Is there a role for activated platelets in progression of aortic valve calcification?

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This editorial refers to ‘Activated platelets promote an osteogenic program and the progression of calcific aortic valve stenosis’, by R. Bouchareb et al., on page 1362.

Because of population aging in most developed countries, the number of patients with degenerative calcific aortic valve stenosis (AS) will continue to grow. AS progresses asymptotically until the amount of restricted blood flow becomes significantly reduced, at which time the prognosis without intervention (surgical or transcatheter aortic valve replacement) is dismal. Also, the risk of stroke from embolized valvular debris (thrombus or endocarditis) is perhaps the most feared complication of this condition. While interventional therapies for AS have made tremendous progress, they remain costly and are not without significant risk. Pharmacotherapy to prevent disease progression would seem to be the ideal solution but, thus far, no agent has been shown to inhibit the progression of AS. In order to discover effective preventative therapies, more basic and translational research is needed to clarify the precise mechanisms of aortic valvular calcification with the goal of delaying or preventing calcific AS progression.

The aortic valve is typically composed of three leaflets, each consisting of three layers: the fibrosa, the spongiosa and the ventricularis. The cellular population of these aortic valve layers involves valvular endothelial cells, valvular interstitial cells (VICs), and fibroblasts. Endothelial cells cover the valvular surface and provide an interface between blood and valve. VICs, the major cell type, are present predominantly in the spongiosa and play a crucial role in valvular remodelling, regulating both the synthesis and degradation of the extracellular matrix. Prior evidence has suggested that AS and atherosclerosis share parallel mechanisms and risk factors including age, male gender, hypertension, dyslipidaemia, smoking, and diabetes. Previously, AS was thought to be a passive degenerative process; however, it is now considered to be an active and multifaceted process involving lipoprotein deposition, inflammation, oxidative stress, and osteogenic differentiation of VICs. Indeed, a prior pathological study including 256 surgically excised calcified aortic valves reported that mature lamellar bone formation, with haematopoietic elements and active bone remodelling, was identified in 34 cases (13.3%) (Take home figure, A–C). Histological studies have demonstrated lipoprotein accumulation and retention in calcifying aortic valves. Similar to the process of atherosclerotic calcification, infiltration of oxidized lipids into the valvular interstitium is reported to promote chronic inflammation and stimulate intracellular osteogenic signalling cascades, such as bone morphogenetic protein-2 (BMP2), runt-related transcription factor-2, msh homeobox 2, and the Wnt pathway in VICs. Transforming growth factor-β (TGF-β) and extracellular matrix overgrowth are also considered to be important contributors in the pathogenesis of aortic valve fibrosis and subsequent calcification. Possible other triggers for VICs’ osteogenic differentiation involve haemodynamic shear stress, reactive oxygen species, and inflammatory cytokines, e.g. tumour necrosis factor-α and interleukin-6 (IL-6).

In this issue of the European Heart Journal, Bouchareb et al. examine whether activated platelets might also directly promote valvular leaflet calcification during high shear stress in the setting of severe AS, using multiple experimental models including human aortic valve samples, isolated human VICs, and a calcified aortic valve calcification mouse model. Before addressing the paper, it should be made clear that the effect of AS on platelet function is not straightforward. On one hand, high shear created by the stenotic aortic valve promotes the proteolysis of high-molecular weight multimers of von Willebrand factor, which is associated with mucosal haemorrhage and acquired type 2A von Willebrand syndrome. Conversely, elevated shear may also increase the release of thrombin and activate platelets in patients with moderate to severe AS, though this finding has not been reproduced by others.11,12 Thus, the interplay between stenotic aortic valves and platelet function is not straightforward.

