TRANSLATIONAL PHYSIOLOGY

Impact of mitral regurgitation on left ventricular anatomic and molecular remodeling and systolic function: implication for outcome

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Pu M, Gao Z, Zhang X, Liao D, Pu DK, Brennan T, Davidson WR Jr. Impact of mitral regurgitation on left ventricular anatomic and molecular remodeling and systolic function: implication for outcome. Am J Physiol Heart Circ Physiol 296: H1727–H1732, 2009. First published March 27, 2009; doi:10.1152/ajpheart.00882.2008.—The aim of the study was to assess the impact of mitral regurgitation (MR) on left ventricular (LV) anatomic and molecular remodeling and function and to determine whether early LV remodeling and function predict long-term outcome in experimental organic MR. A new rodent model of chronic MR was created. Twenty-eight rats had surgically induced MR, twelve rats had a sham operation, and twelve rats had no operation. LV diameters, volume, and mass and LV ejection fraction (LVEF) and LV fractional shortening (LVFS) were assessed using echocardiography in the early stage of MR (6 wk) after induction of MR. LV hemodynamics was assessed invasively. Cardiac α- and β-myosin heavy chains and sarco(endo)plasmic reticulum Ca2+-ATPase 2 (SERCA2) were measured to assess molecular remodeling and contractility. Cox’s proportional hazard ratios (HR) were used to identify outcome predictors. Early LV dilation was demonstrated in rats with MR when LVEF and LVFS were still normal. LV remodeling was associated with an increase in LV end-diastolic pressure and decrease in maximal change in pressure volume, and mass) is observed in asymptomatic patients (24) and experimental animal models (6). However, the impact of chronic MR on anatomic and molecular LV remodeling, systolic function, and the time course of those changes and their association with the outcome have not been systematically studied in experimental organic MR. Early LV remodeling may be considered as a compensatory mechanism for volume overload. We hypothesized that LV remodeling might be a precursor of the development of LV dysfunction. If so, LV remodeling could possibly predict long-term outcome before impaired LV function is detected by conventional measures. If our hypothesis is correct, there should be a molecular basis for LV remodeling and myocardial contractility impairment in chronic MR. To validate our hypothesis, we, for the first time, created a rat model of chronic organic MR and carried out a long-term study using this novel experimental model. The study was particularly designed to 1) evaluate the relationship between early LV remodeling, function, and mortality; 2) investigate the impact of chronic MR on myocardial molecular remodeling and contractility impairment by measuring myocardial α- and β-myosin and sarco(endo)plasmic reticulum Ca2+-ATPase 2 (SERCA2); and 3) assess the relationship between LV remodeling and systolic function invasively and noninvasively.

MATERIALS AND METHODS

Animal Preparation

The study protocol was approved by the Institution’s Animal Care and Use Committee of the Pennsylvania State University College of Medicine (assurance number A3045-01). The study was performed according to the guidelines of the American Society of Physiology. The rats were first induced with 3%–5% of isoflurane and then intubated. The lungs were ventilated using a respirator (Hallowell AWS, Pittsfield, MA), and anesthesia was maintained by mixing isoflurane (1% to 2% isoflurane) with oxygen. Heart rate and oxygen saturation were monitored throughout the study (Ohmeda 5250 RGM).

Creation of Chronic MR

After the animals were prepared, an intracardiac echocardiographic catheter (Acuson/Siemens, Mountain View, CA) was inserted into the rat’s esophagus to obtain transesophageal echocardiographic images (8). A left thoracotomy was performed through the fifth or sixth intercostal space. A fine needle (0.36 mm in diameter) was then inserted into the LV through the LV apex under the guidance of transesophageal echocardiography. The needle was advanced into the mitral valve to perforate and/or tear mitral leaflets to create MR. The severity of MR was assessed in real time by transesophageal eco-
cardiology. MR was considered significant if a regurgitant jet area occupied more than 40% of the left atrial area and/or reversed pulmonary venous flow pattern was detected with a visible vena contracta. After significant MR had been induced, the needle was then withdrawn and the chest was closed. In the sham group, a left thoracotomy was performed and the needle was inserted into the LV, but the mitral valve was not damaged and no MR was produced. The needle was then withdrawn, and the chest was closed. In the control group, no operation was performed. After the operation the rats were returned to the animal facility for recovery. All rats were cared for equally after the MR operation and were observed for mortality rate up to 1 yr. A total of 34 Sprague-Dawley rats underwent the MR operations, and chronic organic MR was created in 28 rats (MR group), which survived perioperatively. In addition, 12 rats had sham operations (sham group) and 12 rats had no operation (control group).

