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VALVULAR HEART DISEASE (V NKOMO, SECTION EDITOR)

Mechanisms of Calcification in Aortic Valve Disease: Role of Mechanokinetics and Mechanodynamics

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Abstract The aortic valve is highly responsive to cyclical and continuous mechanical forces, at the macroscopic and cellular levels. In this report, we delineate *mechanokinetics* (effects of mechanical inputs on the cells) and *mechanodynamics* (effects of cells and pathologic processes on the mechanics) of the aortic valve, with a particular focus on how mechanical inputs synergize with the inflammatory cytokine and other biomolecular signaling to contribute to the process of aortic valve calcification.

Keywords Calcific aortic valve disease · Calcification · Mechanobiology · Mechanokinetics · Mechanodynamics

Introduction

Aortic valve (AV) disease is a leading cause of cardiovascular morbidity in the USA and the developed world [1], and will likely increase in prevalence as life expectancy continues to rise in coming decades [2]. Biomechanical forces imposed during each cardiac cycle play a role in both the progression and the consequences of AV disease. In this Report, we borrow terms from the pharmacology literature to delineate AV *mechanokinetics* (how mechanical forces affect the valve cells and tissue) from AV *mechanodynamics* (how valve cells and resultant pathologic processes in the tissue affect the mechanics), to provide a snapshot of the key evolving concepts related to the biomechanical influences on the causes and consequences of calcific aortic valve stenosis and potential strategies to prevent the implicated mechanobiologic pathways. Although the emphasis will be on recently published science, technology and conceptual analysis, limited older work will be included when pertinent and necessary to provide context.

This article is part of the Topical Collection on *Valvular Heart Disease*

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AV Structure-Function: Cells, Matrix, and Whole Valve Dynamics in Health

The AV, situated at the junction of the left ventricle and the aorta, provides the check valve for blood leaving the heart for the systemic circulation. Because of high peripheral resistance that causes a substantial diastolic back pressure on the valve in the closed position, the AV exists in high-demand mechanical environment that requires a specialized architecture. The AV is made up of 3 equal sized leaflets or cusps that are shaped like half moons, hence the name 'semi-lunar' valve. Each cusp is a few hundred microns thick and is made up of 3 layers: fibrosa (near the aortic side), spongiosa, and ventricularis (near the ventricular side). Valvular structural specializations and tissue dynamics have been described in detail in several recent reviews [3••, 4••]. Valve function is enabled by a complex, highly differentiated, and highly responsive (both mechanically and biologically), dynamic tissue macro- and microstructure, consisting of a layered architectural pattern composed of cells (valvular endothelial cells [VECs] at the blood-contacting surfaces and deep valvular interstitial cells [VICs]) and extracellular matrix (ECM) (including collagen, elastin, and glycosaminoglycans [GAGs]). The fibrosa layer close to the outflow surface is the principal load-bearing layer and is enriched in type I collagen, which is highly aligned and

imparts specific and anisotropic (ie, different in the radial and circumferential directions) biomechanical responses [4••, 5]. The central layer (the spongiosa) is made up primarily of loose connective tissue rich in GAGs; this layer has been hypothesized to provide cushioning and lubrication between the 2 outer layers [6]. The layer facing the ventricular chamber (the ventricularis) is rich in elastin, which is believed to provide some recoil that assists cuspal closure [7].

The single layer of VECs lining the cuspal surfaces are distinct from endothelial cells that populate the inner surfaces of the aorta [8–11], and those covering the fibrosa have a different gene expression profile from those covering the ventricularis [12]. VECs may contribute to the regulation of valve calcification. Indeed, VECs covering the fibrosa have upregulated proteins expected to promote calcification, while VECs subjected to the ventricular hemodynamic waveform (ie, experienced in vivo by the VEC lining the ventricularis) have increased expression of the "atheroprotective" transcription factor Kruppel-like factor2 (KLF2) [13], expected to downregulate calcification.

In contrast, VICs comprise a heterogeneous collection of cells with variable and dynamic features characteristics of fibroblasts, smooth muscle cells, and myofibroblasts [3••, 14]. In the normal AV, most VICs exhibit a fibroblast-like phenotype. However, when VICs are subjected to certain biomechanical or biochemical stimuli, they become activated, demonstrating a myofibroblast phenotype, with contractile features and increased protein synthesis [15, 16]. Furthermore, under certain stimuli, VICs assume a phenotype that resembles that of osteoblasts (see below). ECM architecture and VIC phenotype are dynamic not only in response to altered environmental conditions, but also throughout in-utero maturation, growth and adulthood, and disease [17]. Relevant to the present discussion, aged valves have markedly decreased VIC cell density. Indeed, VIC cell density of aged adult valves is only approximately 10 % that of fetal valves [17]. Additionally, collagen fibers become progressively more aligned with age (ie, more characteristic of a diastolic configuration) suggesting that there is an ongoing "creep" of aortic valve structure during adult life, consistent with the progressive stiffening of valve cusps with increasing age [18, 19].