Bouchareb et al. examined scanning electron microscopy images of explanted mineralized and non-mineralized human AVs, and show that in areas of endothelial denudation on the aortic side of the valve, platelet–fibrin microaggregates were present on mineralized valves but not on non-mineralized valves. In vitro experiments showed that collagen type I, an important component of human AV-activated platelets, induced the osteogenic differentiation of VICs. More...
Take home figure (A–C) Bone formation within advanced aortic valve stenosis. Degenerative aortic valve leaflet from a 70-year-old woman who underwent surgical valve replacement for severe aortic valve stenosis. Low-power images of Movat Pentachrome (A) and haematoxylin and eosin staining (B) show calcific nodules on the aortic surface (black arrow). Top and bottom surfaces indicate the aortic and ventricular sides, respectively. (C) High-power image of haematoxylin and eosin stain from the boxed area in (B). Ossification (black arrow) and cartilaginous metaplasia (white arrow) are found at the edge of the calcific nodule. (D–E) Early aortic valve calcification resembles atherosclerotic changes. Aortic valve leaflet from a 47-year-old old man who died of accidental head trauma. (D) Low-power image of left coronary leaflet (Movat Pentachrome stain). (E) High-power images of the site of closure of the valve showing lipid insudation from the black box in (D). Haematoxylin and eosin stain image (E-1) shows atheromatous changes with lipid insudation (*) in the fibrosa. Von Kossa stain (E-2) highlights microscopic calcifications as black dots. Immunohistochemical stain for von Willebrand factor (E-3) and CD61 (E-4) showing the presence of endothelium with an absence of platelets in the areas of early calcification. (F–H) Early valve calcification is associated with an intact endothelial lining. Aortic valve from a 44-year-old man with a history of hypertension, diabetes, and hyperlipidaemia, who died suddenly from hyperglycaemic crisis. (F) Three-dimensional reconstructed micro-computed tomography image of the aortic valve. Calcification is observed at hinge area of the left and right coronary leaflets as high-density deposition (black arrows). (G) Low-power scanning electron microscopy image of the left coronary leaflet (aortic side) showing the smooth surface of the leaflet. (H) High-power scanning electron microscopy image of the left coronary leaflet surface from the black box area in (G) showing confluent endothelial cells without any areas of denudation. No platelets or inflammatory cells are observed. H&E, haematoxylin and eosin; LCL, left coronary leaflet; NCL, non-coronary leaflet; RCL, right coronary leaflet; vWF, von Willebrand factor.
specifically, adenosine diphosphate produced by platelets stimulated VICs through P2Y1 purinergic receptor (P2RY1) and enhanced autotaxin (ATX) release, which catalyzes lysophosphatidylcholine to lysophosphatic acid (LysoPA), consequently stimulating VIC osteogenic mineralization via binding to the lysophosphatic acid receptor-1 (LPAR1). Furthermore, administration of collagen-activated platelets to LDLR−/− apoB100/100 IGFII mice, which develop AS when fed a high-fat, high-sucrose diet, accelerated AS progression as assessed by Doppler echocardiography and fibro-calcification of the aortic valve leaflet. Although intracellular signalling mechanisms, by which P2RY1 enhances ATX production and LysoPA turns on the switch for osteogenic differentiation of VICs, were not deeply explored, previous work from this group has shown that LysoPA derived from oxidized low-density lipoprotein (LDL) and lipoprotein(a) promote inflammation and osteogenic mineralization by stimulating the nuclear factor-kappa B pathway, leading to IL-6 production and subsequent BMP2 signalling enhancement in VICs.13,14

ATX is a member of the ectophosphodiesterase/nucleotide phosphohydrolase (ENPP) family. ENPP1 and 3 catabolize extracellular ATP to produce inorganic pyrophosphate, a potent inhibitor of valvular and vascular calcification.15 Prior in vitro analysis of ENPP substrate hydrolysis indicated that ATX, also known as ENPP2, is the only enzyme in the ENPP family showing lysophospholipase D activity to synthesize LysoPA, but less pyrophosphatase activity.16

Thus, there is an important distinction between ENPP1/3 and ENPP2, because LysoPA is known as an important lipid mediator that exerts various physiological effects, particularly as an inducer of cell migration, proliferation, and survival through binding to six specific guanine nucleotide-binding protein (G protein)-coupled receptors (termed LPAR1-6) on the surface of various types of cells. In mammals, LysoPA is constantly produced and degraded by multiple metabolic pathways in the intra- and extracellular spaces, and its metabolic abnormality is presumed to be a cause of pathological conditions such as cancer invasion, atherosclerosis, and fibrosis. As mentioned above, the authors have previously showed that LysoPA derived from oxidized LDL promotes the mineralization of VICs. These implications: ATX, also known as ENPP2, is the only enzyme in the ENPP family showing lysophospholipase D activity to synthesize LysoPA, but less pyrophosphatase activity.16

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How much does this specific mechanism contribute to the initiation and/or progression of aortic valvular calcification? Our experience from human autopsy cases suggests that an early predilection of lesion development on the aortic side of valve leaflets occurs at two distinct anatomical locations: the valve attachment hinge region and near the line of valve closure, which may both be related to mechanical stress and strain due to the movement of leaflets. Like the atherosclerotic process, lesion progression appears to occur through the accumulation of lipids and free cholesterol; however, the infiltration of inflammatory cells, e.g. macrophages and T lymphocytes, is rarely observed in the early phase. In fact, lipid infiltration is the earliest event occurring within the fibrosa, giving an appearance resembling pathological intimal thickening (lipid pools), and not in the valve stroma. At this point, microcalcification is detected by calcium staining (von Kossa and/or Alizarin Red). In all early cases showing microcalcification, no endothelial denudation, platelet attachment, or aggregation is observed. (Take home figure, D–H). Thus, it is logical to conclude that the mechanism described in this paper applies not to the initial stages of valve mineralization but to the advanced stage of valvular calcification progression. As pointed out by the authors, one of the questions that it is logical to ask, but currently lacks an answer, is whether currently available antiplatelet agents could inhibit valvular and atherosclerotic calcifications. Further studies are needed to understand whether agents that specifically suppress platelet pathways, including P2RY1 and/or LPAR1, could affect the progression of AS and calcification. Although the current study suggests that such an approach may be useful, the practicality of putting patients, especially those of advanced age, on long-term antiplatelet therapies seems not to be a clinically viable strategy because of the bleeding risk. Given the complex relationship between platelet function and AS, and the relative paucity of animal models resembling human AS, the relative importance of this mechanism vs. the others discussed earlier remains uncertain.

In summary, the work by Bouchareb et al. suggests novel avenues for the treatment of degenerative valvular calcification: platelet activation, which promotes osteogenic differentiation in VICs. Without such efforts to understand the basic molecular mechanisms that underlie this progressive disease, specific targets for the treatment of calcific valvular degeneration will not be feasible and we will continue to rely on more invasive therapies as our only means of treatment.

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