**Echocardiography**

Two-dimensional, color Doppler, and pulsed-wave Doppler imaging was performed using a special (14 MHz) probe for small animals (Acuson/Siemens). Parasternal long- and short-axis two-dimensional views were recorded as previously described (18). M-mode images were obtained at the level of the chordae tendineae of the mitral valve in the short-axis view under the guidance of two-dimensional images. To assess the time course of LV remodeling and changes in LV systolic function, transthoracic echocardiography was performed preoperatively and at 1, 6, and 12 wk after the MR operation. All images were stored digitally and on SVHS tape. Assessment of LV remodeling. To characterize LV remodeling, LV end-diastolic diameter (LVEDD), LV end-systolic diameter (LVESD), interventricular septal thickness (IVS), and LV posterior wall thickness (PW) were measured from M-mode echocardiography as recommended by the American Society of Echocardiography (20). Relative wall thickness was calculated as (PW + IVS)/LVEDD (11), and LV mass was calculated as 1.04 × [(LVEDD + PW + IVS)3 – LVESD3] (13). LV mass index was calculated as LV mass divided by the rat’s body weight. LV end-diastolic volume (LVEDV) was calculated as LVEDD3 × π/3, and LV end-systolic volume (LVESV) was calculated as LVESD3 × π/3 (7). LV volume-to-mass ratio was calculated as LV volume divided by the LV mass.

**Assessment of systolic function.** LV fractional shortening (LVFS) and LV ejection fraction (LVEF) were measured for the assessment of LV systolic function. The LVFS was calculated as 1 – LVEDD/LVESD × 100%. LVEF was calculated as (LVEDV – LVESV)/LVEDV × 100%.

**Myocardial Biomarkers**

After completion of the echocardiography study, eight sham rats and four MR rats chosen randomly were euthanized. The heart and lungs were rapidly excised. The atria were removed, and the right ventricle and the LV (including interventricular septum) were separated on the ice. The LV was cut into three transverse sections: apex, middle, and basal rings. LV myocardium was immediately placed in liquid nitrogen and stored at −80°C for the molecular study. Only the middle ring was used for measurement of cardiac α- and β-myosin heavy chain. Myocardium was thawed. Myocyte suspensions were then centrifuged. Proteins were determined using a Bio-Rad DC protein assay kit. The myocyte suspension homogenates (1 μl/lane) were subjected to SDS-PAGE in 4–9% gradient gels (4°C) that were stained with silver to visualize myosin heavy chain α- and β-myosin. Myocyte lysates were subject to 8% PAGE. The fractionated proteins were transferred onto Immun-Blot polyvinylidene fluoride membranes. SERCA2 was detected with a mouse monoclonal antibody (MA3–919; Affinity Bioreagents, Golden, CO), and a sheep anti-mouse antibody was used for the second antibody. Immunoreactive proteins were detected with an enhanced chemiluminescence-Western blotting system. Protein band signal was quantitated by scanning autoradiograms of the blots with a phosphorimeter.

**Hemodynamic Assessment**

Hemodynamic studies were performed in 12 rats with chronic MR at 36–38 wk. Rats were sedated and intubated. The carotid artery was isolated. A Millar catheter was inserted into the carotid artery and advanced into the aorta for aortic pressures recordings. The Millar catheter was advanced farther into the LV through the aortic valve under the guidance of transthoracic echocardiography. LV pressures were recorded and transferred to the PowerLab. Arterial blood pressures and LV end-diastolic pressures (LVEDP) were measured. The maximal LV change in pressure over time (dP/dtmax) was derived from LV pressure recordings.