AV Mechanokinetics Leading to Calcification: Role of Interstitial Cell Plasticity in AV Pathobiology

Most recent work in AV disease has focused on *mechanokinetics*, or how mechanical forces alter cellular function. The mechanical environment of the cusps can directly affect VIC differentiation [3••, 14, 20]; mechanical forces relevant to the valve function are shear stress from passing blood, buckling stresses during opening and closing, and planar stress when valve is closed [4••]. These forces are effectively translated from the structural proteins of the valvular ECM to the cells as demonstrated by differences in VIC phenotype for the left-sided (aortic) and right-sided (pulmonary) semilunar valves [21]. Multiple studies have probed the effect of planar stress on VIC phenotype and function, primarily in the form of applied strain via in vitro bioreactors [22–24, 25••]. Studies have also examined the role of shear stress on VEC function and also studies of VEC-VIC communication [26, 27].

The hallmark of calcific aortic valve disease is the formation of nodules of calcific minerals (largely calcium phosphate, similar to the hydroxyapatite of bone) in the affected valvular tissue, and generally beginning in and most severely in the fibrosa [28], with frequent formation of bone within the calcific deposits (called osseous metaplasia) [29]. VICs associated with these calcific nodules exhibit an osteoblast-like phenotype and express extracellular bone matrix proteins. The cellular mechanisms involved in valvular calcific nodule morphogenesis are important and highly controversial; the current understanding is summarized in the recent publication from the NHLBI Aortic Stenosis Working Group on calcific aortic valve disease [30••].

Two hypotheses of calcific nodule morphogenesis from the resident VIC population dominate current thinking: the apoptotic/dystrophic calcification theory and the ossification theory (Fig. 1) [31, 32••]. Evidence suggests that these 2 approaches comprise a range of responses of VICs to biomechanical and biochemical stimulation and are not mutually exclusive. The apoptotic/dystrophic calcification theory describes a calcification mechanism in which cell injury is an important and early event. This mechanism is epitomized by the failure of bioprosthetic heart valves, in which calcification is initiated primarily within residual porcine aortic

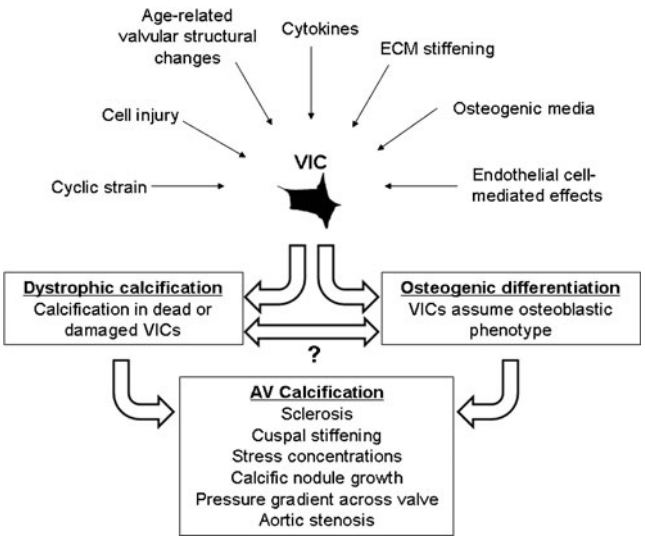

Fig. 1 Current hypotheses of aortic valve calcification mechanisms involving VICs. It is unclear if these pathways are mutually exclusive or if they are overlapping, and to what extent

