Mitral Leaflet Remodeling in Dilated Cardiomyopathy

Tomasz A. Timek, MD; David T. Lai, FRACS; Paul Dagum, MD, PhD; David Liang, MD, PhD; George T. Daughters, MS; Neil B. Ingels Jr, PhD; D. Craig Miller, MD

Background—Normal mammalian mitral leaflets have regional heterogeneity of biochemical composition, collagen fiber orientation, and geometric deformation. How leaflet shape and regional geometry are affected in dilated cardiomyopathy is unknown.

Methods and Results—Nine sheep had 8 radio-opaque markers affixed to the mitral annulus (MA), 4 markers sewn on the central meridian of the anterior mitral leaflet (AML) forming 4 distinct segments S1 to S4 and 2 on the posterior leaflet (PML) forming 2 distinct segments S5 and S6. Biplane videofluoroscopy and echocardiography were performed before and after rapid pacing (180 to 230 bpm for 15 ± 6 days) sufficient to develop tachycardia-induced cardiomyopathy (TIC) and functional mitral regurgitation (FMR). Leaflet tethering was defined as change of displacement of AML and PML edge markers from the MA plane from baseline values while leaflet length was obtained by summing the segments between respective leaflet markers. With TIC, total AML and PML length increased significantly (2.11 ± 0.16 versus 2.43 ± 0.23 cm and 1.14 ± 0.27 versus 1.33 ± 0.25 cm before and after pacing for AML and PML, respectively; *P* < 0.05 for both), but only segments near the edge of each leaflet (S4 lengthened by 23% and S5 by 24%±18%; *P* < 0.05 for both) had significant regional remodeling. AML shape did not change and no leaflet tethering was observed.

Conclusion—TIC was not associated with leaflet tethering or shape change, but both anterior and posterior leaflets lengthened because of significant remodeling localized near the leaflet edge. Leaflet remodeling accompanies mitral regurgitation in cardiomyopathy and casts doubt on FMR being purely “functional” in etiology. (Circulation. 2006; 114[suppl I]:I-518–I-523.)

Key Words: cardiomyopathy ■ mitral regurgitation ■ mitral valve

Dilated cardiomyopathy and heart failure are frequently associated with mitral regurgitation, which is an ominous harbinger of increased morbidity and mortality.1,2 Mitral insufficiency of heart failure has been considered “functional” (FMR), and research efforts have focused mainly on ventricular and annular remodeling as the underlying cause. It is now widely held that FMR in heart failure is a “ventricular” disease,3 because the valve tissue appears grossly normal; however, there may be more than meets the eye. The normal mitral leaflets have rich afferent and efferent innervation,4 intrinsic contractile properties,5 and a gradient of collagen fiber orientation for optimal stress distribution.6 Thus, mitral leaflet tissue may be an active participant in mediating timely and efficient valve closure rather than a passive, inert bystander as held by current dogma. Recent studies of human mitral valves from patients with end-stage heart failure lend support to this notion. Leaflet tissue from these patients was found to have significant biochemical and structural derangements7 as well as altered mechanical properties.8 Therefore, leaflet remodeling may contribute to the pathogenesis of heart failure-associated FMR such that the cause of FMR may not entirely be “functional.”

Although biochemical and mechanical data suggest inhomogeneous structure and deformation of the mitral leaflets in normal valves9 and significant microscopic remodeling in heart failure,7 in vivo assessment of leaflet remodeling has not been reported. These data are lacking because current imaging techniques are still unable to assess and track changes in regional and global leaflet geometry and shape. We used the myocardial marker method to evaluate anterior and posterior leaflet geometry changes during the evolution of heart failure in an ovine model of tachycardia-induced cardiomyopathy (TIC).

