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Evidence of a Role for Tensile Loading in the Pathogenesis of Mitral Valve Degeneration

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Abstract

Degenerative mitral valve disease (DMVD) is significant cause of cardiovascular morbidity and mortality in humans and dogs. Diseased valves present altered architecture, and distinct pathological characteristics including cell proliferation with phenotype transformation and extracellular matrix turnover with net deposition of proteoglycans, disorganization of collagen and fragmentation elastin. The specific triggers and mediators of leaflet degeneration and chordal rupture are largely unknown. Heart valves are very active tissues, capable of sustaining heavy cyclical loads. Indirect clinical evidence and direct experimental evidence support a hypothesis that DMVD might be initiated by abnormal tensile loading on valvular cells that in turn respond by inappropriate remodeling of the valve matrix. In this review, we present *in vivo* and *in vitro* studies linking leaflet or cellular strain to extracellular matrix turnover and expression of myxomatous markers similarly to DMVD. In addition, we discuss additional forces and stimuli that can act as mediators of myxomatous degeneration. Future studies elucidating mechanosensing signaling pathways involved in DMVD will be important to advancing understanding of its pathogenesis.

Keywords: Degenerative mitral valve disease; Tensile loading; Serotonin

Introduction to Degenerative Mitral Valve Disease

Valvular heart disease (VHD) accounts for 20,000 deaths and over 90,000 hospitalizations at a cost of over $9 billion annually, according to the National Institutes of Health. According to the American Heart Association, mitral valve disease was the direct cause of death in 2,759 patients and a contributing cause in 6,600 patients in 2003 [1]. Degenerative mitral valve disease (DMVD) is regarded as an important VHD and is the most common reason for mitral valve surgery [2]. About 65,000 open heart valve repair or replacements are performed annually in the US, with a third of these being performed on the mitral valve. By any measure, DMVD is a significant cause of cardiovascular morbidity and mortality in humans. DMVD is also a common cardiac disease in dogs[3], accounting for approximately 40% to 75% of all canine heart disease [4,5]. Small breed and older dogs (older than 5 years of age) have a higher incidence of myxomatous pathology [4]. The prevalence of DMVD in dogs 5 to 8 years of age on postmortem exam is approximately 10% and in dogs 9 to 12 years of age is approximately 20% [6]. Despite the importance of DMVD, studying the pathogenesis of DMVD has been challenging as the disease develops over a very long time course.

A formal definition of what exactly constitutes DMVD is lacking [7,8]. DMVD is grossly characterized by: 1) mitral regurgitation usually secondary to leaflet prolapse or flail, 2) gross thickening of the leaflets with chordal lengthening or rupture, and/or 3) myxomatous pathology in the valve apparatus. DMVD is further described based on valve architecture. Barlow [9] described the importance of specific differences in nomenclature. In billowing mitral valves the chordae are lengthened and the leaflets "billow" into the left atrium. Billowing mitral valves can also occur with valve prolapse, where the opposing leaflet edges do no close and cause valve regurgitation and incompetency. If billowing is extreme with very voluminous leaflets and elongated chordae, valves are floppy or have undergone myxomatous degeneration. Floppy or myxomatous valves can occur with prolapse or flail (chordal rupture, causing the leaflet edges to prolapse in the atrium). Lastly, mitral regurgitation can happen without valve billowing due to papillary muscle dysfunction, chordal rupture causing prolapse and/or annular dilation.

It is also important to understand differences between normal and degenerative valves at both the tissue and cellular level. Normal and degenerative valves are fundamentally different with regards to pathology and morphology (which can include valvular redundancy, prolapse and dysfunction). The normal mitral valve is structurally complex. It is composed of the leaflets, the annulus, the chordae tendineae and papillary muscles [10]. The valve is attached proximally at the left atrioventricular junction and distally, through papillary muscles, to the ventricular wall. The leaflets constitute a continuous veil inserted around the perimeter of the atrioventricular junction. The anterior leaflet is also called the septal, medial, or aortic leaflet, while the posterior leaflet is referred to as the lateral, marginal, or mural leaflet (distinctive nomenclature based on the positions). The annulus is a fibrous ring composed of dense connective tissue, which extends into the leaflets to form the fibrous layer (fibrosa). The chordae tendineae and papillary muscles constitute the tension apparatus of the valve. The chordae originate from the apical portions of the papillary muscles or directly from the ventricular wall. Degenerative valves typically present increased diameter, leaflet thickness and surface area. Nodules of myxomatous tissue start forming along the leaflet free edges and chordal elongation and rupture can be observed. Changes in degenerative valve leaflets are shown in Figure 1.

