Angiotensin-Converting Enzyme Inhibitor Helps Prevent Late Remodeling After Left Ventricular Aneurysm Repair in Rats

Takuya Nomoto, MD; Takeshi Nishina, MD; Senri Miwa, MD; Hiroshi Tsucheyoshi, MD; Izumi Maruyama, BS; Kazunobu Nishimura, MD, PhD; Masashi Komeda, MD, PhD

Background—We reported in a previous study that the initial effects of left ventricular (LV) repair (LVR) for LV aneurysm were not long lasting. Angiotensin-converting enzyme inhibitor (ACE-I) is known to attenuate remodeling after myocardial infarction, and could be effective after LVR.

Methods and Results—Left ventricular aneurysms were developed in rats after left anterior descending artery ligation. Rats were divided into 3 groups: sham operation with ACE-I (lisinopril 10 mg/kg/d) (n=10; group A), LVR (by plicating the LV aneurysm) with placebo (n=8; group R), and LVR with ACE-I (n=10; group RA). LV function was evaluated by echocardiography and catheterization. Oxidative stress in the myocardium was estimated by immunohistochemistry for 8-hydroxy-2′-deoxyguanosine. One week after LVR, LV end-diastolic area was smaller and fractional area change was better in the 2 LVR groups. Four weeks after LVR, LV end-diastolic area, and fractional area change deteriorated in group R but not so much in group RA; E-max was higher in group RA (0.79±0.20 mm Hg/mL) than in groups A (0.25±0.03 mm Hg/mL; P<0.01) and group R (0.27±0.03 mm Hg/mL; P<0.01). Oxidative stress was much lower in the 2 ACE-I groups.

Conclusions—LVR improved LV size and systolic function only in the early phase. Adjuvant use of ACE-I was useful for preventing redilation and maintaining LV systolic function, was associated with suppressed oxidative stress, and may make LVR a more effective surgical procedure for LV aneurysm. (Circulation. 2002;106[suppl I]:I-115-I-119.)

Key Words: surgery ■ myocardial infarction ■ aneurysm ■ remodeling ■ angiotensin

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ecently, left ventricular (LV) repair surgery (LVR) for LV aneurysm after myocardial infarction (MI) has been widely performed as a surgical treatment. Many studies have been done about LVR with good results especially for discrete LV aneurysms.1−3 However, long-term results or appropriate postoperative treatments have not been clarified. In fact, there have been some reports of LV redilation and LV progressive remodeling. Angiotensin-converting enzyme inhibitor (ACE-I) is well known to attenuate remodeling after MI7,8 and could be effective after LVR. The purpose of this study was to investigate the medium- and long-term effects of LVR with adjuvant use of ACE-I in an LV aneurysm rat model.

Methods

Experimental Design

The animal model, which was employed in this study, has been described previously.5 Forty-six male Sprague-Dawley rats (Harlan Sprague-Dawley, weighing 290 to 310 g) underwent general anesthesia with 1% isoflurane on a volume-cycled ventilator for small animals. Anterior MI was introduced by ligation of the left anterior descending artery (LAD) near the main pulmonary artery. Sixteen rats died of heart failure within 1 day after LAD ligation and the thirty surviving rats went into the study. Four weeks after LAD ligation, the rats developed LV aneurysms with some hypokinesis at nonischemic LV sites. They were randomized into 3 groups (n=10 each group): sham operation with ACE-I administration, lisinopril (group A), LVR with placebo (group R), and LVR with adjuvant use of ACE-I (group RA). LVR was performed by plicating the akinetic area of the LV, as previously described,6 whereas the sham operation consisted of only thoracotomy. All rats in groups A and RA survived. In group R, 2 rats died (1 died of bleeding and another died of lung injury), leaving only 8 rats in group R. In groups A and RA, oral lisinopril administration (10 mg/kg/d) was started the day following surgery and was continued for 4 weeks. Lisinopril was given in a volume of 1.0 mL/kg of physiological saline by gastric gavage once

From the Department of Cardiovascular Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan.

Correspondence to Masashi Komeda, MD, Professor and Chairman, Department of Cardiovascular Surgery, Kyoto University, 54 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto, Japan 606-8507. E-mail masakom@kuhp.kyoto-u.ac.jp

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per day. In group R, the same amount of physiological saline was given as placebo.

