Initial Effects of the Left Ventricular Repair by Plication May Not Last Long in a Rat Ischemic Cardiomyopathy Model

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Background—Long term effects of left ventricle (LV) repair surgery (LVR) for ischemic cardiomyopathy are not well understood.

Methods and Results—Sixty-nine rats developed ischemic cardiomyopathy with large akinetic LV area 4 weeks after the left anterior descending artery was ligated. In a second surgery 4 weeks later, 33 rats underwent LVR by plication of the akinetic LV area (LVR group), and 36 underwent rethoracotomy alone (sham group). No medication was used in either group. All rats survived the second surgery. LV end-diastolic dimension as measured by echocardiography, LV fractional shortening, and the maximal end-systolic pressure-volume relationship (Emax) as calculated from the data by catheter-tipped manometer and echocardiography improved in the LVR group after the second surgery, but LV end-diastolic dimension and Emax gradually deteriorated as time passed. LV end-diastolic pressure improved 1 week after LVR but rose significantly 4 weeks after LVR. Brain natriuretic peptide mRNA was lower in the LVR group than in the sham group 1 week after LVR but not 4 weeks postoperatively.

Conclusions—Initial improvement in LV function and neurohormonal status after LVR did not last for 4 weeks in this rat model when untreated medically. The mechanism of deterioration should be elucidated to improve long-term results of LVR. (Circulation. 2001;104[suppl I]:I-241-I-245.)

Key Words: surgery ■ ischemia ■ cardiomyopathy ■ infarction ■ natriuretic peptides

The definitive treatment for chronic cardiac failure, including ischemic and nonischemic cardiomyopathy, is cardiac transplantation. However, it is not widely available because of the shortage of donors and adaptable limitations. Recently, left ventricle (LV) volume reduction surgery or LV repair surgery (LVR) for dilated cardiomyopathy1–3 or ischemic cardiomyopathy4 has been introduced as a surgical treatment for patients with dilated LV and chronic heart failure. For ICM, improved ejection fraction in the early phase after LVR has been reported.4 Many studies have been done for LV aneurysm repair with good results, especially for discrete LV aneurysm.6–7 However, indications, method of LVR, and long-term results have not been clarified for ICM. In fact, there have been some reports of late redilation of the LV after LVR.8,9 The objectives of this study were to investigate medium and long-term effects of the LVR by physiological study and molecular effects with the use of our ICM rat model.

Methods Producing the Animal Model

The animal model used in this study has been described elsewhere (abstracts of The Seventh Antwerp-La Jolla-Kyoto Research Conference on Cardiac Function 2000 and American Heart Association 2000). Sixty-nine male Sprague-Dawley rats (weight, 290 to 310 g) underwent general anesthesia with 1% isoflurane on a volume-cycled ventilator for small animals (Rodent Ventilator, model 683).10 The left anterior descending artery (LAD) was ligated near the main pulmonary artery with 6–0 polypropylene suture (Prolene, Ethicon, Inc; first surgery). The rats with ligated LADs developed an ICM with large akinetic aneurysm with some hypokinesis at nonischemic LV sites. Four weeks after the LAD ligation, all rats were randomized to 2 groups. Thirty-three rats underwent LVR by plicating the akinetic area of the LV with a pledgetted 5–0 polypropylene suture (Prolene, Ethicon Inc) under general anesthesia (LVR group), and the remaining 36 underwent rethoracotomy and closure of the chest alone (sham group; second surgery). During LVR, a polypropylene suture with the pledget the same length as the akinetic area was applied to the border zone of the LV wall between the scar and normal area to minimize the akinetic area. As shown in Figure 1, the akinetic area was completely excluded by plication without excision of the scar. The complete plication of scar area in the LVR group was confirmed by echocardiography immediately after the second surgery.

