Calcific aortic valve disease and hypertension

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Abstract:

This review addresses the role of hypertension in precipitating Calcific aortic valve disease (CAVD) and the therapeutic potential of anti-hypertensive interventions to ameliorate CAVD. CAVD was originally considered to be a degenerative disease representing the "wear and tear" of the aortic valves. More recently both conceptually and experimentally, CAVD has come to be considered the result of an active disease process. Whilst, there are some common factors in the pathology and risk factors for atherosclerosis and CAVD there are also some distinct differences. Hypertension is an established risk factor for coronary artery disease and has been recognised as a risk factor for CAVD. Angiotensin converting enzyme inhibitors have been found to have beneficial effects in CAVD and as in atherosclerosis such effects may be due to the blood pressure lowering action but also to direct pleiotropic effects on the biochemical and cellular mechanisms of disease progression in the respective tissues. The very high prevalence of hypertension in the community coupled with an aging population, a risk factor associated with both hypertension and CAVD, infers that hypertension will be one of the predominant factors that increase the impact of CAVD on human health in the coming decades.

INTRODUCTION

Calcific aortic valve disease (CAVD) is a progressive condition which leads to significant symptomatology and death if untreated. The prevalence of CAVD is increasing because of our increasingly elderly population. Currently 2-5% of very elderly patients are diagnosed with CAVD [1, 2] which carries an 80% 5 year risk of progression to heart failure, valve replacement or death [3]. CAVD is also the second most common indication for cardiac surgery in the Western world [4].

Initially thought to be a degenerative disorder of aging, increasingly specific risk factors are being identified in association with CAVD. Age, male gender, hypertension and current smoking have all been found to be associated with CAVD in a large population cohort study. Correlations have also been found between CAVD and elevated levels of lipoprotein and low density lipoprotein cholesterol. Other identified risk factors are diabetes, and elevated body mass index [5] and end stage renal disease [6].

As stated above, CAVD was originally considered to be a degenerative disease representing the "wear and tear" of the hard working aortic valves [7]. More recently conceptually and experimentally CAVD has come to be considered the result of an active disease process with the implication that it may be amenable to therapeutic intervention [8, 9]. The major observations indicating that CAVD is an active disease process are the deposition of lipid in early lesions, specifically lipids associated with atherosclerosis, the presence of monocyte macrophages and other cells of the immune system [9], the presence and involvement of proteins associated with calcification [10] and the presence of the components of the Renin Angiotensin System (RAS) including angiotensin converting enzyme (ACE), angiotensin II (AII) and angiotensin receptors [9, 11]. CAVD has a catastrophic impact on the appearance

of the valve leaflets (Fig. 1). Current concepts suggest an analogy between the pathological mechanisms of atherosclerosis [8] and CAVD however only 50% of patients with severe aortic stenosis have appreciable coronary disease, so clearly some differences must be present in the aetiology [12]. There are some common factors as well as distinct differences in the pathology and risk factors for atherosclerosis and CAVD. In atherosclerosis, the arterial neo-intima is the location of the formation of atherosclerotic plaques whereas CAVD disease commences within the boundaries of the valve and ultimately forces an expansion of the valve tissue [13]. Also, angiotensin receptors are expressed in VSMCs in the wall of normal vessels [14] but are not expressed by aortic valve myofibroblasts (avMs) in normal aortic valves [8].

There are however common factors as well. There is a large amount of literature on the relationship between hypertension and cardiovascular disease but very little on the mechanisms through which hypertension accelerates atherosclerosis. There is also only a small amount of information on the association and underlying mechanisms linking CAVD and hypertension. Hypertension is a strong and well-established risk factor for coronary artery disease and has been recognised as a risk factor for CAVD. Multiple trials have demonstrated the benefits of blocking RAS in preventing atherosclerosis-related cardiovascular disease [15-17]. However, as in the case that the hyperglycaemia of diabetes is more than just elevated blood glucose [18], hypertension seems to be more than just elevated blood pressure [19]. Studies on the relationship of systolic blood pressure and atherosclerosis show that more than pressure *per se* is involved [19] and by analogy with the role of insulin resistance in diabetes, the stimulus for the hypertension is strongly implicated. The major stimulus is the RAS and manipulations of the RAS system have a direct impact of atherosclerotic lesion size [19]. Angiotensin converting enzyme (ACE) inhibitors

have been found to have beneficial effects, but not unequivocally, in CAVD. As in atherosclerosis, such beneficial effects in CAVD may be due to the anti-hypertensive action and also pleiotropic effects directly on the biochemical and cellular mechanisms of disease progression [20]. The very high prevalence of hypertension in the community [21] coupled with the aging of the population which is associated with both hypertension and CAVD, infers that hypertension will be one of the major underlying factors that increase the impact of CAVD on human health in coming decades. This review addresses the role of hypertension in precipitating CAVD and the potential for therapeutic benefit of anti-hypertensive interventions to ameliorate CAVD.