**Noninvasive Studies**

In each group, the rats underwent echocardiography, heart rate (HR), and blood pressure (BP) measurements before surgery and 1 and 4 weeks post surgery. HR and BP were measured using a Soft Ron tail-cuff without anesthesia, as previously described. Echocardiography was taken using a 7.5-MHz phased-array transducer (HP SONOS 1000; Hewlett Packard Company, Andover, Mass). For echocardiography, the rats were anesthetized with ether and placed in a supine position. Two-dimensional echocardiographic short-axis images of the LV were obtained at the papillary muscle level from left parasternal windows. According to the recommendations of the American Society of Echocardiography for two-dimensional echocardiography, the endocardium was traced by covering the innermost edge of that surface. The endocardial borders were traced frame by frame throughout the entire cardiac cycle. LV end-diastolic area (LVEDA) and LV end-systolic area (LVESA) were determined as the minimum and maximum values for these tracings, respectively. Systolic function was evaluated as the fractional area change (FAC). The percentage of FAC was calculated as:

$$\text{FAC} \% = \left( \frac{\text{LVEDA} - \text{LVESA}}{\text{LVEDA}} \right) \times 100$$

Five measurements were averaged during each examination to calculate LVEDA, LVESA, and FAC.

**Cardiac Catheterization**

All of the rats underwent cardiac catheterization for measurement of functional parameters 4 weeks after surgery (LVR or sham), as previously described. A micromanometer-tipped catheter (Edwards Life Science Corp) was inserted via the right carotid artery into the LV to measure LV pressure. A 3F Fogarty balloon catheter (Millar Instruments) was inserted via the right jugular vein into the inferior vena cava (IVC) for caval occlusion. Two-dimensional echocardiography using a 12-MHz phased-array transducer (HP SONOS 5500; Hewlett Packard Company) and LV pressure were measured at the same time, before, and after balloon inflation in the IVC. The maximal end-systolic pressure-volume relationship (E-max) as a systolic functional parameter was then calculated from the recorded data.

**Histology and Immunohistochemistry**

One day after cardiac catheterization, the rats were sacrificed. The hearts were excised and weighed. The LV were transversely sliced into 2-mm-sections in each section of the base of the papillary muscles and fixed in Bouin’s solution. These transverse sections were stained with hematoxylin eosin and Masson trichrome stains.

Immunohistochemistry for 8-hydroxy-2'-deoxyguanosine (8-OHdG) was carried out to investigate oxidative stress in myocardium, because 8-OHdG has been established as a marker for oxidative stress. We used the avidin-biotin complex method as reported by Toyokuni et al. The following formula was used for the densitometric quantification of immunohistochemistry: $\text{8-OHdG Index} = \frac{\text{Area} \times \text{Density}}{\text{Total Cell Number}}$.

**Brain Natriuretic Peptide Measurement**

After slices were taken, the areas of the MI or LVR were excised and the LV myocardium remaining was divided into 4 segments. Two were defined as “adjacent” areas from MI (or LVR) and the others as “remote” areas. These pieces of LV were frozen at −80°C, and tissue concentrations of brain natriuretic peptide (BNP) mRNA were measured by Northern blot analysis, as reported previously.

**Statistical Analysis**

All data are expressed as the means±SEM. Comparisons of data of noninvasive studies among the groups were performed by two-way repeated measures analysis of variance (ANOVA). Comparisons of other data among the groups were conducted by one-way factorial ANOVA. If significance was found for a group, a time effect, or a group-by-time interaction, post hoc comparisons were performed, when appropriate, among the groups, or between groups or time points, using paired or unpaired Student t test. Statistical analyses were performed with Statview for Windows version 5.0 (SAS Institute Inc., Cary, NC). A probability value $P<0.05$ was considered statistically significant.

**Results**

**Noninvasive Studies**

Results from the noninvasive studies are summarized in Table 1 and Figure 1. There were no differences in HR or systolic BP between the groups before surgery. HR in groups undergoing LVR increased but not significantly after week 1. Systolic BP in groups A and RA (ie, groups with ACE-I) were significantly lower than in group R after both weeks 1 ($P<0.001$) and 4 ($P<0.0001$) postoperatively.