Noninvasive Study

In both groups, the rats (n=20 in both groups) underwent echocardiography and heart rate (HR) and blood pressure (BP) measurements before and after LVR every week up to 4 weeks after the

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second surgery. HR and BP were measured with a Soft Ron (BP-98A, Soft Ron Corp) tail cuff without anesthesia before echocardiography. After the whole body of the rat was warmed to 40°C, the cuff was attached to the tail to measure HR and systolic BP. The measurements were repeated ≥3 times on each occasion. Echocardiography was done with a 7.5-MHz phased-array transducer (HP SONOS 1000, Hewlett Packard Co) soon after the systolic BP measurement. For echocardiography, the rats were anesthetized with ether; their chests were shaved; and they were placed in the supine position on a specially designed table. A 2D targeted M-mode echocardiogram was obtained along the short-axis view of the LV at the level of the papillary muscles. The end-diastolic dimension (EDD), end-systolic dimension (ESD), and fractional shortening (FS) of the LV were measured by M-mode echocardiography over ≥3 consecutive cardiac cycles according to the American Society of Echocardiography leading-edge method.11,12

Invasive Study
The 55 rats were used in an invasive study for measurement of functional parameters immediately before and after the second surgery. HR and BP were measured with a Soft Ron (BP-98A, Soft Ron Corp) tail cuff without anesthesia before echocardiography. After the whole body of the rat was warmed to 40°C, the cuff was attached to the tail to measure HR and systolic BP. The measurements were repeated ≥3 times on each occasion. Echocardiography was done with a 7.5-MHz phased-array transducer (HP SONOS 1000, Hewlett Packard Co) soon after the systolic BP measurement. For echocardiography, the rats were anesthetized with ether; their chests were shaved; and they were placed in the supine position on a specially designed table. A 2D targeted M-mode echocardiogram was obtained along the short-axis view of the LV at the level of the papillary muscles. The end-diastolic dimension (EDD), end-systolic dimension (ESD), and fractional shortening (FS) of the LV were measured by M-mode echocardiography over ≥3 consecutive cardiac cycles according to the American Society of Echocardiography leading-edge method.11,12

Invasive Study
The 55 rats were used in an invasive study for measurement of functional parameters immediately before and after the second surgery (LVR group, n=5; sham group, n=5), 1 week after the second surgery (LVR group, n=6; sham group, n=7), and 4 weeks after the second surgery (LVR group, n=13; sham group, n=13). The 3F Fogarty balloon catheter (Edwards Life Sciences Corp) was inserted via the right internal jugular vein into the inferior vena cava. Then, the maximal end-systolic pressure-volume relationship (E<sub>max</sub>) as a systolic functional parameter was calculated from the recorded data.16

Brain Natriuretic Peptide Measurement Study
The rats were killed 1 or 4 weeks after the second surgery to measure brain natriuretic peptide (BNP) mRNA in the myocardial cell on the day after the invasive study (n=4 each). Thoracotomy was performed under general anesthesia with intraperitoneal injection of pentobarbital, and the heart was excised immediately. The right and left atria, right ventricle, and area of myocardial infarction or LVR were excised, and the remaining LV muscle was divided into 4 regions. These regions were defined as “near” or “remote” as shown in Figure 2. The pieces of cardiac tissue were frozen in liquid nitrogen quickly and stored at −80°C until use. Total RNA of cardiac tissues was extracted by 4 mol/L guanidinium thiocyanate buffer, and tissue concentration of rat BNP mRNA was measured by Northern blot analysis as previously reported.17

The investigation conformed to the Guidelines for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Statistical Analysis
All values are expressed as mean±SD. The statistical analysis was performed with ANOVA (StatView, SAS Institute Inc), and differences were considered statistically significant at a value of P<0.05.

Results
Noninvasive study
As shown in Table 1, echocardiography revealed similar EDD, ESD, and FS values between the LVR and sham groups before the second surgery (ie, LVR). In the LVR group, EDD decreased significantly after the second surgery (P<0.001), at the same time before and after balloon inflation in the inferior vena cava. Then, the maximal end-systolic pressure-volume relationship (E<sub>max</sub>) as a systolic functional parameter was calculated from the recorded data.16

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and a corresponding fall in ESD was seen \((P<0.001)\). No such changes were observed in the sham group. In the LVR group, FS increased significantly after the second surgery \((P<0.001)\). No such improvement was observed in the sham group. In the LVR group, however, EDD and ESD gradually increased and FS gradually decreased as time passed at each measurement point; 4 weeks after the second surgery, EDD, ESD, and FS were at levels similar to those in the sham group and the status before the second surgery.