Materials and Methods

Surgical Preparation

The current data are derived from an experiment previously published,10 and details are only briefly summarized here. Nine adult sheep (79 ± 10 kg) were premedicated with ketamine (25 mg/kg intramuscularly) for venous and arterial line placement, monitored, and anesthesia was induced with sodium thiopental (6.8 mg/kg intravenous). The animals were intubated, mechanically ventilated (Servo Anesthesia Ventilator Siemens-Elema AB), and anesthesia was maintained with inhalational isoflurane (1% to 2.2%). A left...
thoracotomy was performed. Eight miniature tantalum were inserted beneath the left ventricular (LV) epicardial surface along 4 equally spaced longitudinal meridians for LV volume determination with one marker placed at the LV apex. After establishment of cardiopulmonary bypass and cardiopлегic arrest, 4 markers were sewn along the central meridian of the anterior leaflet (1 to 4; Figure 1) and 2 along the central meridian of the posterior leaflet (5 and 6; Figure 1). Eight tantalum markers were sutured to delineate the circumference of the mitral annulus (7 to 14; Figure 1, with marker 7 at the mid-septal position of the leaflet edges, distance from each leaflet edge marker should be least affected by LV dilatation. To further assess the extent and width of the regurgitant jet and categorized as none (0), mild (+1), moderate (+2), moderate to severe (+3), or severe (+4).

Data Acquisition
Images were acquired with the animal in the right lateral decubitus position with a Phillips Optimus 2000 biplane Lateral ARC/Poly DIAGNOST C2 system (Phillips Medical Systems, North America, Pleasanton, Calif) with the image magnification set in the 9-inch fluoroscopic mode. Marker image positions from the 2 simultaneous fluoroscopic views were digitized and merged to yield 3-dimensional coordinates for each radiopaque marker every 16.7 ms using custom-designed software.11 Synchronized ascending thoracic aortic pressure, LV pressure, and ECG voltage signals were recorded simultaneously during videographic data acquisition.

Pacing Protocol
After baseline data acquisition, while still sedated, a rapid pacing pulse generator (Prodigy S 816; Medtronic Medical, St. Paul, Minn) was inserted into a subcutaneous pocket and connected to the previously externalized LV electrode, and the animal recovered. Rapid pacing was initiated 24 hours later. During the pacing period, interval transthoracic echocardiography was performed to assess LV dimensions, systolic LV performance, and MR (with the pacemaker temporarily off) to guide pacing rate adjustments. The end point for cessation of pacing was development of clinically significant MR, left ventricular dilatation, and heart failure. The first 2 animals were paced at 180 min \(^{-1}\) and subsequent animals at 230 min \(^{-1}\), which resulted in faster development of heart failure and MR, pacing time averaged 15±6 days. Then, the animals were returned to the catheterization laboratory with the pacemaker turned off before the study. Hemodynamic, marker, and echocardiographic data were again acquired.

All animals received humane care in compliance with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health (DHEW NIH publication 85-23, revised 1985). This study was approved by the Stanford Medical Center Laboratory Research Animal Review Committee and conducted according to Stanford University policy.

Data Analysis
Three consecutive steady-state beats during normal sinus rhythm before and after rapid pacing were averaged and defined as “baseline” and “TIC” data for each animal. During each cardiac cycle, the time of end-systole was defined as the videofluoroscopic frame containing the point of peak negative LV rate of pressure fall (−dP/dt), whereas the time of end-diastole was defined as the image frame containing the peak of the ECG R-wave. Instantaneous LV volume was calculated from the epicardial LV markers with the use of a space-filling multiple tetrahedral volume method for each frame, ie, every 16.7 ms.12

Mitrail Leaflet Geometry
Mitrail leaflet length was calculated as the sum of individual leaflet segments (4 for anterior leaflet [S1 to S3 from annulus to leaflet edge] and 2 for posterior leaflet [S6 and S8 leaflet edge and annular segment, respectively]), whereas each segment length was compared before and after pacing to assess for regional leaflet remodeling. Angles between leaflet segments were also calculated as surrogates for regional leaflet shape. Three angles were calculated for the origin located at the mid-septal annulus (marker 7), with the Y-axis passing through the LV apex (positive toward the apex), positive X-axis directed toward the mid-lateral annulus such that marker 11 was contained in the X-Y plane, and positive Z-axis toward the posterior commissure (Figure 2). The mid-septal annulus was chosen as the origin because it is at the center of the fibrous annulus, which should be least affected by LV dilatation. To further assess the position of the leaflet edges, distance from each leaflet edge marker (4 and 5) to the least-square mitral annular plane was also calculated.