Histologically, the normal mitral leaflet is comprised of 3 distinct layers, atrialis, spongiosa, and fibrosa. The chordae tendineae are extensions of the fibrosa [10]. Each of these structures has been ascribed

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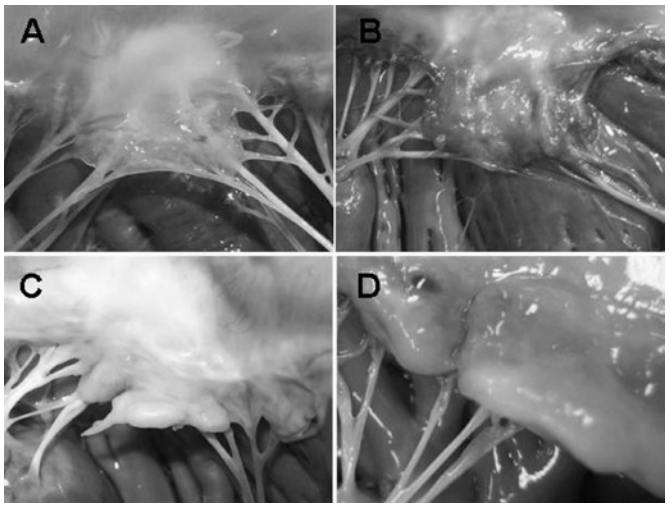

Figure 1: Mitral valve anatomy showing the anterior leaflet and chordae tendineae. Each box represents a different disease stage: (a) normal valve; (b) mild degeneration; (c) intermediate degeneration; and (d) severe degeneration.

functional roles that counter mechanical loading on the valve including flow-generated shear (atrialis), compression and flexure (spongiosa), and tensile loading (fibrosa and chordae tendineae) [11]. Each valve layer is enriched in a specific extracellular matrix (ECM) component that counters these biomechanical forces including elastin (atrialis), proteoglycan/glycosaminoglycan (spongiosa) and collagen type I (fibrosa and chordae tendineae). Degenerative valves present a large central zone of loose connective tissue with the main characteristics of intense disorganization and fragmentation of the collagen and elastin layers, in addition to increased thickness of the glycosaminoglycan layer. The valve interstitial cell (VIC) is the principal cell type in the heart valve responsible for maintenance of the ECM [12,13]. VICs present two known characteristic phenotypes: fibroblast and myofibroblast [14,15], each of which has an important and specific role in pathologic and developmental processes [16,17]. Myofibroblasts or "activated VICs" have been implicated as mediators of the pathologic changes associated with DMVD [15], however direct evidence is lacking. Recent literature suggests that there are five identifiable phenotypes of VICs, including embryonic progenitor cells, quiescent VICs (qVICs), activated VICs (aVICs), progenitor VICs (pVICs), and osteoblastic VICs (obVICs) [18]. It has also been proposed that altered mechanical forces induce an aVIC phenotype via activated valve endothelial cell (VEC) Mechanotransduction [18].

Myxomatous pathology is generally described by VIC proliferation, net ECM degradation (with collagen bundle disorganization, elastic fiber fragmentation, and PG/GAG deposition) and phenotype transformation to aVIC [8,13]. Myxomatous leaflets show gross abnormalities in the collagen layer, with increasing deposition of myxomatous lesions along the free edge and maximal destruction around the areas of chordal insertion [10]. Mitral valve flail can be explained as an overall weakness of the collagen layer, which allows for leaflet redundancy and chordal rupture in abnormal hemodynamic conditions [10]. Known mediators of of ECM remodeling in DMVD involve: 1) deposition of decorin and other PGs and hyaluronan [19,20]; and 2) degradative enzymes including matrix metalloproteinases (MMPs) [16, 21] and elastases (cathepsins K and S) [16]. Markers of phenotype transformation [16, 21] are desmin, α-SMA (alpha smooth muscle actin) and SMemb (nonmuscle myosin heavy chain B).