ANATOMY AND PATHOLOGY OF CAVD

The processes causing the initiation and progression of aortic valve disease have not been clearly identified. Aortic valves have three distinct anatomical layers. These layers commencing from the upper or aortic side of the leaflet are the fibrosa, the spongiosa and the ventricularis and these are comprise predominantly collagen, glycosaminoglycans and elastin, respectively (see Fig. 2A). Pathologically, there are similarities but distinct differences between CAVD and atherosclerosis. The most important similarity is the co-localization of lipids with the proteoglycans, biglycan and decorin, in early CAVD and atherosclerosis [22-24]. Extracellular matrix (ECM) changes are characteristic of both atherosclerotic vessels and diseased aortic valves. Lipid-rich lesions accumulate predominately on the aortic surface of the valve [9], the basement membrane/elastic lamina beneath these lesions appears to be damaged or absent. The collagenous fibrosa becomes thickened [9], partially due to the infiltration of lipids. Proteoglycans, specifically the chondroitin/dermatan sulfate proteoglycans, biglycan, decorin, and versican, show increased abundance in early through late aortic valve lesions and biglycan and decorin are co localized with apoE and apoB [22, 23] (Fig. 2B). A fundamental difference is the development of atherosclerosis in the neointima of coronary arteries whereas in aortic valve pathology the neo-intima does not have an anatomical equivalent [13]. Other differences between aortic valve disease and atherosclerosis are the deposition of lipid in the sub-endothelial fibrosa layer in aortic valves but not in deeper layers, and calcification instead of necrosis as a response to lipid trapped in the valvular tissue [23, 25, 26]. Lipids, oxidized lipids, lipoproteins (Low Density Lipoproteins (LDL) and Lipoprotein (a)), and cholesterol are present in lesions of human sclerotic and stenotic

aortic valves, as well as in underlying regions of collagenous fibrosa [8, 9, 26, 27] characterized by elastic lamina fragmentation, macrophages, and accumulation of glycosaminoglycans (GAG) and proteoglycans [22, 23, 27].

Some signaling studies have provided evidence on the pathways involved in CAVD that may represent therapeutic targets. Interestingly these pathways are distinct from those associated with vascular smooth muscle and atherosclerosis and further studies in this area may provide for interesting new leads possibly in both pathologies. A pathway identified in CAVD pathology but only associated with vascular development and not yet vascular disease is the canonical Wnt pathway [28, 29]. Low density receptor-related protein (Lrp) binds to the secreted glycoprotein Wnt (a key regulator of bone formation) and frizzled receptors that subsequently activate β-catenin as part of the known signaling pathway in bone mineralization [30]. Wnts are highly secreted, cysteine-rich glycoproteins that are highly conserved among species. They bind to frizzled receptors (Fz) and can be inhibited by frizzled-related proteins (FRP) [29]. VSMCs have been found to express Wnt-5a, Fz-1,2,3 receptors and two inhibitors, FRP-1 and FRP-3 [29]. Although not much is known about the role of Wnt signalling in the vasculature, some studies have demonstrated its role in normal and pathological vessel growth and VSMC proliferation, where Wnt signalling requires the co-receptor, LRP6 [31].

There is substantial evidence that CAVD involves inflammatory processes as has been demonstrated for atherosclerosis, the latter especially in the presence of diabetes [32]. Lesions from early aortic valve sclerosis as well as from advanced calcific stenosis contain macrophages, T-lymphocytes, and mast cells [9, 33]. VCAM-1, which is not normally expressed in aortic valve leaflets, is expressed on the endothelium of calcific aortic valves [34]. Inflammatory cytokines, including IL-1β,

TNF-α, IL-2, HLA-DR and C-reactive protein are also present in valve lesions [8, 35].

The aortic valve tissue response to injury is similar to other tissues with initial elevated TGF-β levels that have been shown in aortic valves myofibroblasts (avM)s *in vitro* to increase SM α-actin expression, stress fibre formation, contraction, collagen and hyaluronan (HA) synthesis, and secretion of sulfated GAGs [36]. TGF-β can signal through several pathways, classically via high affinity binding to TGF-βRII which recruits the kinase ALK5 (TGF-βRI). ALK5 phosphorylates the carboxy terminal Ser residues of the intracellular signal transducers, SMAD-2 and -3, leading to the formation of phospho-SMADs which form complexes with co-Smads and translocate to the nucleus where they act as transcription factors to activate or repress gene expression. TGF-β is also able to activate the MAP-kinases ERK, JNK and p38. We have recently demonstrated that the action of TGF-β on VSMCs in atherosclerosis involves Smad-2 phosphorylation and activation of p38 MAP kinase [37]. The specific TGF-β pathways in avMs and particularly those participating in CAVD have yet to be elucidated.

The Renin Angiotensin System (RAS) also contributes to the development of aortic valve sclerosis and stenosis [8]. ACE and angiotensin II are co localized with ApoB in valve lesions [11]. The angiotensin-1 receptor, present in normal healthy arteries but not normally expressed in heart valves, is also present in these lesions. There is *in vitro* evidence that these peptides can be synthesized within the valve [38].

The formation of calcific nodules is a defining pathological occurrence in advanced late-stage valvular sclerotic lesions and results in the leaflets becoming stiff and the valve stenotic which precipitates the clinical condition of CAVD. Calcific nodules are found in association with oxidized lipids both in diseased human valves

and in animal models of CAVD. Not surprisingly, calcific leaflets contain osteoblast-like cells [33] and an abundance of several osteogenic mediators, including alkaline phosphatase, osteocalcin, osteopontin, BMPs, and MMP2 [39, 40].