Statistical Analysis
All data are reported as mean plus or minus 1 standard deviation (±1SD), unless otherwise stated. Hemodynamic and marker-derived data from 3 consecutive steady-state beats were averaged for each of the nine animals and calculated at end-systole. Because of the loss of leaflet edge markers during pacing, data were used from only 5 animals to determine distal anterior leaflet segment length and angle S6. Data were compared using Student \(t\) test for paired comparisons with the level of statistical significance set at \(P<0.05\). The authors had full access to the data and take full responsibility for their integrity. All authors have read and agree to the manuscript as written.

Results
The average cardiopulmonary bypass time was 90±27 minutes, with a mean aortic cross-clamp time of 55±7 minutes.
Hemodynamic variables before and after development of cardiomyopathy are shown in Table 1. Rapid pacing markedly increased LV end-systolic and end-diastolic size and end-diastolic pressure, consistent with the development of clinical heart failure. Overall MR grade increased from 0.2 to 2.2 (P=0.0001) after the development of cardiomyopathy.

Individual leaflet segment lengths and total leaflet lengths at end-systole before and after pacing are summarized in Table 2. Leaflet remodeling was significant only in the distal-most segment of each leaflet (ie, leaflet edge) with very similar degree of segmental lengthening being observed. Total leaflet length increased significantly after pacing mostly because of distal segment remodeling. As shown in Figure 2, anterior leaflet shape was not altered by the induction of cardiomyopathy and angles between individual leaflet segments did not change after pacing (Table 3). Posterior leaflet, however, flattened out with the onset of heart failure and the angle between its 2 segments increased significantly thus decreasing curvature of the leaflet. With TIC, the distance from the anterior leaflet edge marker (marker 4) to the annular plane did not change (0.77±0.31 versus 0.50±0.33 cm before and after pacing, respectively; P=0.17), whereas the posterior leaflet edge marker (marker 5) moved closer to the annular plane (0.80±0.38 versus 0.57±0.31 cm before and after pacing, respectively; P=0.03), suggesting mild prolapse.

**Discussion**

Mitral regurgitation associated with dilated cardiomyopathy and heart failure has been attributed to ventricular remodeling leading to reduced leaflet coaptation and subsequent valvular insufficiency. The bulk of current experimental and clinical research has focused on altered annular and subvalvular geometry as the cause of functional MR, while the valvular leaflet tissue properties have been ignored. The current experiment demonstrates that both anterior and posterior mitral leaflets remodel in an ovine model of cardiomyopathy, suggesting that leaflet tissue is a contributor to the pathophysiology of FMR and by virtue of such leaflet remodeling may limit the severity of FMR. Furthermore, the exact location of leaflet remodeling was consistent with the previously described heterogeneous composition and mechanical properties of the mitral valve.

Anatomical and biochemical studies of the mitral valve have shown normal mitral valvular tissue to be richly innervated, having a complex blood supply, heterogeneous biochemical composition, and nonhomogeneous deformation. These investigations suggest that mitral leaflets are not inert flaps strictly bound to the hemodynamic conditions of the left ventricle but rather active components of the valvular–ventricular complex capable of regulating valvular function in health and disease. Important recent observations by Grande-Allen et al have

<table>
<thead>
<tr>
<th>TABLE 1. Hemodynamics</th>
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<td></td>
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<tr>
<td><strong>Baseline</strong></td>
</tr>
<tr>
<td>HR, min⁻¹</td>
</tr>
<tr>
<td>dP/dt, mm Hg/s</td>
</tr>
<tr>
<td>EDV, mL</td>
</tr>
<tr>
<td>ESV, mL</td>
</tr>
<tr>
<td>LVESP, mm Hg</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
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<td>LVP, mm Hg</td>
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</tbody>
</table>

dP/dt indicates maximum positive rate of change of LV pressure; EDV, LV end-diastolic volume; ESV, LV end-systolic volume; HR, heart rate; LVESP, LV end-systolic pressure; LVEDP, LV end-diastolic pressure; LVP, maximum systolic LV pressure.