**Statistical Analysis**

All data are expressed as means ± SD. A Student’s t-test was used to compare the means of continuous variables between the MR group versus the sham group or the sham group versus the control group. One-way ANOVA was also used to compare the means of continuous variables for the MR, sham, and control groups. The 1-yr survival rate was evaluated by the Kaplan-Meier method. Cox’s proportional hazard models were used to calculate the relative risks [hazard ratios (HR)] and 95% confidence intervals (CI) of mortality associated with LV remodeling parameters and LV functional parameters. The study objective was to identify the strongest predictors of mortality following the creation of chronic MR. Early LV remodeling parameters (LVEDD and LVESD, LV mass index, LV volume, LV ventricular volume-to-mass ratio) and LV function parameters (LVEF, LVFS) all had different scales. Therefore, we reported the associated HR and 95% CI in terms of one SD increment.

**RESULTS**

**Natural History and Mortality**

Among 28 rats with MR, 16 rats were followed up for 1 yr to obtain a long-term outcome data (1-yr all-cause mortality) and 12 rats were electively euthanized for hemodynamic study at 36–38 wk. Figure 1 shows 1-yr Kaplan-Meier survival curves in the MR, sham, and control groups. At the first 12 wk after the MR operation, survival rates were identical among the MR, sham, and normal control groups. However, the survival curves started diverging after 12 wk when LVESD started increasing. The 1-yr survival rate was significantly lower in the chronic MR group (38%) than in the sham (83%; P < 0.001) or the control (100%; P < 0.001) groups.

![Fig. 1. Kaplan-Meier survival analysis in rats with and without mitral regurgitation (MR). One-year survival rate was significantly lower in the MR group than in the sham and control groups.](http://ajpheart.physiology.org/10.220336/10172323)
Time Course of LV Remodeling

Table 1 shows the LV remodeling parameters before and at 6 and 12 wk after the MR operation. The LV mass, LV mass index, LVEDV, and LV volume-to-mass ratio increased significantly in the MR group compared with the sham and control groups. However, LVESD and LVESV did not increase significantly in the MR group compared with the sham and control groups. These findings indicated significant volume overload shifted cardiac myosin heavy chains from phenotype to fetal type. Cardiac α-myosin heavy chain was significantly reduced in the MR group (84% vs. 95% \(P < 0.001\)). In contrast, cardiac β-myosin heavy chain was significantly higher in the MR group than in the sham group (45 ± 8% vs. 5 ± 4%; \(P < 0.001\)). SERCA2 was significantly reduced in the MR group (55% ± 8% vs. 5 ± 4%; \(P < 0.001\)). SERCA2 was significantly reduced in the MR group (55% ± 8% vs. 5 ± 4%; \(P < 0.001\)). SERCA2 was significantly reduced in the MR group (55% ± 8% vs. 5 ± 4%; \(P < 0.001\)). SERCA2 was significantly reduced in the MR group (55% ± 8% vs. 5 ± 4%; \(P < 0.001\)). SERCA2 was significantly reduced in the MR group (55% ± 8% vs. 5 ± 4%; \(P < 0.001\)). Servomodulating heavy chain was significantly higher in the MR group than in the sham group (157 ± 15 mmHg LVEDP or 15 mmHg LVEDP 10 0.23 7 0.30 5 0.002 578 8; \(P < 0.001\)). SERCA2 was significantly reduced in the MR group (84 ± 8) in comparison with the normal group (157 ± 8; \(P < 0.001\)).

Relationship Between LV Remodeling and Function

To assess the relationship between the LV remodeling and LV systolic function, 12 rats with chronic MR underwent invasive hemodynamic study. These rats were divided into the two groups according to the LVEDP of \(\geqslant 15\) mmHg or <15 mmHg. Table 2 compares aortic pressure, LV pressure, LVEDV, and dP/dt max between the high and low LVEDV groups. LV size is significantly larger, LV mass is significantly higher, and the dP/dt max is significantly lower in the group with an elevated LVEDP (\(\geqslant 15\) mmHg). Figure 3 illustrates LV systolic, diastolic, and LV end-diastolic pressure and dP/dt max in rats with high (Fig. 5A) and low (Fig. 5B) LVEDV, respectively.

