Atrial Natriuretic Peptide Helps Prevent Late Remodeling After Left Ventricular Aneurysm Repair

Hiroshi Tsuneyoshi, MD; Takeshi Nishina, MD; Takuya Nomoto, MD, PhD; Hideo Kanemitsu, MD; Rika Kawakami, MD; Oriyanhan Unimonh, MD; Kazunobu Nishimura, MD, PhD; Masashi Komeda, MD, PhD

Background—Left ventricular aneurysm repair (LVR) reduces LV wall stress and improves LV function. However, as we reported previously, the initial improvement of LVR was short-term because of LV remodeling but could be maintained longer with postoperative use of an angiotensin-converting enzyme (ACE) inhibitor. Atrial natriuretic peptide (ANP) has been used to treat patients with heart failure by natriuretic and vasodilatory actions. Recent reports have suggested that ANP inhibits the rennin-angiotensin system. In this study, the effects of ANP after LVR were evaluated.

Methods and Results—Rats that had an LV aneurysm 4 weeks after left anterior descending artery ligation underwent LVR by plicating the LV aneurysm and were randomized into 2 groups: LVR+A group was intravenously administrated with 10 μg/h of carperitide, recombinant α-hANP, by osmotic-pump for 4 weeks, and the LVR group was given normal saline. Echocardiography revealed better LV remodeling and function in LVR+A group than in LVR group. Four weeks after LVR, left ventricular end diastolic pressure (LVEDP) and Tau were significantly lower in LVR+A group (LVEDP: 10±4 mm Hg in LVR+A group versus 18±6 mm Hg in LVR group, Tau: 13±2 versus 17±2ms). End-systolic elastance (Ees) was higher in LVR+A group (Ees: 0.34±0.2 versus 0.19±0.11 mm Hg/μL). The levels of myocardial ACE activity in LVR+A group was significantly lower than in LVR group. The mRNA expressions of brain natriuretic peptide and transforming growth factor β1 inducing fibrosis significantly decreased in LV myocardium in LVR+A group. Histologically, myocardial fibrosis was significantly reduced in LVR+A group.

Conclusions—Intravenous administration of ANP had beneficial effects on LV remodeling, function, and fibrosis after LVR. ANP could be a useful intravenous infusion drug for postoperative management after LV repair surgery. (Circulation. 2004;110[suppl II]:II-174–II-179.)

Key Words: remodeling natriuretic peptides surgery fibrosis myocardial infarction cardiomyopathy

Left ventricular (LV) aneurysm repair surgery (LVR) after myocardial infarction (MI) has been widely performed as a surgical treatment. LVR reduces LV wall stress and improves LV systolic function. Many studies of LVR have been performed with good results, but long-term results or appropriate postoperative treatments are not well known. In fact, there have been some clinical reports on LV remodeling indicating LV redilation and functional deterioration after LVR in both linear closure and patch plasty. We initially reported that the initial improvements of LVR were short-term in a rat LV aneurysm model, mainly because of postoperative LV remodeling. Ratcliffe et al also show the same results in a sheep LV aneurysm model. In clinical cases, LV remodeling after LVR may contribute to the development of cardiac failure and late mortality. Accordingly, it is important to prevent progressive LV remodeling after LVR. We have previously reported that an adjuvant therapy by angiotensin-converting enzyme inhibitors (ACE-I) after LVR may prevent the postoperative LV remodeling and maintained better LV function, and the rennin-angiotensin system (RAS) may play an important role in LV remodeling after LVR. Moreover, to prevent LV remodeling and improve mortality after acute MI, early administration of ACE-I from the onset of MI is recommended by the evidence of clinical trials. Therefore, we believe that the inhibition of RAS immediately after LV repair surgery is important in the same way as the management after MI. However, in clinical situations, it is often difficult to use oral drugs such as ACE-I immediately after surgery, because some patients who underwent LV repair surgery are seriously ill and need prolonged respirator management after surgery. ACE-I is an oral drug and is not able to be used intravenously. Therefore, intravenous medication to prevent LV remodeling is more convenient and useful in postoperative management of patients in this category.

Atrial natriuretic peptide (ANP), which is a circulating hormone of cardiac origin, can be used intravenously to treat heart failure for natriuretic and vasodilatory activities. We

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have previously reported that infusion therapy of ANP improved hemodynamics in patients with various conditions of heart failure.\textsuperscript{8,9} Recent studies demonstrate that ANP also inhibits RAS and has a beneficial effect on LV remodeling after acute MI.\textsuperscript{10} However, no reports are available regarding the effects of ANP infusion after LVR. The purpose of this study was to evaluate the effects of infusion therapy of ANP on LV remodeling after LVR.

**Methods**

**Experimental Design**

All experimental procedures were conducted according to Kyoto University’s guidelines for animal care. MI was induced in male Sprague-Dawley rats (Harlan Sprague-Dawley, Ind; weighing 290 to 310 grams) through ligation of the left anterior descending artery as previously described.\textsuperscript{3} In brief, Sprague-Dawley rats were anesthetized with 1% isoflurane on a volume-cycled ventilator for small animals. Anterior MI was introduced by ligation of the left anterior descending artery (LAD) to measure MI size. Rats with an infarct size of more than 30% of the LV circumference were excluded from this study because they did not show typical LV remodeling. All measurements were made by an observer blinded to the study group. Thirty rats with induced large MI were randomly divided into 2 groups (n=15 each group): LVR with infusion therapy of ANP (LVR+A group) and LVR with saline (LVR group). LVR was performed by plicating the akinetic area of the LV as previously described\textsuperscript{3,5} (Figure 1). These procedures were less technically demanding and reproducible. All rats survived and the operative mortality of LVR procedure was 0%. Just after LVR, the rats were subcutaneously implanted with an osmotic pump (Alzet model 2ML4) filled with carperitide, a recombinant alpha-human ANP (Daiichi Pharmaceutical, Japan), dissolved in distilled water to release 10 \textmu g/h of the peptide for 4 weeks in the LVR+A group. The pump was connected to the left jugular vein by a small polyethylene catheter and positioned in a pocket constructed in the subcutaneous tissue just below the subscapular region. 0.9% saline, as the placebo, was infused in the same manner in the LVR group.

