which encodes the Smad 6 protein). A targeted insertion of LacZ reporter demonstrated that Smad 6 expression is largely restricted to the heart and blood vessels and that Madh-6 mutants have multiple cardiovascular abnormalities. These abnormalities include hyperplasia of the cardiac valves and outflow track septal defects. Interestingly, these mutant mice developed aortic ossification and high blood pressure, thus supporting the hypothesis that a decrease in BMP inhibitors may predispose to ossification.[73]

RENIN-ANGIOTENSIN SYSTEM

The renin-angiotensin system controls blood pressure and fluid and electrolyte balances through coordinated effects on blood vessels, kidneys, and the heart, but, if unregulated, it may have proatherothrombotic effects.[74–78] Angiotensin-converting enzyme, angiotensin II, and bradykinin receptor binding were observed in aortic valves from Sprague-Dawley rats.[79] Interestingly, high-density angiotensin-converting enzyme binding was anatomically coincident with sites of fibrosis and attenuated by lisinopril in these animals.[80] Members of the renin-angiotensin system and perhaps bradykinin are likely responsible for normal physiologic repair of the aortic valve due to routine wear and tear. However, if the renin-angiotensin system becomes consistently dominant, pathologic fibrosis and calcification may ensue in the aortic valve.

Angiotensin II, the downstream product of this system, has been implicated in different proinflammatory effects that potentially contribute to the atherosclerotic process. O'Brien et al[81] found that angiotensin-converting enzyme is present in aortic valve sclerosis and aortic valve stenotic lesions. This enzyme was primarily distributed extracellularly, where it co-localized with apolipoprotein B. In addition, angiotensin II co-localized with angiotensin-converting enzyme in aortic valve lesions. The angiotensin-1 receptor, a major receptor for angiotensin II, was also found in advanced aortic valve lesions.[81] These results suggest that angiotensin-converting enzyme may be concentrated in aortic valve lesions through retention of plasma lipoproteins, such as low-density lipoprotein cholesterol. Thus, the renin-angiotensin system may contribute to the inflammation observed in aortic valve lesions and may promote lipid-laden macrophage development in the interstitium of the valve leaflet.

OBSERVATIONAL STUDIES OF THERAPEUTIC INTERVENTIONS

There is no currently approved medication by the United States Food and Drug Administration for medical management of aortic valve stenosis. Recent, nonrandomized, clinical studies have indicated that medical therapy may slow progression of aortic valve calcification. Two retrospective studies[82,83] and 1 prospective population-based study[84] noted echocardiographically that patients who used statin drugs had a significantly decreased annualized rate of aortic stenotic progression, but the rate of progression was not explained simply by changes in plasma lipids. Although echocardiography accurately measures aortic valve area, it is not useful for quantification of valve calcium content. Electron beam computed tomography (EBT) affords quantification of tissue calcium and has been used to measure aortic valve calcification.[85] Two retrospective clinical studies of aortic valve calcification using EBT found that calcium content did not increase as quickly for patients who use statin drugs.[86,87] Studies have indicated that statin drugs inhibit secretions of MMP-1, MMP-2, MMP-3, and MMP-9 from vascular smooth muscle cells and macrophages, which may represent a pleiotropic effect of statin drugs that favorably affects aortic valve disease.[88–90] Statin drugs may stabilize "atherosclerotic" cardiac valve lesions and retard calcification and ossification. However, statin drugs stimulate bone for-

mation in vitro,[91] and some data have indicated that they may decrease the risk of hip fracture.[92–94] The effect of statins on bone in calcified aortic valves is unknown. Randomized controlled trials are needed to evaluate the efficacy and safety of statins on ectopic calcification and ossification in aortic valves. There are no observational studies or randomized controlled trials of other therapeutic interventions such as angiotensin-converting enzyme inhibition or anti-inflammatory therapies in aortic valve calcification. Such trials are warranted, because of recent pathologic data that invoke inflammation and the renin-angiotensin system in aortic valve calcification.

Summary: The calcified aortic valve lesion develops in the setting of endothelial injury and inflammation and displays hallmarks of atherosclerosis, including lipid accumulation, MMP activation, and interaction with the renin-angiotensin system. In addition to ectopic calcification, bone formation may occur in severely diseased valves. Our understanding of the molecular mechanisms that participate in an aortic valve lesion has lagged behind atherosclerotic disease of arteries. Further understanding of atherosclerotic disease will assuredly yield new insights into valvular heart disease. Current evidence indicates that modification of atherosclerotic risk factors will slow progression of aortic valve calcification, and valve risk factors should be addressed in all patients who have aortic valve calcification. Prospective trials are needed to evaluate therapeutic approaches to prevent progression of aortic valve disease.

Acknowledgment: I thank Robert Zimmerman, MD, for providing the photomicrograph shown in Figure 4 and Frederick S. Kaplan, MD, for insightful comments.

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