Impact of LV Remodeling on Survival

The HR for mortality and their 95% CI are presented in Fig. 4. In the first week, neither the LV remodeling nor the LV

Table 2. Hemodynamic study in rats with chronic MR

<table>
<thead>
<tr>
<th>LVEDP &lt; 15 mmHg</th>
<th>LVEDP (\geqslant 15) mmHg</th>
<th>(P) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP, mmHg</td>
<td>118±5</td>
<td>112±10</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>86±6</td>
<td>82±7</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>10±3</td>
<td>18±5</td>
</tr>
<tr>
<td>dP/dt max, mmHg/s</td>
<td>3,967±557</td>
<td>2,756±110</td>
</tr>
<tr>
<td>LVEDV, cm</td>
<td>1.09±0.09</td>
<td>1.33±0.04</td>
</tr>
<tr>
<td>LVEDV, cm</td>
<td>0.69±0.10</td>
<td>0.91±0.04</td>
</tr>
<tr>
<td>LV mass index, mg/g</td>
<td>1.68±0.36</td>
<td>2.79±0.17</td>
</tr>
<tr>
<td>LVFS, %</td>
<td>38±6</td>
<td>35±9</td>
</tr>
</tbody>
</table>

Values are means ± SD. LVEDP, LV end-diastolic pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; dP/dt, change in pressure over time; LVEDV, LV end-diastolic volume; LVFS, LV fractional shortening.
function parameters predicted later death. However, at 6 wk, LVEDD and LVEDV were associated with mortality. At 12 wk all parameters of LV remodeling (LVEDD, LVESD, LVEDV, LV mass, and LV mass index) were associated with increased mortality. However, neither LVEF nor LVFS measured at any stage of chronic MR (1–12 wk) predicted death. LV mass index was significantly higher in nonsurviving rats than those surviving in the MR group (Fig. 5).

DISCUSSION

The determination of the best timing of mitral valve surgery for asymptomatic patients with chronic severe MR and normal LV function is important (4). Currently, expert consensus is that mitral valve surgery should be performed before LV dysfunction (LVEF < 60%) occurs. However, altered loading condition imposed by chronic MR often results in high levels of LVEF (5). Clinically, it is difficult to determine the time course of LV remodeling and LV dysfunction in chronic MR. Therefore, it may not be able to accurately predict when LVEF falls. Clinical decision of mitral valve surgery for patients with severe MR and normal LVEF may rely on the development of clinical symptoms. This may be problematic because patients with chronic MR may have occult LV dysfunction without notable clinical symptoms.

The current experimental study prospectively reveals the time course and relationship between progressions in LV remodeling and LV dysfunction. Although a high LVEF and LVFS was observed in the early stage of MR (within the first 6–12 wk), decrease in LVEF and LVFS was seen in the late stage. Using a pig model of chronic organic MR, Neilan and colleagues (17) showed that the LV was progressively dilated at 1 and 3 mo induction of MR, but LVEF did not decrease. LV remodeling is associated with systolic impairment. MR rats with increased LVEDP had larger LV size and higher LV mass and lower dP/dt max than the rats with low LVEDP. This is consistent with previous experimental studies in dogs with chronic organic MR. Using invasive methods, both Carabello’s group (16) and Starling’s group (21) found early myocardial contractility abnormality in chronic MR. In the current study all death occurred after LVEF and LVFS decreased. Therefore, the time course and pathophysiological consequences of chronic organic MR followed the sequence of initial LV
diastolic remodeling (end-diastolic LV dilation, increased LV mass) with normal or high LVEF or LVFS, with subsequent LV end-systolic remodeling (end-systolic LV dilation) with LV function impairment, and finally congestive heart failure and death. Therefore, significant early LV remodeling would be considered evidence of LV intolerance for volume overload with a risk of development of LV dysfunction. LV remodeling would then be a marker for early intervention (surgical or medical) before LV function is reversibly damaged. On the other hand, a decline in LVEF as an indicator for mitral valve surgery could put some MR patients at risk for unfavorable outcome, particularly in patients who are not closely followed due to a lack of symptoms.