Data shown as mean±1 SD.
demonstrated that mitral leaflets from explanted recipient hearts with FMR have a much different biochemical profile than control leaflets, having significantly higher DNA content and collagen concentration with greatest deposition in the anterior leaflet free edge. Overall, the human heart failure leaflets were 28% to 41% longer than control values, which is almost double the leaflet lengthening observed in our study, a discrepancy that is most likely caused by the much more chronic nature of the human condition. Further mechanical analysis revealed that leaflet tissue was 61% stiffer in the radial direction and 50% stiffer in the circumferential direction than control leaflets, reflecting the microstructural alterations described previously. These data suggest prominent biochemical and ultrastructural remodeling of leaflet tissue, particularly in the region of the free edge, which is consistent with the in vivo measurements reported in the current study. The anterior leaflet edge and the entire posterior leaflet have been found to have a relatively thin collagenous layer, and these regions may be particularly prone to greater remodeling as annular dilatation increases leaflet stresses and collagen synthesis is upregulated in response. Greater remodeling in the coaptation region of the leaflets may not be surprising because Chen et al demonstrated nonhomogeneous deformation of the anterior leaflet in and ex vivo porcine leaflet model. These investigators found that the anterior leaflet in normal porcine valves stretches significantly after valve closure along a radial gradient from the mitral annulus to the leaflet edge. Thus, it is not unexpected that the greatest remodeling occurred in the area of maximum deformation, ie, the leaflet leading edge or free margin. Based on the segmental lengthening pattern of posterior leaflet tissue observed in our experiment, one may suspect that a similar gradient of deformation is present in the posterior leaflet. Similar radial stretch of the posterior leaflet mid-belly and anterior leaflet free edge have been observed in other experimental preparations.

With the onset of cardiomyopathy, the shape of the anterior leaflet did not change from baseline although the leaflet lengthened significantly. All angles between the leaflet segments remained unchanged, and neither prolapse nor restriction of the anterior leaflet edge was observed. It is therefore less likely that the leaflet remodeling observed in our study, particularly at the leaflet edge, was caused by leaflet tissue “unfurling” and represents actual tissue lengthening as leaflet shape remained unchanged. The posterior leaflet, however, flattened by ~9° after the development of heart failure and FMR, and part of leaflet lengthening could be attributed to this phenomenon. Tibayan from our group recently described leaflet shape in a chronic ovine ischemic MR model in which radii of curvature of both leaflets were significantly increased 7 weeks after posterolateral myocardial infarction. In that study, the anterior and posterior leaflets lengthened only by ~1.4 mm and 0.8 mm, respectively, and flattening or “unfurling” of leaflet tissue could have accounted for significant portion of the observed “lengthening.” In our study, tissue lengthening was >2-fold greater and was limited mainly to the free edge region of both leaflets. Annular septal-lateral diameter dilatation and altered papillary muscle position were reported by Tibayan to be independent predictors of anterior leaflet shape in sheep, whereas Grande-Allen found leaflet length to correlate with annular diameter in human heart transplant recipient. Annular dilatation along with subvalvular changes can be expected to stretch leaflet tissue across the valve orifice and alter loading conditions.

### TABLE 2. Leaflet Segment Length at End-Systole

<table>
<thead>
<tr>
<th>At End-Systole</th>
<th>Baseline</th>
<th>TIC</th>
<th>% Change</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML length, cm</td>
<td>Total</td>
<td>2.11±0.16</td>
<td>2.43±0.23</td>
<td>15±6%</td>
</tr>
<tr>
<td></td>
<td>S1</td>
<td>0.60±0.16</td>
<td>0.64±0.18</td>
<td>8±12%</td>
</tr>
<tr>
<td></td>
<td>S2</td>
<td>0.43±0.08</td>
<td>0.45±0.07</td>
<td>6±10%</td>
</tr>
<tr>
<td></td>
<td>S3</td>
<td>0.55±0.15</td>
<td>0.60±0.21</td>
<td>10±14%</td>
</tr>
<tr>
<td></td>
<td>S4</td>
<td>0.54±0.17</td>
<td>0.71±0.24</td>
<td>23±17%</td>
</tr>
<tr>
<td>PML length, cm</td>
<td>Total</td>
<td>1.14±0.27</td>
<td>1.33±0.25</td>
<td>19±17%</td>
</tr>
<tr>
<td></td>
<td>S1</td>
<td>0.56±0.15</td>
<td>0.69±0.17</td>
<td>24±18%</td>
</tr>
<tr>
<td></td>
<td>S2</td>
<td>0.58±0.21</td>
<td>0.64±0.22</td>
<td>11±24%</td>
</tr>
</tbody>
</table>

Anterior (AML) and posterior (PML) leaflet length and length of each individual leaflet segment before and after tachycardia-induced cardiomyopathy (TIC).