There were no differences in HR and systolic BP between the groups before the second surgery. After the second surgery, HR in the LVR group increased significantly \((P<0.05)\); this phenomenon was not seen in the sham group. Except for increased HR after the second surgery in the LVR group, HR and BP in both groups were at the same level up to 4 weeks.

### Invasive Study

EDD, ESD, and FS values were similar to those obtained during noninvasive study. As shown in Table 2, in the LVR group, \(E_{\text{max}}\) increased significantly after the second surgery \((P<0.005\) versus the sham group) but decreased 1 week thereafter. Four weeks after the second surgery, \(E_{\text{max}}\) decreased to the same level as the sham group and the status before the second surgery. End-systolic pressure did not change in either group throughout the time course of this study. There was no difference in end-diastolic pressure between the groups throughout the course of the study. There was no significant difference in \(\tau\) between the groups at each time point.

### BNP mRNA Expression

As shown in Figures 3 and 4, the expression of BNP mRNA as measured by Northern blot analysis in the LVR group 1 week after the second surgery was lower than in the sham group in both the near and remote areas \((P<0.05)\). Four weeks after the second surgery, however, there was no difference in the BNP mRNA level between the groups in either the near or remote area.

### Discussion

Right after the LVR, the rats showed smaller LV EDD and LV ESD, as well as larger FS and similar BP, findings that basically are the same as the clinical data reported.\(^5,18\) At that point, neither \(\tau\) nor LV end-diastolic pressure significantly increased, but \(E_{\text{max}}\) did. Thus, LVR improved LV systolic function without deteriorating LV diastolic function. This initial improvement in LV function may be caused by smaller LV diameter after LVR, which means decreased LV wall stress according to Laplace’s law and improved shortening of the LV wall.\(^19\)

At a later phase after the LVR, however, both LV EDD and LV ESD kept increasing as time passed, whereas FS kept falling to the range close to that of the sham group (ie, no LVR performed). \(E_{\text{max}}\) followed the same time course, and 4 weeks after the LVR, the initial improvement almost disappeared. It is important to note that the animals did not have any medications such as ACE inhibitors or \(\beta\)-blockers; in this study, we purposely avoided medication to investigate the net effects of LVR at both the early and late phases after LVR. The data suggest that with no medication to protect the repaired LV, the LV cannot maintain the restored function right after LVR.

In an attempt to understand the late results of LVR from a neurohormonal viewpoint, we studied BNP expression in the LV wall. The BNP mRNA was lower in both the near and remote areas 1 week after LVR but not in either area 4 weeks postoperatively.

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### Table 2. Hemodynamics Changes by Invasive Study

<table>
<thead>
<tr>
<th>Group</th>
<th>Before Second Surgery</th>
<th>After Second Surgery</th>
<th>1 wk</th>
<th>4 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>(E_{\text{max}})</td>
<td>LVR 2.3±2.0</td>
<td>14.0±5.0*</td>
<td>8.0±3.0*</td>
<td>3.0±2.0</td>
</tr>
<tr>
<td></td>
<td>Sham 2.1±1.8</td>
<td>2.0±1.0</td>
<td>1.0±0.6</td>
<td>2.0±1.0</td>
</tr>
<tr>
<td>(\tau), ms</td>
<td>LVR 21±4</td>
<td>28±6</td>
<td>17±3</td>
<td>22±6</td>
</tr>
<tr>
<td></td>
<td>Sham 21±5</td>
<td>21±5</td>
<td>20±5</td>
<td>21±6</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>LVR 10±4</td>
<td>14±9</td>
<td>9±2</td>
<td>18±10</td>
</tr>
<tr>
<td></td>
<td>Sham 12±7</td>
<td>10±6</td>
<td>16±8</td>
<td>14±8</td>
</tr>
</tbody>
</table>

*\(P<0.01\) vs sham group.