Rat models have been widely used in the study of the pathophysiology of cardiovascular diseases and testing effects of medical therapies (1, 9, 19). One of important findings of this study is to demonstrate for the first time that a rat model of chronic organic MR expresses a pathophysiology similar to humans. One of advantages of this novel experimental model is to allow prospective study of the natural history of LV remodeling, alterations in LV function and mortality in a relatively large number of animals with long-term follow-up. Obviously, such a national history study cannot be carried out in patients. Furthermore, the current study provides basic scientists with a new experimental model for studying the cellular and molecular biological mechanisms of congestive heart failure caused by chronic MR and for testing different therapeutic strategies. With successful development of gene knockout rats (23), MR research can be further expanded from traditional observations of changes in gross anatomy (LV size, volume, mass, etc.) and hemodynamic (LVEF, LVFS, dP/dt, etc.) to molecular and genetic mechanisms.

At the present time, it is unknown when and how LV myocardium transitions at the cellular and molecular levels from normal contractility to abnormal contractility in chronic organic MR. Beeri and colleagues (2) demonstrated the molecular impact of moderate MR in a sheep model of myocardial infarction. They reported decreased remote-zone SERCA2 levels and decreased isolated myocyte contractility in sheep with MR. Changes in several molecules included downregulation of prohypertrophic Akt and gp130 in the MR group and dynamic changes in extracellular matrix metalloproteinase (MMP)-13, membrane-type MMP-1, MMP tissue inhibitors, and proapoptotic caspase-3 (2). To our knowledge, the current study is the first to investigate the molecular changes associated with LV remodeling and function impairment caused by chronic organic MR in a small animal model. In a normal heart, myocytes contain mostly cardiac α-myosin heavy chain and a small amount of β-myosin heavy chain. Increased relative expression of the slow molecular motor of the heart (β-myosin heavy chain) is well known to occur in rodent models of cardiovascular disease and in human heart failure. Replacement of α-myosin heavy chain with β-myosin heavy chain attenuates contractility (10). In failing human hearts, downregulation of α-myosin heavy chain has been correlated with systolic dysfunction. Krenz and Robbins (12) showed that the expression of β-myosin heavy chain appears to be disadvantageous under severe cardiovascular stress, implying a maladaptive response. The current study demonstrated a significant shift in myosin heavy chain isoform content from α- to β-myosin heavy chain (molecular remodeling) in the rats with chronic MR. Reduced SERCA2 activity is a major determinant of reduced contractility in heart failure (14). Significant decrease in SERCA2 was observed in the rats with chronic MR, indicating that chronic organic MR impairs myocardial contractility as determined by molecular markers. Previous studies showed that β-blockade increased SERCA2 gene expression in patients with congestive heart failure (15) and that mechanical unloading improves intracellular Ca\textsuperscript{2+} regulation with an increase in SERCA2 protein levels in the rats with doxorubicin-induced cardiomyopathy (22). Correction of MR in a sheep model of myocardial infarction resulted in reversal of anatomic LV remodeling (less increase in LVEDV and LVESV) with a decrease in metalloproteinase-2 and an increase in tissue inhibitor of MMP-4. Elimination of MR in the setting of myocardial infarction activated intracellular signals promoting hypertrophy and opposing apoptosis and reduced matrix proteolytic activity (2).

Further study of the molecular pathogenesis of heart failure caused by organic MR would be an important step toward understanding this distinctive pathophysiology and investigating potential biological treatment targets (blockade of receptors or pathogenetic pathways).

**Implications**

In the clinical setting, the optimal timing of surgery for chronic MR has not been well established in asymptomatic patients with normal LV function. In the laboratory, biological changes in the myocardium in chronic organic MR are not fully understood. In this study, we created the first rat model of chronic organic MR and revealed the unique time course of LV remodeling and LV dysfunction and mortality. A high 1-yr mortality in this experimental study further supports the emerging concept that once significant LV remodeling develops in chronic severe MR, early surgical intervention should be considered even if LV systolic dysfunction is not detected by conventional measures (LVEF, LVFS). Significant changes in myocardial myosin composition and impaired Ca\textsuperscript{2+} handling (decreases SERCA2) indicate that chronic organic MR results in myocardial molecular remodeling as well as functional impairment.

**GRANTS**

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