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Research Paper

Evidence of adaptive mitral leaflet growth

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ABSTRACT

Ischemic mitral regurgitation is mitral insufficiency caused by myocardial infarction. Recent studies suggest that mitral leaflets have the potential to grow and reduce the degree of regurgitation. Leaflet growth has been associated with papillary muscle displacement, but role of annular dilation in leaflet growth is unclear. We tested the hypothesis that chronic leaflet stretch, induced by papillary muscle tethering and annular dilation, triggers chronic leaflet growth. To decipher the mechanisms that drive the growth process, we further quantified regional and directional variations of growth. Five adult sheep underwent coronary snare and marker placement on the left ventricle, papillary muscles, mitral annulus, and mitral leaflet. After eight days, we tightened the snares to create inferior myocardial infarction. We recorded marker coordinates at baseline, acutely (immediately post-infarction), and chronically (five weeks post-infarction). From these coordinates, we calculated acute and chronic changes in ventricular, papillary muscle, and annular geometry along with acute and chronic leaflet strains. Chronic left ventricular dilation of 17.15% ($p<0.001$) induced chronic posterior papillary muscle displacement of 13.49 mm ($p=0.07$). Chronic mitral annular area, commissural and septal–lateral distances increased by 32.50% ($p=0.010$), 14.11% ($p=0.007$), and 10.84% ($p=0.010$). Chronic area, circumferential, and radial growth were 15.57%, 5.91%, and 3.58%, with non-significant regional variations ($p=0.868$). Our study demonstrates that mechanical stretch, induced by annular dilation and papillary muscle tethering, triggers mitral leaflet growth. Understanding the mechanisms of leaflet adaptation may open new avenues to pharmacologically or surgically manipulate mechanotransduction pathways to augment mitral leaflet area and reduce the degree of regurgitation.

1. Introduction

Mitral regurgitation, the inability of the mitral valve to close properly, is associated with increased morbidity and mortality (Yiu et al., 2000). Functional mitral regurgitation, as seen in patients with ischemic or idiopathic dilated cardiomyopathy, is a result of annular and subvalvular alterations secondary to left ventricular remodeling (Komeda et al., 1997; Otsuji et al., 1997).

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A recent study has shown that mitral leaflets of patients with ischemic or idiopathic dilated cardiomyopathy are significantly larger than leaflets of normal patients (Chaput et al., 2008). The same study demonstrated that patients with enlarged leaflets display a smaller degree of regurgitation than patients with normal leaflets. These findings motivate the hypothesis that adaptive mitral leaflet growth might be a compensatory mechanism for left ventricular remodeling.

In a controlled ovine model of tachycardia-induced dilated cardiomyopathy, mitral leaflets elongated chronically in the radial direction (Timek et al., 2006). In an ovine model with inferior myocardial infarction, the entire leaflet area increased in response to left ventricular remodeling (Chaput et al., 2009). In an ovine model of isolated apical papillary muscle displacement, not only leaflet area but also leaflet thickness increased chronically in response to elevated mechanical stresses induced by papillary muscle tethering (Dal-Bianco et al., 2009). The same study reported significant changes in cell phenotype, secondary to the reactivation of embryonic gene profiles. Taking advantage of mechanotransduction is a powerful approach to endogenously engineer new tissue (Jaalouk and Lammerding, 2009; Zöllner et al., 2012). This concept has been applied successfully to induce controlled in situ growth of thin biological membranes in plastic and reconstructive surgery (Buganza Tepole et al., 2011; De Filippo and Atala, 2002). The potential to manipulate leaflet area by stretch would open exciting new avenues for a pharmacologically or surgically targeted reduction in the degree of mitral regurgitation.