Mechanical Environment of the Mitral Valve

Heart valves are subjected to four mechanical force s [11]. These are imposed cyclically as the valve opens (flexure), permits blood to pass (shear), closes (flexure), and prevents the reverse flow of blood (tension and compression). The closed valve maintains a balance of tensile and compressive loads, where the chordae and the flat central region of the anterior leaflet are in tension, and the free edge of the anterior leaflet and the posterior leaflet are in appositional compression. Valve leaflets cycle between a fully unloaded to a fully loaded state every cardiac cycle. Biomechanical characterization of stress-strain in *in vivo* normal mitral valves has been widely studied and reviewed [22, 23]. Normal mitral valve leaflets present heterogeneous deformations and large anisotropic strains. The central region usually demonstrates more consistent and homogeneous strains, and the leaflet edge shows more inconsistent deformations and mechanics. Surface strains are heterogeneous due to different regions having different material properties related to ECM composition and regions of chordal insertion [22]. Peak strain rates typically occur near full closure or full opening. Mean peak circumferential strains range from 2.5% to 3.3%, areal strains range from 15% to 20%, and mean peak radial strains range from 16% to 22% [23].

The magnitude of tensile strain on anterior mitral valve leaflets are substantial and mostly noted during valve closure[24]. As the mitral valve closes and is subjected to high transvalvular pressure, the leaflet is stretched to the point where collagen fiber undulations are removed. This results in substantial stiffening of the tissue, to the point where further leaflet deformations are prevented, suggesting that the anterior leaflet does not function as a simple coapting membrane structure; but rather deforms in a complex manner [24].At the molecular level, the collagen fiber network of the valve is finely tuned to allow for sufficient initial compliance to allow for adequate leaflet coaptation, followed by a dramatic stiffening to prevent further deformation, which would lead to valve billowing. Abnormal valve strains generated by increased transvalvular pressures can be a leading cause of collagen imbalance and consequent valve billowing, prolapse and regurgitation. These may ultimately translate to the molecular level as myxomatous remodeling.

DMVD is fundamentally a disease of dysfunction of ECM homeostasis. Heart valves are the most heavily loaded tissues in the human body, flexing between opened and closed 30 million times per year, which subjects the leaflets to large tissue strains and hemodynamic stresses. It is a well-established biological principle that mesenchymal cells respond to mechanical stimuli [25] by remodeling their ECM. It is equally clear that inappropriate mechanical loading can mediate pathological processes. Any or all of the above-mentioned mechanical forces, if inappropriate, could play a role in the pathogenesis of DMVD. A current general hypothesis is that inappropriate tensile strain on heart valve cells contributes to initiation/propagation of myxomatous gene expression patterns through unidentified mechanosensing signaling mechanisms.

Indirect Evidence for Tensile Strain and DMVD

Empirical evidence for the tensile strain hypothesis comes in part from clinical data. Hypertension [26,27] and heritable connective tissue (CT) disorders [28,29] (e.g. Marfan's syndrome) are known risk factors for VHD, including DMVD. Each can be reasoned to cause increased tensile strain on valve interstitial cells (VIC); the former by increasing total mechanical stress in the hemodynamic environment and the later because of inherent weakness of the supporting matrix. Moreover, there is evidence that cell proliferation and TGFβ signaling

are activated in Marfan syndrome [30], which can be directly correlated with enhanced myxomatous signaling mechanisms due to a weakened ECM. Progressive weakening of ECM associated with aging could explain its association with degenerative heart valve disease [31]. Thus, pathologic tensile stain is a logical candidate for an initiating mechanism of DMVD.