Before surgery all LVEDA, LVESA and FAC values were similar between the groups. One week after surgery, LVESA decreased and FAC skyrocketed in the 2 groups undergoing LVR, and there were significant differences from those in group A. After week 4, LVESA increased significantly more than those after week 1 in groups R and RA (Figure 1). However, although LVESA in group R returned to the preoperative value, LVESA in group RA remained smaller than it was before LVR. Besides, LVESA in group R was less than that in group A at the late period. Similar results were recognized in LVEDA. FAC showed no significant deterio-

| Table 1. Hemodynamic Changes by Noninvasive Studies |
|---|---|---|---|---|
| **Group** | **Before Surgery** | **1 Week After Surgery** | **4 Weeks After Surgery** |
| LVEDA (mm²) | A | 68±2 | 67±2 | 68±3 |
| R | 73±3 | 57±4* | 77±3 |
| RA | 70±2 | 49±2* | 63±3† |
| LVESA (mm²) | A | 47±2 | 44±2 | 43±2 |
| R | 50±2 | 30±2* | 46±2 |
| RA | 47±2 | 26±2* | 35±2† |
| FAC (%) | A | 31±2 | 35±2 | 36±3 |
| R | 31±1 | 44±2* | 41±2 |
| RA | 34±2 | 47±2* | 45±3* |
| SBP (mm Hg) | A | 136±5 | 103±7† | 89±4† |
| R | 130±1 | 123±1 | 125±2 |
| RA | 130±4 | 108±4† | 88±4† |
| HR (bpm) | A | 329±14 | 349±11 | 351±14 |
| R | 342±9 | 371±14 | 331±11 |
| RA | 327±8 | 374±12 | 344±7 |

LVEDA indicates left ventricular end-diastolic area; LVESA, left ventricular end-systolic area; FAC, fractional area change; SBP, systolic blood pressure; HR, heart rate.

*P<0.05 vs group A; †P<0.05 vs group R.
ration in group RA between the weeks 1 and 4, and was highest in group RA after week 4.

Cardiac Catheterization
As shown in Table 2, E-max in group RA was significantly higher than that in the other 2 groups after week 4. LV end-systolic pressure was smaller in the 2 groups with ACE-I administration.

Heart Weight
Four weeks after surgery, LV weight was significantly lower in the 2 groups who received ACE-I administration (P<0.05). Right ventricular weight was also smaller in the 2 groups with ACE-I but there was no significant difference.

Histology and Immunohistochemistry
Histological findings of the transverse LV slices in the 3 groups are shown in Figure 2. The LV chamber size was much smaller in group RA than in the other groups, which was in concordance with echocardiography. Severe fibrosis around the Teflon felt developed in group R, whereas there was only a little fibrosis seen in group RA.

Concerning oxidative stress, 8-OHdG was strongly stained in the nuclei of myocytes in group R (Figure 3), whereas myocytes in groups A and RA showed only trivial nuclear immunostaining. The densitometric quantitation of 8-OHdG immunohistochemistry showed that the 8-OHdG index was 23.2±9.6 in group A, 267±68 in group R, and 24.5±5.2 in group RA. There was a significant difference between group R and the other groups with ACE-I (groups A and RA; P<0.0001 for each). This result implies that oxidative stress was lower in the 2 groups with ACE-I administration.

BNP mRNA Expression
As shown in Figure 4, the expression BNP mRNA in group RA was lower than those in group R in both "adjacent" (P<0.01) and "remote" areas (P<0.05) 4 weeks after LVR. The BNP mRNA level in group RA was similar to that in group A in the remote area, but was lower than that in group A in the adjacent area.

Discussion
Soon after LVR in a rat LV aneurysm model, as previously reported, LV systolic function was improved mainly because...
LV scar was minimized and LV wall stress decreased by the reduced LV size. However, at late phase, LV redilation developed and LV systolic function deteriorated. Ratcliffe et al performed LVR as we did in a sheep MI model. They also showed that the initial improvement of LV function, followed by LV remodeling 6 weeks after LVR. The remodeling caused LV size and LV function to return to preoperative values. These negative results after LVR prompted us to consider adjuvant therapies to improve operative outcomes.

After MI, the heart develops remodeling as follows: during the early phase (in the first few days), there is expansion of the infarcted tissue leading to early LV chamber dilation and increased mechanical stress on the remaining viable myocardium. In the late phase, there is ongoing progressive remodeling of the remote areas of the LV, characterized by further chamber dilation, myocardial hypertrophy, and progressive deterioration in systolic pump function. The tissue renin-angiotensin system (RAS) is activated after MI, and plays an important role not only in early but also in late remodeling. ACE-I is well known to prevent remodeling after MI, both clinically and experimentally. Mulder et al have shown that ACE-I exerted markedly beneficial effects even when treatment was started late into the evolution of heart failure after MI. The LV aneurysm model in our study should be one in which in part prevented LV remodeling after LVR, partly due to its effect reducing oxidative stress.