Prolonged *in vitro* culture of avMs leads to the spontaneous formation of calcific nodules and again TGF-β plays a prominent role in this stage of disease development [41]. In a specific association with a manifestation of hypertension, avMs exposed to elevated cyclic pressures downregulate the expression of osteopontin; since this agent is protective against mineralization, its down regulation may potentiate calcification [42].

ASSOCIATION OF HYPERTENSION AND CAVD

The association between CAVD and clinical and biochemical factors has been investigated in several studies [1, 43]. Lindroos *et al.* [43] compared the occurrence of aortic valve disease in elderly subjects as established by echocardiographic and Doppler techniques with the existence of various biochemical and clinical parameters. They investigated 577 subjects ranging from 55-86 years of age. Twenty one subjects (4%) had severe aortic stenosis but 40% and 13% had slight and severe calcification, respectively [43]. Multivariate analysis identified several factors including hypertension which was shown to be an independent and highly statistically significant predictor of CAVD. The odds ratio for hypertension was 1.74 (95% confidence limits of 1.19-2.55) indicating a 74% higher rate of association when hypertension is present. This compares with an 89% increase for a 10 year increase in age and interestingly a 39% increase for each 5 $kg.m^{-2}$ decrease in body mass index, the latter associating leanness with predisposition to CAVD. In this study, factors including gender and diabetes were not correlated with aortic valve calcification [43].

In the Cardiovascular Health Study, Otto and colleagues [1] investigated the prevalence of aortic sclerosis and stenosis in the elderly and the clinical factors associated with CAVD. The study covered a randomly chosen group of more than 5000 elderly subjects ($\geq$ 65 years) who underwent 2D echocardiographic investigation and these results were analysed by stepwise logistic regression analysis with a range of clinical parameters [1]. In the entire group, aortic sclerosis and stenosis were present at 26% and 2%, respectively. Disease rates were approximately 50 per cent higher in the group $\geq$ 75 years of age. A history of hypertension established on a self-reported basis was associated with an excess odds ratio of 1.23 (95% confidence

limits of 1.14-1.32) establishing a highly statistically significant 23% increase in risk. This compares with an increase of 118 % for each 10 year increase in age, 103% for male gender and 35 % increase for smoking as independent predictors of aortic valve disease. LDL cholesterol, a strong predictor of coronary artery disease had an odds ratio for association with aortic sclerosis and stenosis of 1.12 (95% confidence limits of 1.03-1.23) indicating modest but statistically significant association of this parameter for both coronary artery disease and CAVD [1].

HYPERTENSION AND ATHEROSCLEROSIS: RELEVANCE TO CAVD

Hypertension is recognised as one of the strongest independent risk factors for cardiovascular disease [44, 45] and treatments for hypertension are highly efficacious in the prevention of cardiovascular events [15-17]. Hypertension, like other risk factors such as hyperglycaemia, accelerates atherosclerosis leading to increased rates of cardiovascular disease [13, 46]. It is surprising but there is little information on the mechanisms by which hypertension accelerates atherosclerosis. Historically, studies in animal models in the 1980s particularly those that used the spontaneous hypertensive rat (SHR) and its normotensive Wistar Kyoto (WKY) controls, focused on the mechanism of hypertension *per se* and specific parameters such as the development of vascular hypertrophy [47, 48]. Rats are particularly resistant to the development of atherosclerosis and SHR do not develop atherosclerotic lesions. More recent studies have focused on the genetically modified mice ($ApoE^{-/-}$ and $LDLR^{-/-}$) which develop atherosclerosis on high fat diets and the mechanisms of the development of lesions in those mice but the impact of hypertension has not attracted great attention [49]. A major reason for the lack of mechanistic studies on the

association of hypertension and atherosclerosis is due to the fact that hypertension is obviously an *in vivo* phenomenon which cannot be reproduced in cell culture. There have been studies of blood flow and shear stress and also of cell stretching which partially mimic the parameters associated with hypertension but none is a suitable comprehensive model of hypertension [50, 51].

One of the strongest factors linking hypertension and the accelerated development of atherosclerosis is the activation of the RAS [11]. The RAS is up regulated in hypertension and cardiovascular disease and blocking this system at multiple levels abrogates the development of cardiovascular disease. There is considerable evidence that the actions of the major hormonal effector of the RAS, AII, not only increase blood pressure but also has a range of direct actions which are deleterious to the cardiovascular system. However, based on the multiple roles of AII in causing hypertension, the actions of AII can somewhat narrowly be used as a surrogate for studies of hypertension and the development of atherosclerosis and also potentially for considering the role of hypertension in the development of aortic sclerosis and stenosis.

AII is a potent activator of a wide range of actions on VSMCs which are associated with the development of atherosclerosis [52, 53]. AII stimulates the production and modifies the structure of proteoglycans by vascular smooth muscle cells (VSMCs) [54, 55]. These proteoglycans play a critical early role in atherosclerosis through the trapping of lipoproteins in the vessel wall [24, 56, 57]. AII stimulates the production of reactive oxygen species (ROS) and also many facets of the inflammatory response including the secretion of cytokines and adhesion molecules [58].