Segments labeled as distances between corresponding marker pairs. S1 = distance from marker 1 to 2 (see Figure 1).

P determined by t test for dependent comparisons.

### TABLE 3. Leaflet Angles at End-systole

<table>
<thead>
<tr>
<th>At End-Systole (Degrees)</th>
<th>Baseline</th>
<th>TIC</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S1</td>
<td>147±12</td>
<td>152±15</td>
<td>0.29</td>
</tr>
<tr>
<td>S2</td>
<td>156±15</td>
<td>150±11</td>
<td>0.10</td>
</tr>
<tr>
<td>S3</td>
<td>149±12</td>
<td>153±10</td>
<td>0.36</td>
</tr>
<tr>
<td>PML</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S6</td>
<td>140±14</td>
<td>149±15</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Anterior (AML) and posterior (PML) leaflet inter-segmental angles (S) before and after development of tachycardia-induced cardiomyopathy (TIC). Angles labeled according to angle vertex marker number. S1 = angle between markers 7-1-2.

P determined by t test-dependent comparisons.
thus leading to leaflet remodeling as a compensatory mechanism that becomes exhausted as ventricular and annular pathophysiological changes progress. Perhaps as suggested by Grande-Allen, anterior leaflet edge and the entire posterior leaflet regions that usually experience compressive stress relief during coaptation, and leaflet stretching brought on by annular and subvalvular remodeling applies unusually high loads to these areas, leading in turn to the regional lengthening observed in the current study. This notion is supported by our data as both anterior and posterior leaflet segments near the leaflet edge lengthened by almost the same degree (23% versus 24%), suggesting similar ultrastructural composition and load milieu of the leaflet tissues. Furthermore, DNA content of anterior leaflet free edge and the posterior leaflet were much greater in human cardiomyopathy valves, whereas the central region of the anterior leaflet showed only a mild increase. In summary, the present experiment for the first time to our knowledge illustrates regional mitral leaflet remodeling in vivo in an ovine model of FMR that is consistent with previous biochemical and ultrastructural studies. Thus, the mitral regurgitation associated with dilated cardiomyopathy and congestive heart failure is associated with both ventricular and valvular remodeling, casting a shadow on the concept that FMR is purely “functional” in genesis.

Limitations

The FMR in this ovine TIC model may not be representative of FMR in humans because of interspecies anatomical differences. Leaflet occlusion and coaptation area relative to the mitral orifice in sheep is smaller than in humans. Although annular dilatation may exhaust this redundant leaflet area in an ovine heart, such may not be true in human hearts. Pacing cardiomyopathy, however, exhibits similar hemodynamic and neurohumoral changes, as seen in human heart failure. It is also possible that the sutured markers affected leaflet geometry and remodeling, but this seems unlikely as they are very small (aggregate mass 20±6 mg). Even when we grossly overloaded the anterior leaflet with a larger number of excessively heavy markers (total mass 184 mg) in another experiment, the peak anterior leaflet opening velocity by epicardial pulse wave Doppler echocardiography was 0.47±0.05 m/sec compared with 0.45±0.06 m/sec for leaflets without any markers implanted. The peak E-wave velocities ranged from 0.55 to 0.60 m/sec (unpublished data). Thus, the markers per se do not alter normal leaflet dynamics acutely; however, we admit that we do not have long-term data to discount completely the possibility of the markers affecting leaflet geometry and mobility. Our limited leaflet marker array does not permit us to extrapolate leaflet geometry and remodeling near the commissures; it is possible that tissue remodeling at these locations differs, although clinical studies suggest that geometric leaflet perturbations are symmetric across the valve orifice in dilated cardiomyopathy. To investigate this question further, a denser marker array would be needed, which in turn might have more of an effect on leaflet dynamics. Lack of ex vivo leaflet measurements and histological analysis is yet another limitation of this study that will be addressed in forthcoming experiments designed specifically to elucidate changes in the mitral leaflets in the pathogenesis of “functional” mitral regurgitation.

Acknowledgments

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Disclosures

None.

References


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