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Figure 3. BNP mRNA expression in rat LV was shown by Northern blot analysis 1 week after second surgery. BNP mRNA expression in LVR group (VRS) was lower than in sham group in both near and remote areas.

Figure 4. Quantitative analysis of BNP mRNA levels in LV with LVR group (VRS) and sham group. In LVR group, BNP mRNA levels were higher than in sham group in both near and remote areas 1 week after LVR but not in either area 4 weeks postoperatively.
remote LV area in the LVR group 1 week after LVR but not 4 weeks after the surgery. This suggests that the LV wall tension may be lower in the LVR group but only soon after surgery, such as 1 week postoperatively, because BNP is secreted mainly from the LV and the secretion is regulated by LV wall tension.\textsuperscript{20,21}

We speculate that the possible mechanism of the late deterioration of the LV systolic function after LVR occurs as follows. First, the remaining ischemia of the LV myocardium around the border zone between the infarcted and intact areas possibly caused another remodeling of the repaired LV. In this model, we placed the plication suture close to the border zone (ie, some ischemic area may possibly have been left unplicated) to eliminate the scar area as much as possible and to maximize the viable myocardium at the same time. From their clinical data, Dor et al\textsuperscript{13} and Suma et al\textsuperscript{21} pointed out that redilation of the LV volume may be due to improper identification of the excised area. Second, the way of repairing the LV may not be compatible for the long-lasting and good performance of the LV.\textsuperscript{22} In this series, the scar area of the LV was not excised but plicated by pledgeted sutures, because small animals such as rats are not suitable for cardiopulmonary bypass and plication appears very similar to the excision from the viewpoint of hemodynamics. In fact, the initial results after LVR in this series are compatible with clinical results. Third, the amount of the remaining myocardium may not be enough.\textsuperscript{24,25} LAD ligation caused considerable myocardial infarction in this model, and LVR itself cannot salvage the loss of myocardium. To examine the above speculations, we are currently investigating postoperative LV status from the viewpoint of local oxidative stress, downregulation of adrenergic receptors, etc. We are also comparing other ways of repairing LV and combined treatments of LVR and medications.

In clinical reports of surgical treatment of LV aneurysm, the linear closure technique had not shown adequate improvement in LV function because of remaining scar area in the septal portion and decreasing LV wall curvature.\textsuperscript{26,27} Thereafter, many surgeons recognized the importance of LV geometry in LV function. A new surgical technique advocated by Dor et al.,\textsuperscript{4} called endoventricular circular patch (EVCPP), has been widely accepted. The EVCPP enabled surgeons to completely exclude the scar area, including the septum, and yet maintain LV geometry with a patch. In clinical reports of EVCPP, LV cavity has been slightly redilated 1 year after the operation.\textsuperscript{22,28} In this study, scar area was almost completely excluded by plication without excising any myocardium or scar tissue. In that sense, this surgical model was close to linear closure repair. In addition, scar formation by LAD ligation in a rat model was not only at the anterolateral portion, but in the septal portion. We attempted to completely exclude all scar area in this study, to mimic EVCPP. However, unlike EVCPP, this model failed to maintain the LV geometry with a patch because of difficulty in such a small animal model. Thus, this study suggested that sole exclusion of scar area caused redilation at the long term after LVR.

This study has some limitations. First, we used a small animal model that has a slightly different anatomy of coronary vessels and ventricles. Second, as described above, a plication method was used to repair the LV, which is hemodynamically similar but not identical to excision and linear closure in the clinical setting. Third, 2D or 2D guided M-mode echocardiography was used to measure the dimension of the LV in this study. Especially when the LV is repaired, use of 3D is important, and either 3D echo or some method with a fixed reference point is desirable if available. Despite the limitations, we believe that this study offers important information on improvement of LVR for ICM. It also provides control data for assessing future combined therapies between various surgical and medical treatments.

In conclusion, initial improvement of the LV function and neurohormonal status after LVR did not last well 4 weeks in this rat model. Further investigation is warranted to elucidate the mechanism of the late deterioration after LVR.

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