There is one major difference between the potential role of AII in atherosclerosis and CAVD. VSMCs in the vessel wall express high levels of angiotensin receptors (associated with vessel tone) and cultured VSMCs express angiotensin receptors to variable levels. In contrast, the avMs of normal aortic valves do not express angiotensin receptors. Unlike VSMCs, early passage avMs do not respond to AII by increasing proteoglycan production (P.J. Little, unpublished observations) [54]. Diseased aortic valves express angiotensin receptors [11]. Thus there would appear to be a clear difference that AII may contribute to early atherosclerosis but as previously proposed its role is likely to be later in the CAVD process [8]. There may be considerably different opportunities for therapeutic intervention in atherosclerosis and CAVD and the underlying pathology will often render earlier intervention preferable in preventing disease initiation and progression. Thus, ACE inhibitors and angiotensin receptor blockers (ARB) may be particularly beneficial in early atherosclerosis (due to effects on blood pressure and direct protective effects on the vasculature). However, the absence of the expression of angiotensin receptors in normal aortic valves might imply that these agents may not have beneficial effects on valves in the early stages of disease development and this is one issue that needs to be resolved clinically [8]. However, it should be appreciated that other potentially beneficial effects of RAS blockade on cardiovascular pathology should not be excluded and the role of blocking the RAS system in preventing CAVD and its consequences should be further investigated and clarified.

Rosenhek *et al*. reported that ACE inhibitors do not appear to slow the progression of aortic stenosis at a time when statin therapy does have an impact [59]. In view of the expression of angiotensin receptors in avMs in diseased aortic valves this result is quite surprising. Furthermore, the effect of statins may not be related to

plasma cholesterol lowering and may relate to the anti-inflammatory actions of statins. As ACE inhibitors also have anti-inflammatory actions [60], perhaps the anti-inflammatory profiles of ACE inhibitors and statins are sufficiently different to provide some efficacy for statins over ACE inhibitors in CAVD? Clearly further *in vitro* and *in vivo* studies as well as clinical trials are required to determine the impact of drugs on the development and progression of aortic valve sclerosis and stenosis. This includes in clinical setting consideration of the timing of any therapeutic intervention which may not necessarily mean only early therapeutic intervention.

ANTI-HYERTENSIVE THERAPY AND PREVENTION OF CAVD

The conjunction of hypertension and CAVD was initially thought to represent a therapeutic conundrum and historically ACE inhibition was considered to be contraindicated in patients with aortic stenosis. It could be reasoned that therapeutic peripheral vasodilatation coupled with compromised cardiac output due to aortic valve stenosis could potentially lead to circulatory collapse. However, although the reasons are unclear, the results of numerous studies indicate that this is a theoretical rather than practical issue [61]. Furthermore, many patients with aortic valve stenosis or at least severe aortic valve sclerosis will already be on blood pressure lowering therapy without apparent compromise. Considerable evidence conclusively indicates that inhibition of the RAS with ACE inhibitors or ARBs has favourable effects on the cardiovascular system, particularly the prevention of left ventricular hypertrophy and cardiac remodelling, both of which are strong risk factors for cardiac disease. Although further information is required before a definite position can be adopted it

would appear that the cardiovascular benefits of RAS inhibition may outweigh the potential for negative outcomes in patients with aortic valve stenosis.

There are no prospective studies available only retrospective observational studies of RAS inhibition and CAVD progression. A recent observational study investigated the relationship between the rate of change of volumetric aortic valve calcium (AVC) scores of patients receiving or not receiving ACE inhibitors [62]. The study investigated 123 patients of whom 43 were on ACE inhibitors and the mean study time was 2.5 years [62]. The patients had undergone two serial electron beam computed tomography (CT) investigations. The rates of change (increase) of AVC scores were statistically significantly higher in the group of patients not receiving ACE inhibitors. The rates of increasing calcium deposition for patients not receiving and those receiving ACE inhibitors were 28.7 %/yr and 11.0 %/yr, respectively. The adjusted odds ratio was a highly significant 29 % in favour of the group on ACE inhibitors. Of interest the prevalence of hypertension was high and the baseline AVC score was statistically significantly higher (146.3 *versus* 69.5) in the group of patients on ACE inhibitors [62]. It was noted that the association of decreased rates of AVC progression with ACE inhibitors therapy was maintained after adjustment for the higher baseline level of AVC in the ACE inhibitor group. Although a careful interpretation is appropriate this study in total suggests a role for hypertension in precipitating CAVD and a potential role of ACE inhibitors in slowing the progression of disease even in the presence of ongoing hypertension.

Rosenhek *et al.* have retrospectively evaluated the impact of ACE inhibitors and statins on the progression of aortic stenosis [59]. Of 211 patients, average age 70 years, with confirmed aortic stenosis (peak velocity >2.5 m/s) and otherwise normal cardiac physiology receiving two echocardiograms separated by an average of 24±18

months 102 were on ACE inhibitors, 50 patients on statins and 32 patients on both agents [59]. The increase in annualised peak aortic jet velocity for the entire study group was 0.32±0.44 $m.s^{-1}.y^{-1}$. ACE inhibitors had no effect on this rate of disease progression either alone or in combination with statin therapy. For comparison, statin therapy markedly and statistically significantly reduced the rate of progression to 0.10±0.41 $m.s^{-1}.y^{-1}$. and this effect of statins was present in patients with both mild-to-moderate and severe aortic stenosis. In this study 80 percent of the patients had hypertension possibly pointing to a role of hypertension in the development of CAVD notwithstanding the lack of efficacy of ACE inhibitor therapy [59].