While recent technologies enable the precise characterization of leaflet area in vivo using three-dimensional echocardiography (Dal-Bianco et al., 2009), these methods are not suited to quantify chronic regional and directional leaflet growth on a local level. Since leaflet valves display strong regional and directional variations (Prot et al., 2010; Smuts et al., 2011), this regional and directional information could provide valuable insight into the mechanistic origin of leaflet growth. Here, rather than using global information from standard imaging technologies, we follow discrete anatomic landmarks over an extended period of time (Bothe et al., 2011; Kvitting et al., 2010). This allows us to precisely quantify regional and directional growth across the entire anterior mitral leaflet (Göktepe et al., 2010). Using a chronic infarct ovine model, we test the hypothesis that chronic leaflet stretch, induced by papillary muscle tethering and annular dilation, triggers a chronic increase in leaflet area. We further hypothesize that this leaflet area growth is a result of both circumferential and radial leaflet elongation.

2. Materials and methods

All animals received humane care in compliance with the Principles of Laboratory Animals Care formulated by the National Academy of Sciences and published by the National Institute of Health. This study was approved by the Stanford Medical Laboratory Research Animals Review Committee and conducted according to Stanford University policy.

2.1. *Surgical preparation*

We premedicated five Dorsett hybrid sheep (71±5 kg) with ketamine (25 mg/kg intramuscularly), anesthetized them intravenously with sodium thiopental (6.8 mg/kg IV), and maintained anesthesia with inhalational isoflurane (1–2.5%). Through a left thoracotomy, we established access to the heart. We placed eight markers onto the epicardial surface of the left ventricle along four equally spaced longitudinal meridians and added a ninth marker at the apex. To induce controlled myocardial infarction, we placed polypropylene 2-0 sutures around the second and third obtuse marginal branches of the left circumflex coronary artery and snared them loosely (Llaneras et al., 1993).

On cardiopulmonary bypass with the heart arrested, we sewed additional markers onto the tips of both papillary muscles, two markers each, to the anterior, lateral, and posterior portions of the endocardium in the equatorial plane. Last, we sewed a total of 17 markers to the mitral valve, 8 to the annulus, 5 to the anterior leaflet, and 4 to the posterior leaflet, see Figs. 1 and 2. To measure the left ventricular pressure, we used a micromanometer pressure transducer (PA4.5-X6, Konigsberg Instruments Inc., Pasadena, CA). Once the animals were weaned off cardiopulmonary bypass, we externalized the tourniquets for the coronary artery snares through the fifth intercostal space and buried them in a subcutaneous pocket.

2.2. *Experimental protocol*

After 8±2 days, we took the animals to the cardiac catheterization laboratory, where we sedated them intravenously with ketamine (1–4 mg/kg/h) and diazepam (5 mg), and maintained sedation with inhalational isoflurane (1–2.5%). With the animal in the right decubitus position, we acquired baseline marker coordinates via biplane videofluoroscopy at a sampling frequency of 60 Hz (Philips Medical Systems, Pleasanton, CA). Simultaneously, we recorded aortic pressure, left ventricular pressure, and ECG signals. Following medication with lidocaine (100 mg IV), bretylium (75 mg IV), and magnesium (3 g IV), we tightened the coronary snares and verified complete vessel occlusion angiographically. Immediately post-infarction, we

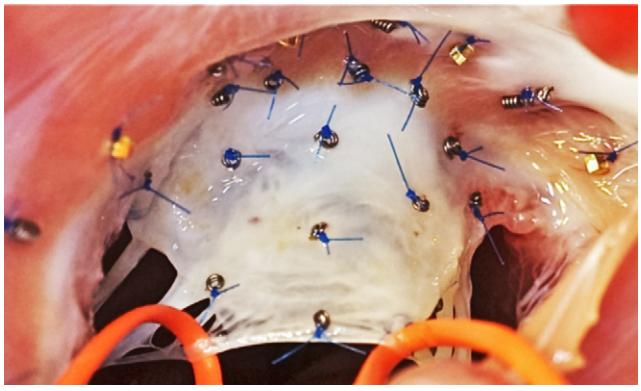

Fig. 1 – Intraoperative photograph of the mitral annulus with the anterior mitral leaflet. Biplane videofluoroscopic imaging allows a precise spatial and temporal reconstruction of mitral valve dynamics both acutely and chronically.