valve or bovine pericardial cells that have been devitalized by glutaraldehyde pretreatment [3••, 33]. The key event involves reaction of calcium-containing extracellular fluid with the phosphorus-containing membranes of the non-functional cells, which are incapable of excluding calcium ions. Calcification of dead or damaged cells is called dystrophic calcification, and is considered a passive phenomenon. Calcification is accelerated by young valve recipient age and increased mechanical stress. In calcific AV disease, the increased mechanical stress on resident VICs induced by aging-related valvular remodeling and other mechanical and biochemical processes could play an important role in cell injury [3••, 30••]. The cytokine transforming growth factor-β1 (TGF-β1) induces rapid dystrophic calcification in vitro through an apoptosis mediated process [34–37]. Conversely, bone morphogenetic proteins 2 and 4 (BMP-2 and BMP-4) are potent osteogenic morphogens that are associated with the ossification theory, which specifies regulated osteogenic differentiation of VICs rather than cell injury as the key event, and thus an active calcification process [32••, 38–41]. It is unclear if these calcification processes either can occur independent of one another yet concurrently, or if they are somehow linked or interdependent, or if they are mutually exclusive. Moreover, there is debate on whether VICs can attain osteoblastic function directly or only by progressing through a myofibroblast phenotype first [42]. Rodriguez et al. found that the core of nodules contain different types of mineralization as well as apoptotic cells [43]. Also, Chen et al. described ossifying nodules that did not contain apoptotic cells, noting that different types of nodules may be forming [44]. It is evident that a more detailed description and understanding of the mechanisms of calcific nodule morphogenesis are needed, and further investigation of the behavior of VICs and their interactions (such as cell-cell tension) in nodule formation is essential in clarifying the mechanisms involved.

This notion of cell-cell tension and cell-ECM interactions implicates a key role for mechanics in calcification, and there is synergism between the cytokines mentioned above and mechanical inputs, which complicates the debate of dystrophic calcification vs ossification even further. While the complex effects of cytokines on VIC proliferation, repair, and injury have been explored [45, 46•, 47, 48], mechanokinetic studies of calcific nodule formation are in their infancy. In vitro studies have found clear dependence of VIC phenotype and/or calcific nodule formation on substrate chemistry [50, 51], substrate stiffness [32••, 52], and applied strain [37, 53], similar to the seminal work that describes the mechano-dependent differentiation of stem cells [49]. The majority of these studies focus on dystrophic calcification as a result of substrate chemistry or strain; however, Yip et al. successfully generated calcific nodules without apoptosis by culturing VICs on substrates of low stiffness [32••]. The model developed in this study is the only in vitro model available for generating ossific nodules.

AV Mechanodynamics Following Calcification: What do we Know About Stenosis Mechanisms?

Calcification, regardless of the mechanism by which it arises, restricts the motion of the leaflets, causing sclerosis (defined as early calcification without significant pressure gradient across the valve) and ultimately stenosis (with a significant pressure gradient). In addition, early calcification is likely to lead to increased mechanical loading on the non-calcified portion of the leaflet, which could initiate calcification in these regions via the mechanisms described above. This theory is supported by clinical observations in patients who are diagnosed with AV sclerosis by echocardiography or computed tomographic imaging, estimated to be approximately 25 % of patients at age 65 [54•]. Moreover, AV sclerosis is associated with an an approximately 50 % increased risk of myocardial infarction and cardiac death over 5 years, probably primarily because AV sclerosis serves as a marker for coronary artery atherosclerosis. Once even mild valve obstruction is present, progression to AV steniosis is virtually inevitable, sometimes occurring in as little as 4–8 years after initial diagnosis [55], although progression is variable among patients. Once a nidus of sclerosis emerges within a leaflet, the stiffening of the leaflet follows, likely due to 'strain magnification' experienced by the surrounding, initially healthy, tissue (as described mathematically in [37]). Briefly, if the leaflets were a supple, highly compliant sheet (like latex), then a focal area that becomes stiff would in turn lead to greater than normal (ie, concentrated) strain in the region directly outside of the focal area. If strain leads to mechanobiologic changes to the VICs, then this strain magnification would proceed to change the VICs in a ripple effect emanating from the focal area. Over a few years, the ripple effect alters the leaflet properties and decreases compliance, likely leading to stenosis. An initial attempt at a computational model of the gross progression of disease has been published [56].

Congenital bicuspid aortic valve (BAV), the most frequent gross developmental cardiovascular malformation in humans, has an increased propensity to calcification and stenosis. BAV is usually uncomplicated in early life but frequently eventuates in calcific aortic stenosis or regurgitation in later life [57]. The vulnerability of BAV to calcification is so great that although the prevalence of BAV is (only) approximately 1 % in the overall population, congenital BAV is the anatomic substrate in approximately half of adults who have surgery for calcific aortic stenosis. In addition, calcification of a BAV occurs approximately a decade earlier in these patients than in those who have an anatomically normal valve. The mechanistic basis for the propensity for accelerated calcification of a BAV is uncertain. Nevertheless, in a finite element model, bicuspid valve geometry presented much higher stresses compared with the tricuspid model, particularly in the central basal region of the conjoint cusp (+800 %) [57]. However, another

computational model has suggested that the cellular deformations are not significantly different in the 2 geometries in the calcification-prone region [58]. Interestingly, not only have mutations in the transcriptional regulator NOTCH1 been linked to human developmental valve abnormalities but also NOTCH1 signaling represses osteoblast-like calcification pathways [59, 60].