CONCLUSIONS

CAVD is now appropriately recognised as a formal disease process and not a result of nonspecific wear and tear based aging of the leaflets. Hypertension is one of the most potent risk factors for atherosclerotic cardiovascular disease and a limited amount of data strongly suggests that hypertension is also associated with the development of CAVD. The development of CAVD has similarities with that of the known processes underlying the development of atherosclerosis but there are appreciable differences in the anatomy, cell biology and indeed based on a limited amount of data, on the response therapeutic interventions. The use of the many classes of anti-hypertensive agents does not give a uniform or consistent response in terms of slowing the development of atherosclerosis and preventing cardiovascular disease. It is preferable at this time that analogies between CAVD and atherosclerosis not be taken too strongly, lest potential treatments for CAVD, related and unrelated to hypertension, be overlooked. However, as always, account must be taken of the range

of actions of drugs on the whole cardiovascular system and not just the disease process in the aortic valve leaflets with the aim of optimising the patient outcome when hypertension and CAVD are present.

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FIGURES AND FIGURE LEGENDS

Figure 1.

Picture of a complete diseased aortic valve from a patient who underwent aortic valve replacement at The Alfred, Melbourne, Australia in mid 2007. The three leaflets shown are typical and representative of valves from such patients. The view is from the upper or aortic side and the scale shows major divisions in centimetres.

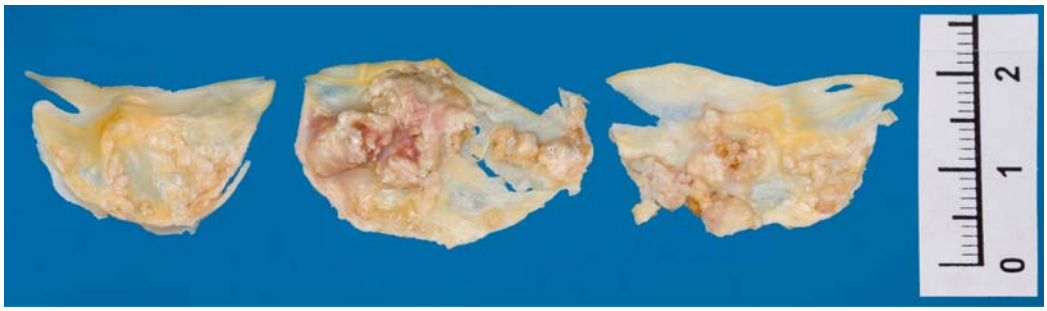


Figure 2A. Stylised structure of a normal aortic valve leaflet

The aortic valve is covered with a layer of endothelial cells which sit on a basement membrane. The elastic lamina lies under the basement membrane. The valve has three layers comprised predominantly of extracellular matrix - the fibrosa layer containing collagen, the spongiosa layer containing glycosaminoglycans (proteoglycans and hyaluronan) and the ventricularis containing elastin. The aortic myofibroblasts are mainly present in the elastin layer, with a random occurrence in the spongiosa. avMs reside mostly across the width of the leaflet but also form perpendicular bundles which make up a cross network in the valve. As drawn, the valve is joined to the heart/aorta at the back of the diagram. The figure here shows a valve leaflet dissected vertically two thirds from the back. The key to the components is shown under Fig. 2B.

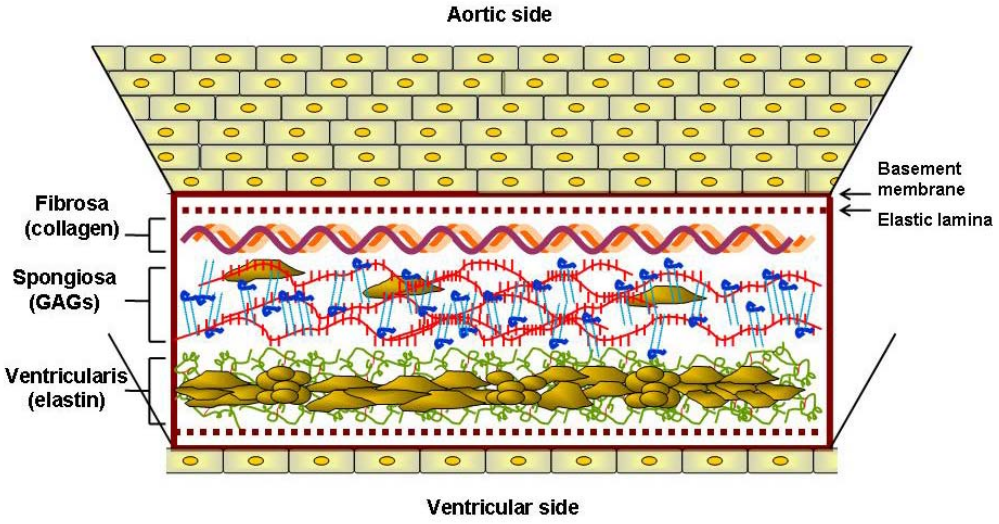
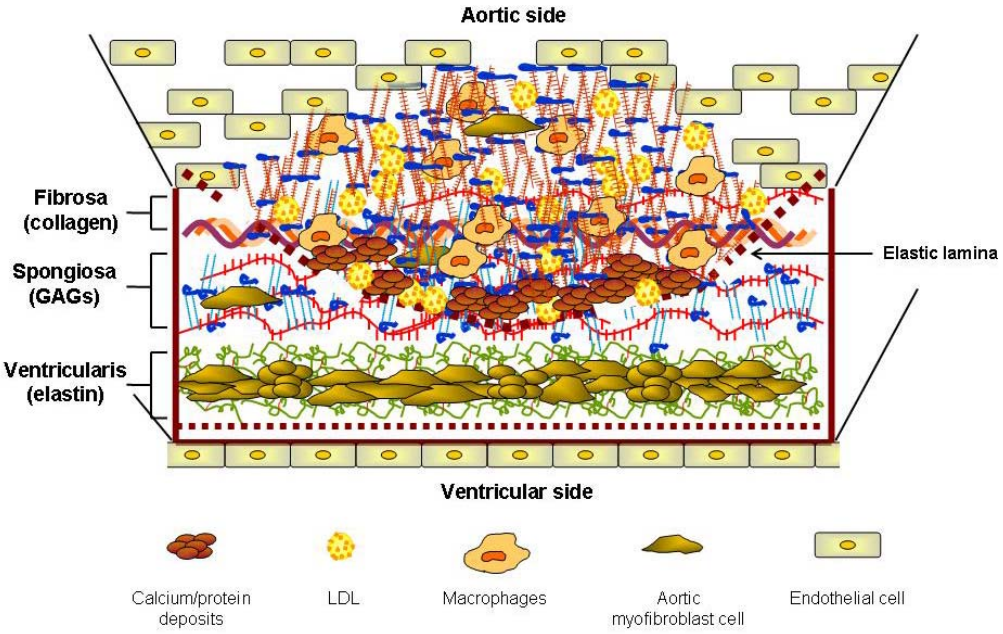