Further support for a tensile stain hypothesis comes from tensile strain analyses of myxomatous valves. Biomechanical studies of myxomatous mitral valve leaflets revealed that they are more extensible and less stiff than normal leaflets [32]. It was also found that the total supported tension was much more compromised in the chordae than the leaflets, suggesting that myxomatous mitral valve disease affects the load-bearing capacity of the chordae more than it does the leaflets. Failure strength of the myxomatous leaflets decreases very little [32], despite the large increase in cross-sectional area, implying that the number or strength of load-bearing fibers changes very little. These results indicate that myxomatous leaflets still need to maintain their load-bearing capability to counteract environmental tensile strains, despite the common observations suggesting the overall collagen network degradation and loss of tissue strength.

A biomechanical study comparing myxomatous mitral valves with mitral valves obtained from patients with congestive heart failure (CHF)found that CHF mitral valves were less extensible, stiffer and less viscous compared to normal mitral valves, whereas myxomatous mitral valves were more extensible, less stiff, and more viscous than normal valves [33]. These biomechanical changes correlated with histological changes of increased fibrosis in CHF mitral valves and increased GAG content in myxomatous mitral valves. It was concluded that remodeling of CHF mitral valves was the result of high tensile loading whereas remodeling of myxomatous mitral valves was the result of low tensile loading. However tensile loading was not measured in this study and a pathophysiologic rationale for why CHF mitral valves might be subjected to high tensile loading and myxomatous mitral valves subjected to low tensile loading was not offered. This study does suggest that biomechanical loading of mitral valves in various pathologic conditions is likely complex..

Valvular Strain Studies and Expression of Myxomatous Markers

Evidence that altered mechanical stimulation of the mitral valve can lead to leaflet remodeling is an *in vivo* study of mitral regurgitation [34]. In this ovine model, regurgitation was created by surgically punching the posterior mitral leaflet. Collagen remodeling was identified with a reduction in collagen type I content and an increase in collagen type III. Consistent with myxomatous remodeling, several other markers were up-regulated in the regurgitation model (the proteoglycan decorin, MMP1, MMP9, and nonmuscle myosin, confirming VIC transformation). The tensile strain hypothesis is that collagen remodeling to flexible reticular collagen along with annular dilation might induce greater stresses to reach coaptation.

In vitro studies of stretched mitral VIC constructs or mitral leaflets have demonstrated that tensile strain can induce synthesis of myxomatous effect or proteins. A study of stretched mitral leaflets used static strain to determine expression of endothelin 1 and its receptors [35]. It has been shown that the release of nitric oxide and endothelin 1 from the vascular endothelium is elevated in association with increased stress loads such as mechanical stretch and shear stress. However, this study only identified increased expression of endothelin receptor B, and no difference in levels of endothelin receptor A and endothelin 1. These results can be attributed to main limitations of this study, such as, short treatment time (6 h) and static strain (not physiologic for a heart valve). In addition, a more comprehensive investigation of myxomatous markers needed to be performed.

VIC mounted on a 3D scaffold and exposed to static tensile strain produce and retain chondroitin/dermatan 4- and 6-sulfate within their scaffold, and secrete hyaluronan, 6-S and unsulfated chondroitin/dermatan into the culture medium in total GAG at magnitudes close to those found in native heart valve tissues [36]. Presence or absence of 10% tensile strain produces distinct effects on the VIC production of different GAG classes. A follow-up study used the same system to characterize the expression of specific proteoglycans during static strain of 3D cell-seeded scaffolds [37]. VICs were found to produce decorin, biglycan and versican, which were retained within the scaffold and secreted into the culture medium. The amount of decorin and biglycan retained in the constructs increased with the application of static strains, whereas versican decreased under the same conditions. With a more refined cyclic strain model, the same research group used cell-seeded constructs subject to different strain levels at 1.17 Hz for 24 h in culture. It was found that VIC from both leaflet and chordae secrete abundant GAG in response to strain [38, 39]. Mitral leaflet and chordal VICs showed stretching induced up regulation of total GAGs, decorin and biglycan, followed by a relaxation-induced decrease in GAGs. Based on findings in this study, the authors proposed that VIC from chordae may be particularly responsive to mechanical strains.