In this study, LV systolic function was restored in the early phase after LVR. Without medication, however, LV redilation developed and LV systolic function deteriorated in the late phase, as in our previous study. In this study ACE-I per se had only a little effect on LV systolic function (eg, a small increase of FAC in ACE-I group). However, when ACE-I was used with LVR, it attenuated LV remodeling after LVR. In this situation, ACE-I not only reduced the afterload to the LV, but also maintained the lower LV wall stress, which had been decreased by LVR. These effects of ACE-I probably improved LV systolic performance. In addition, tissue RAS promotes cardiac hypertrophy and interstitial cardiac fibrosis, and deteriorates LV diastolic function in the process of cardiac remodeling after MI. ACE-I attenuates cardiac hypertrophy and fibrosis, and improves diastolic filling. Probably the smaller LV weight in the 2 groups with ACE administration suggested a reduction of LV hypertrophy, and the lower LVEDP in these 2 groups indicated improvement of LV diastolic function. BNP levels are known to be elevated in patients with symptomatic LV dysfunction and correlate to prognosis. Lubien et al recently reported that BNP levels increased in patients with LV diastolic dysfunction. In our study the BNP mRNA level was lower in group RA than in group R, in both the adjacent and the remote areas 4 weeks after surgery. This indicates that ACE-I maintained LV global function after LVR and could improve operative outcomes. Thus, the combination therapy of LVR and ACE-I showed that it might have a great benefit on the function of the LV with an aneurysm after MI.

We noticed that severe fibrosis developed in the adjacent area in group R, whereas there was only a little fibrosis seen in group RA. Kramer and colleagues showed that the global LV dysfunction characteristic of LV remodeling was associated with increased wall stress in the adjacent noninfarcted region. Computer-assisted planimetry of histologic sections by Moulton and colleagues revealed that the collagen content was as high as 18.5 ± 12% in the adjacent area, although it was only 1.0 ± 0.1% in the remote area. Sakaguchi and colleagues performed LVR in rats and demonstrated a persistently increased level of regional mRNA of pre-pro atrial natriuretic peptide in the adjacent area after LVR. Those findings indicated that the adjacent area played a special role in LV remodeling after MI, and even after LVR. ACE-I is known to reduce myocardial fibrosis, as previously discussed. In the current study, ACE-I prevented the development of fibrosis at least in the adjacent LV area after LVR, which in part prevented LV remodeling after LVR.

We investigated oxidative stress on myocardium by using immunohistochemistry for 8-OHdG. Reactive oxygen species can produce myocardial contractile dysfunction and structural damage. Antioxidant enzyme activities are decreased and reactive oxygen species are increased in postinfarction myocardium. Kinugawa et al reported that a hydroxyl radical scavenger, dimethylthiourea, attenuated myocardial reactive oxygen species after MI and contributed to prevent LV remodeling. In our study, we found that the expression of 8-OHdG was much lower in the 2 groups with ACE-I administration than the group without it. This result suggests that ACE-I attenuated oxidative stress as has been reported even in the LV after LVR. ACE-I administration presumably prevented LV remodeling after LVR, partly due to its effect reducing oxidative stress.

Our study has some limitations. First, we used a rat LV aneurysm model in the study. The rats developed myocardial infarction after LAD occlusion in the anterior LV wall, but...
not in the interventricular septum. This is the main difference from human LV aneurysms after LAD occlusion. Secondly, the LVR method used in this study is similar to that of linear closure, but not identical. In a clinical setting, there are better surgical methods to repair LV aneurysms, such as endoaneurysmorrhaphy, which has a different type of adjacent area dynamics. We believe, however, that, to a certain extent, the model simulated the clinical LVR because of the improved LV size, shape, and function after the repair. Thirdly, although LV function was the best in group RA, it deteriorated between weeks 1 and 4. This may indicate that ACE-I does not prevent LV remodeling after LVR but only delays it. To show that ACE-I is really useful to prevent postoperative LV remodeling, we are doing a similar study in which measurements will be taken 8 and 12 weeks after surgery. Fourthly, we think that ACE-I prevented remodeling after LVR mainly because it suppressed tissue RAS. However, ACE-I also reduced afterload to the LV, which per se can protect the LV to some extent. We are carrying out another study to assess the role of afterload reduction after LVR by using other types of antihypertensive drugs such as alpha-1-receptor blockers or calcium-channel antagonists.

In conclusion, LVR for LV aneurysm improved LV size and systolic function soon after surgery. However, the effects did not last long, probably because of late remodeling. Adjuvant use of ACE-I, which is associated with suppressed oxidative stress, attenuated LV redilation and LV functional deterioration after LVR. Adjuvant ACE-I therapy may prolong the effects of LVR and improve the surgical results.

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References

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