Figure 2B. Stylised structure of a severely effected leaflet from a stenotic aortic valve. In the early stage of CAVD (not shown), the basement membrane is disrupted, endothelial cells are lost and the surface is exposed. The upper elastic lamina gets pushed down as the lesion forms. The lesion contains a large amount of glycosaminoglycans (proteoglycans and hyaluronan). Monocytes in the blood migrate into the valve where they differentiate to form macrophages. Macrophages accumulate lipid leading to foam cell formation. The lesion also contains free lipid droplets. In the end stage of the disease, the thickness of the valve increases. A calcified layer comprising protein and calcium deposits is formed at the base of the lesion. The lesion expands upwards and above the surface of the valve. The top section of the lesion may contain only a few endothelial cells. A photograph of the 3 leaflets of a severely diseased aortic valve is shown in Figure 1.



References

[1] Stewart BF, Siscovick D, Lind BK*, et al.* Clinical factors associated with calcific aortic valve disease. Cardiovascular Health Study. J Am Coll Cardiol 1997;29(3):630-4.

[2] Lindroos M, Kupari M, Heikkila J, Tilvis R. Prevalence of aortic valve abnormalities in the elderly: an echocardiographic study of a random population sample. J Am Coll Cardiol 1993;21(5):1220-5.

[3] Otto CM, Lind BK, Kitzman DW, Gersh BJ, Siscovick DS. Association of aortic-valve sclerosis with cardiovascular mortality and morbidity in the elderly. N Engl J Med 1999;341(3):142-7.

[4] Roberts WC, Ko JM. Frequency by decades of unicuspid, bicuspid, and tricuspid aortic valves in adults having isolated aortic valve replacement for aortic stenosis, with or without associated aortic regurgitation. Circulation 2005;111(7):920-5.

[5] Aronow WS, Ahn C, Kronzon I, Goldman ME. Association of coronary risk factors and use of statins with progression of mild valvular aortic stenosis in older persons. Am J Cardiol 2001;88(6):693-5.

[6] Raggi P, Bommer J, Chertow GM. Valvular calcification in hemodialysis patients randomized to calcium-based phosphorus binders or sevelamer. J Heart Valve Dis 2004;13(1):134-41.

[7] Sell S, Scully RE. Aging Changes in the Aortic and Mitral Valves. Histologic and Histochemical Studies, with Observations on the Pathogenesis of Calcific Aortic Stenosis and Calcification of the Mitral Annulus. Am J Pathol 1965;46:345-65.

[8] O'Brien KD. Pathogenesis of calcific aortic valve disease: a disease process comes of age (and a good deal more). Arterioscler Thromb Vasc Biol 2006;26(8):1721-8.

[9] Otto CM, Kuusisto J, Reichenbach DD, Gown AM, O'Brien KD. Characterization of the early lesion of 'degenerative' valvular aortic stenosis. Histological and immunohistochemical studies. Circulation 1994;90(2):844-53.

[10] Mohler ER, 3rd, Adam LP, McClelland P, Graham L, Hathaway DR. Detection of osteopontin in calcified human aortic valves. Arterioscler Thromb Vasc Biol 1997;17(3):547-52.

[11] O'Brien KD, Shavelle DM, Caulfield MT*, et al.* Association of angiotensin-converting enzyme with low-density lipoprotein in aortic valvular lesions and in human plasma. Circulation 2002;106(17):2224-30.

[12] Exadactylos N, Sugrue DD, Oakley CM. Prevalence of coronary artery disease in patients with isolated aortic valve stenosis. Br Heart J 1984;51(2):121-4.

[13] Ross R. Atherosclerosis--an inflammatory disease. N Engl J Med 1999;340(2):115-26.