Our research group has used a custom-built bioreactor (Figure 2) to cyclically stretch anterior mitral valve leaflet sections radially (unpublished data). We have applied 10%, 20% and 30% strains to freshly harvested ovine and canine anterior mitral valves. We used radial strain data [23] to categorize these groups into low physiological, high physiological and pathological stresses, respectively. We have investigated the effect of different strain levels on myxomatous markers, specifically, markers of phenotype transformation (smooth muscle actin and nonmuscle myosin) and ECM turnover (MMP1, MMP13, cathepsin K, decorin and xylosyl tranferase). Using immunoblotting, we observed that pathologic cyclic strain significantly increases the

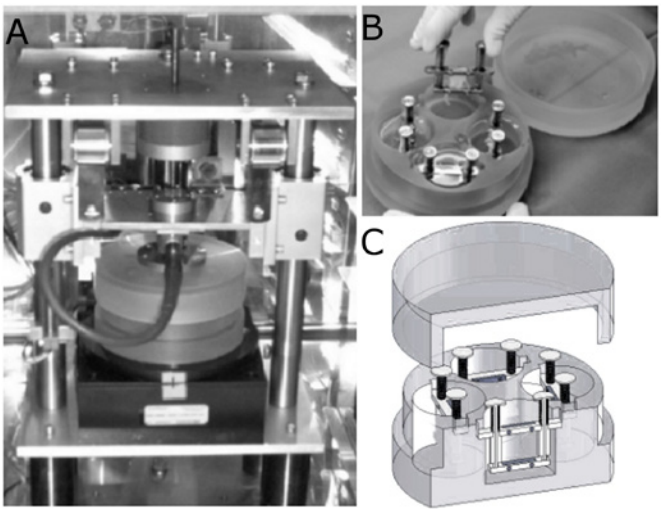

Figure 2: Experimental setup for valve leaflet stretch in culture. (a) Cyclic stretch machine and stretch chamber containing valves in culture. (b) Mitral valve leaflet mounted on leaflet holder and placed into stretch chamber. (c) Stretch chamber drawing with detail of the leaflet holder.

expression of all these markers of myxomatous degeneration. However, we believe that expression of these effector proteins is a downstream consequence of abnormal mechanical loading and that there is a need to identify signaling mechanisms that directly link the sensing of a mechanical signal to tissue remodeling.

Involvement of other Mechanical Forces and Stimuli

This review focuses on the role of abnormal tensile force as an initiator or mediator of DMVD. Although other force components likely act in conjunction with tensile forces, in our view modeling and study of isolated biomechanical forces is useful. Ultimately full understanding of the pathogenesis of myxomatous degeneration will require study of the role of other biomechanical stimuli.

Hypertension increases tensile stresses, but can also affect all other force components in the valve. A few studies are available determining a role of shear stresses in DMVD [40-42]. In addition to mechanical loading, other factors might also act as initiators, each of which deserving its isolated study to determine its role. Other known potential role players in DMVD are pre-existing tissue abnormalities, altered hemostasis, platelet function, and oxidative stress [43]. In any case, it is unknown whether these changes are triggers or propagators of DMVD, or if they predispose the valve to altered mechanical stresses and enhance disease propagation.

In addition, it must be noted that not all modifications in the mitral valve loading conditions will lead, in the long term, to a pathological deficit in mitral valve function. Moreover, not all clinical patients with abnormal intra-cardiac or systemic pressures develop DMVD. It is important to consider that compensatory local and systemic events may play an important role *in vivo*, leading heart valves to adaptively respond to loading patterns by remodeling their microstructure and matrix components. These mechanisms are absent in *in vitro* models, which complicates the direct translation of *in vitro* findings.

While these hypotheses are focused on tensile stresses, they do not exclude the possibility of mechanosensing signaling mechanisms triggered by other forms of inappropriate mechanical loading (e.g. shear strain). In conclusion, there is diverse evidence linking abnormal tensile strain to the initiation of myxomatous degeneration in DMVD. The use of *in vitro* models of cyclic valve strain is a useful tool to unveil signaling mechanisms that mediate expression of key effector proteins associated with myxomatous degeneration. An important next step is to identify the specific cell mechanoreceptors and mechanosensing signaling mechanisms with the ultimate goal of developing drug treatments to prevent or slow progression of DMVD.

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