Quadricuspid aortic valves also occur, and calcific stenosis is unusual, although data are limited. Clinical and pathologic studies suggest that 1 %–2 % of surgically removed aortic valves are quadricuspid and that regurgitation is the predominant pathology [61, 62]. Whether there is a biomechanical reason accounting for the specific pathologic profile of quadricuspid valves is unknown.

Novel Mechanobiological Strategies to Prevent Calcification

While the specific mechanisms of calcification remain unclear, the clinical investigation of drugs and heart valve disease in the past 15 years has been both hopeful and cautionary. The hopeful story has been that lipid-lowering drugs (ie, statins) would prevent valve disease [63, 64], in a similar fashion to their effectiveness against atherosclerosis. In 2001, 2 retrospective observational clinical studies suggested that statins may inhibit progression of AV stenosis [65, 66]. However, prospective, randomized clinical studies appeared much less promising. A small double-blind, placebo controlled study showed no benefit of statins to reduce valve disease and a larger clinical study demonstrated, rather conclusively, that statins do not reduce major cardiovascular outcomes, including AV replacement, in patients with existing AV stenosis [67]. A more recent meta-analysis of 2,344 patients from previous trials shows that statins do not prevent the progression of AV stenosis [68•]. In light of these findings, the enthusiasm for statin therapy as a potential preventive treatment for valve disease has been severely dampened. It is possible, however, that statins initiated earlier in the natural history of valve calcification might slow or prevent disease, but this is a very difficult hypothesis to test. Recent in vitro data demonstrated that C-type natriuretic peptide prevents myofibroblast differentiation of VICs and was also found to be upregulated by Simvastatin [69], suggesting a mechanism by which benefit could accrue from statins. A very recent report found that single nucleotide polymorphisms in the lipoprotein(a) locus was highly correlated with AV calcification and was across all patient populations examined [70•]. Therefore, statin therapy may be more effective in patients with these polymorphisms.

One of the present authors (WDM) recently found 2 distinct pathways that may be selectively targeted to prevent calcification of VICs. First, the serotonin 2B receptor, which was implicated in the drug-induced valve disease [71] that occurred with Fen-Phen, pergolide, and cabergoline, can be antagonized to prevent non-canonical TGF-β1 signaling and calcification in vitro [72•]. Targeting of the serotonin 2B receptor is similar to the much more focused on angiotensin receptors for treatment of valve disease [73]. Jaffre et al. [74] demonstrated that the serotonin 2B receptor works in concert with the angiotensin II type 1 receptor, to mediate hypertrophic signaling in cardiac fibroblasts. Angiotensin II demonstrates a mechano-dependent signaling activation during ventricular pressure overload similar to that noted above for serotonin 2B, and increased angiotensin II signaling at the type 1 receptor results in an increase in TGF-β1 secretion and the subsequent downstream initiation of fibrosis. In the aforementioned study, Jaffre et al. conclusively demonstrated a necessary interdependence between serotonin 2B and angiotensin II type 1 receptors in regulating TGF-β1 secretion. Many of the cardiac-related studies into the molecular signaling pathways of the 2 receptors have focused on their role in ventricular fibroblasts. This strategy needs to be further examined in small animal models of valve disease, but selective targeting strategy combined with the clear arrest of TGF-β1 effects appears promising.

Second, the homophilic cadherin, cadherin-11, was found to be required for TGF-β1-induced calcific nodule formation in vitro [75••]. Because cadherin-11 is involved in bone formation, this provides a plausible common pathway between the ossific and dystrophic calcification pathways, and targeting cadherin-11 with functional blocking antibodies may be an additional strategy in preventing calcification. As the mechanism(s) becomes clear, there may be additional novel mechanobiologic targets that may prevent pathologic differentiation of VICs that leads to calcification.

Conclusions

There is still much to be examined in the mechanokinetics and mechanodynamics of heart valve calcification; however, in the past few years, there has been success in elucidating the early inflammatory mechanisms and potential intervention strategies to prevent these processes. A major challenge is relating in vitro findings to clinical directives through early detection of calcification. We believe that as mechanisms are better understood in the coming years, a molecular signature of early, pre-calcification that can be imaged in vivo may be possible. Further, if inflammatory processes in the VICs can be targeted and halted it may be possible to develop a non-surgical intervention strategy against calcific heart valve disease.

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Conflict of Interest W. David Merryman declares that he has no conflict of interest.

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- •• Of major importance

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