[14] Laurent S, Vanhoutte P, Cavero I*, et al.* The arterial wall: a new pharmacological and therapeutic target. Fundam Clin Pharmacol 1996;10(3):243-57.

[15] Yusuf S, Sleight P, Pogue J, Bosch J, Davies R, Dagenais G. Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. N Engl J Med 2000;342(3):145-53.

[16] Dahlof B, Devereux RB, Kjeldsen SE*, et al.* Cardiovascular morbidity and mortality in the Losartan Intervention For Endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol. Lancet 2002;359(9311):995-1003.
[17] Fox KM. Efficacy of perindopril in reduction of cardiovascular events among patients with stable coronary artery disease: randomised, double-blind, placebo-controlled, multicentre trial (the EUROPA study). Lancet 2003;362(9386):782-8.
[18] Bansilal S, Farkouh ME, Fuster V. Role of insulin resistance and hyperglycemia in the development of atherosclerosis. Am J Cardiol 2007;99(4A):6B-14B.
[19] Lu H, Cassis LA, Daugherty A. Atherosclerosis and arterial blood pressure in mice. Curr Drug Targets 2007;8(11):1181-9.
[20] Chlopicki S, Gryglewski RJ. Angiotensin converting enzyme (ACE) and HydroxyMethylGlutaryl-CoA (HMG-CoA) reductase inhibitors in the forefront of pharmacology of endothelium. Pharmacol Rep 2005;57 Suppl:86-96.
[21] Ong KL, Cheung BM, Man YB, Lau CP, Lam KS. Prevalence, awareness, treatment, and control of hypertension among United States adults 1999-2004. Hypertension 2007;49(1):69-75.
[22] O'Brien KD, Otto CM, Reichenbach DD, Alpers CE, Wight TW. Regional Accumulation of Proteoglycans in Lesions of "Degenerative" Valvular Aortic Stenosis and their Relationship to Apolipoproteins. Circulation 1995;92(8 (Suppl 1)):612.
[23] O'Brien KD, Reichenbach DD, Marcovina SM, Kuusisto J, Alpers CE, Otto CM. Apolipoproteins B, (a), and E accumulate in the morphologically early lesion of 'degenerative' valvular aortic stenosis. Arterioscler Thromb Vasc Biol 1996;16(4):523-32.
[24] Nakashima Y, Fujii H, Sumiyoshi S, Wight TN, Sueishi K. Early human atherosclerosis: accumulation of lipid and proteoglycans in intimal thickenings followed by macrophage infiltration. Arterioscler Thromb Vasc Biol 2007;27(5):1159-65.
[25] Otto CM, Burwash IG, Legget ME*, et al.* Prospective study of asymptomatic valvular aortic stenosis. Clinical, echocardiographic, and exercise predictors of outcome. Circulation 1997;95(9):2262-70.
[26] Olsson M, Thyberg J, Nilsson J. Presence of oxidized low density lipoprotein in nonrheumatic stenotic aortic valves. Arterioscler Thromb Vasc Biol 1999;19(5):1218-22.
[27] Walton KW, Williamson N, Johnson AG. The pathogenesis of atherosclerosis of the mitral and aortic valves. J Pathol 1970;101(3):205-20.
[28] Dale TC. Signal transduction by the Wnt family of ligands. Biochem J 1998;329 (Pt 2):209-23.
[29] Goodwin AM, D'Amore PA. Wnt signaling in the vasculature. Angiogenesis 2002;5(1-2):1-9.
[30] Johnson ML, Rajamannan N. Diseases of Wnt signaling. Rev Endocr Metab Disord 2006;7(1-2):41-9.
[31] George SJ, Beeching CA. Cadherin:catenin complex: a novel regulator of vascular smooth muscle cell behaviour. Atherosclerosis 2006;188(1):1-11.

[32] Saito M, Ishimitsu T, Minami J, Ono H, Ohrui M, Matsuoka H. Relations of plasma high-sensitivity C-reactive protein to traditional cardiovascular risk factors. Atherosclerosis 2003;167(1):73-9.
[33] Mohler ER, 3rd, Gannon F, Reynolds C, Zimmerman R, Keane MG, Kaplan FS. Bone formation and inflammation in cardiac valves. Circulation 2001;103(11):1522-8.
[34] Mohler ER, 3rd. Mechanisms of aortic valve calcification. Am J Cardiol 2004;94(11):1396-402, A6.
[35] Skowasch D, Steinmetz M, Nickenig G, Bauriedel G. Is the degeneration of aortic valve bioprostheses similar to that of native aortic valves? Insights into valvular pathology. Expert Rev Med Devices 2006;3(4):453-62.
[36] Jian B, Xu J, Connolly J*, et al.* Serotonin mechanisms in heart valve disease I: serotonin-induced up-regulation of transforming growth factor-beta1 via G-protein signal transduction in aortic valve interstitial cells. Am J Pathol 2002;161(6):2111-21.
[37] Dadlani H, Ballinger ML, Osman N, Getachew R, Little PJ. Smad and p38 MAP kinase mediated signalling of proteoglycan synthesis in vascular smooth muscle. J Biol Chem 2008.
[38] Katwa LC, Tyagi SC, Campbell SE, Lee SJ, Cicila GT, Weber KT. Valvular interstitial cells express angiotensinogen and cathepsin D, and generate angiotensin peptides. Int J Biochem Cell Biol 1996;28(7):807-21.
[39] Jian B, Narula N, Li QY, Mohler ER, 3rd, Levy RJ. Progression of aortic valve stenosis: TGF-beta1 is present in calcified aortic valve cusps and promotes aortic valve interstitial cell calcification via apoptosis. Ann Thorac Surg 2003;75(2):457-65.
[40] Durbin AD, Gotlieb AI. Advances towards understanding heart valve response to injury. Cardiovasc Pathol 2002;11(2):69-77.
[41] Mohler ER, 3rd, Chawla MK, Chang AW*, et al.* Identification and characterization of calcifying valve cells from human and canine aortic valves. J Heart Valve Dis 1999;8(3):254-60.
[42] Warnock JN, Burgess SC, Shack A, Yoganathan AP. Differential immediate-early gene responses to elevated pressure in porcine aortic valve interstitial cells. J Heart Valve Dis 2006;15(1):34-41; discussion 42.
[43] Lindroos M, Kupari M, Valvanne J, Strandberg T, Heikkila J, Tilvis R. Factors associated with calcific aortic valve degeneration in the elderly. Eur Heart J 1994;15(7):865-70.
[44] Vasan RS, Larson MG, Leip EP*, et al.* Impact of high-normal blood pressure on the risk of cardiovascular disease. N Engl J Med 2001;345(18):1291-7.
[45] Thomas F, Bean K, Pannier B, Oppert JM, Guize L, Benetos A. Cardiovascular mortality in overweight subjects: the key role of associated risk factors. Hypertension 2005;46(4):654-9.
[46] Libby P. Inflammation in atherosclerosis. Nature 2002;420(6917):868-74.
[47] Folkow B. Early versus late onset of therapy. Experiences from animal studies. Hypertension 1984;6(6 Pt 2):III185-7.
[48] Skov K, Mulvany MJ, Korsgaard N. Morphology of renal afferent arterioles in spontaneously hypertensive rats. Hypertension 1992;20(6):821-7.
[49] Wouters K, Shiri-Sverdlov R, van Gorp PJ, van Bilsen M, Hofker MH. Understanding hyperlipidemia and atherosclerosis: lessons from genetically modified apoe and ldlr mice. Clin Chem Lab Med 2005;43(5):470-9.

[50] Sudhir K, Hashimura K, Bobik A, Dilley RJ, Jennings GL, Little PJ. Mechanical strain stimulates a mitogenic response in coronary vascular smooth muscle cells via release of basic fibroblast growth factor. Am J Hypertens 2001;14(11 Pt 1):1128-34.
[51] Stanley AG, Patel H, Knight AL, Williams B. Mechanical strain-induced human vascular matrix synthesis: the role of angiotensin II. J Renin Angiotensin Aldosterone Syst 2000;1(1):32-5.
[52] Berk B, Vallega G, Muslin A, Gordon H, Canessa M, Alexander R. Spontaneously hypertensive rat vascular smooth muscle cells in culture exhibit increased growth and Na+/H+ exchange. J Clin Invest 1989;83:822-9.
[53] Griendling KK, Berk BC, Alexander RW. Evidence that Na+/H+ exchange regulates angiotensin II-stimulated diacylglycerol accumulation in vascular smooth muscle cells. The Journal of Biological Chemistry 1988;263(22):10620-10624.
[54] Figueroa JE, Vijayagopal P. Angiotensin II stimulates synthesis of vascular smooth muscle cell proteoglycans with enhanced low density lipoprotein binding properties. Atherosclerosis 2002;162(2):261-268.
[55] Shimizu-Hirota R, Sasamura H, Mifune M*, et al.* Regulation of vascular proteoglycan synthesis by angiotensin II type 1 and type 2 receptors. J Am Soc Nephrol 2001;12(12):2609-15.
[56] Little PJ, Ballinger ML, Osman N. Vascular wall proteoglycan synthesis and structure as a target for the prevention of atherosclerosis. Vascular Health Risk Management 2007;3:1-8.
[57] Skalen K, Gustafsson M, Rydberg EK*, et al.* Subendothelial retention of atherogenic lipoproteins in early atherosclerosis. Nature 2002;417(6890):750-4.
[58] Plutzky J. Inflammatory pathways in atherosclerosis and acute coronary syndromes. Am J Cardiol 2001;88(8A):10K-15K.
[59] Rosenhek R, Rader F, Loho N*, et al.* Statins but not angiotensin-converting enzyme inhibitors delay progression of aortic stenosis. Circulation 2004;110(10):1291-5.
[60] Egido J, Ruiz-Ortega M. Anti-inflammatory actions of quinapril. Cardiovasc Drugs Ther 2007;21(3):211-20.
[61] Chockalingam A, Venkatesan S, Subramaniam T*, et al.* Safety and efficacy of angiotensin-converting enzyme inhibitors in symptomatic severe aortic stenosis: Symptomatic Cardiac Obstruction-Pilot Study of Enalapril in Aortic Stenosis (SCOPE-AS). Am Heart J 2004;147(4):E19.
[62] O'Brien KD, Probstfield JL, Caulfield MT*, et al.* Angiotensin-converting enzyme inhibitors and change in aortic valve calcium. Arch Intern Med 2005;165(8):858-62.