**Hemodynamic Measurements and Echocardiography**

The measurements of systolic blood pressure and heart rate and echocardiographic studies were performed before and after LVR every week for 4 weeks as previously described in detail.\textsuperscript{3,5} Systolic blood pressure and heart rate were measured using a Softron tail-cuff system. M-mode tracings were recorded through the anterior and posterior LV walls at the level of the papillary muscle to measure LV end-diastolic dimension. LV end-diastolic area (LVEDA) and LV end-systolic area (LVESA) were determined as the minimum and maximum values for tracings of LV cavity. Fractional area change (FAC) (%) was calculated as (LVEDA – LVESA)/LVEDA × 100 and was used as an index of LV systolic function. Akinetic segment (%) was calculated as (akinetiic length in LV diastolic phase)/(LV diastolic circumference) × 100. More than 3 measurements were taken and averaged to calculate these parameters during each examination.

**Cardiac Catheterization**

All rats underwent cardiac catheterization for measurement of functional parameters 4 weeks after LVR surgery as previously described.\textsuperscript{3} A micromanometer-tipped catheter (Millar Instruments) was inserted via the right carotid artery into the LV to measure LV end-diastolic pressure. A 3-French Fogarty balloon catheter (Edwards Life Science Corp) was inserted via the right femoral vein into the inferior vena cava for caval occlusion. M-mode echocardiography using a 12-MHz phased-array transducer (HP SONOS 5500 Hewlett-Packard Company) was taken to calculate LV volume from end-systolic dimension by the cube formula. LV pressure waveforms and M-mode tracings were simultaneously recorded before and after balloon inflation in the inferior vena cava. End-systolic elastance as a systolic functional parameter was then calculated from the recorded data. Tau (τ), the time constant of LV relaxation, was calculated during the continuous pressure monitoring assuming a zero-pressure asymptote.

**Plasma Human ANP Determination**

One day after cardiac catheterization, a blood sample was rapidly withdrawn from the rats under pentobarbital anesthesia and immediately centrifuged to isolate the plasma, which was stored at −15°C until determination of human ANP concentrations with an immunoradiometric assay kit (human ANP IRMA kit; Shionogi, Osaka, Japan). Because this assay kit was specific for human ANP, it had no cross-reactivity with rat ANP. After blood sampling, the rats were euthanized.

**Determination of Heart Weights and Myocardial Fibrosis**

The hearts were dissected out and weighed (right ventricular free wall weight and LV weight). The LV myocardium was transversely sliced into 2-mm-diameter sections at the base of papillary muscles and fixed in Bouin solution. After slices were taken, the areas of LVR were excised and the LV myocardium remaining was divided into 2 segments of the area near LVR or remote area. These pieces of LV were frozen at −80°C until analyzed. The transverse sections were stained with Picrosirius red, a collagen-specific dye, to determine the degree of the myocardial fibrosis. In each section, 20 separate parts of the LV remote area from LVR were scanned under microscopy (magnification ×200), and the images were analyzed using a KS400 image system (Zeiss).\textsuperscript{11} The ratio of occupied area of myocardial fibrosis, which appeared red with Picrosirius red stain, was automatically calculated. Percentage of myocardial fibrosis was obtained by calculating the mean ratio of occupied area of fibrosis in 20 separate parts of the LV remote myocardium.

**Analysis of Tissue ACE Activity**

The frozen LV myocardium of remote area was minced and homogenized in 5 volumes (wt/vol) of 20 mmol/L Tris-HCl buffer, pH 8.3, containing 5 mmol/L Mg(CH\textsubscript{3}COO)\textsubscript{2}, 30 mmol/L KCl, 250 mmol/L sucrose, and 0.5% NP-40. The homogenate was centrifuged at 20 000g for 30 minutes at 4°C. The supernatant was used for the measurement of ACE activity and protein concentration.

The ACE activity in heart tissue extract was measured using a synthetic substrate, hippuryl-His-Leu, specifically designed for ACE (Peptide Institute, Inc, Osaka, Japan). Fifty microliters of tissue extract were incubated for 30 minutes at 37°C with 5 mmol/L hippuryl-His-Leu in 250 \textmu L of 10 mmol/L phosphate buffer, pH 8.3, containing 0.3 mol/L NaCl. The reaction was terminated by addition of 750 \textmu L of 3% metaphosphoric acid, and then the mixture was centrifuged at 20 000g for 5 minutes at 4°C. The supernatant was analyzed using a reversed-phase column (RP-18; IRICA Instrument, Kyoto, Japan).\textsuperscript{12} In analysis of tissue ACE activity and mRNA...
expressions, normal hearts of SD rats served as controls (n=6; same weight and age as this study).

Analysis of mRNA
Total mRNA was prepared from the frozen LV pieces of remote area by use of TRIzol (Life Technologies Inc) and reverse-transcribed and amplified with ABI PRISM 7700 Sequence Detector (Applied Biosystems). Polymerase chain reaction conditions were 40 cycles of denaturing at 94°C for 20 seconds and primer annealing/extension at 62°C for 60 seconds. The nucleotide sequences of polymerase chain reaction primers and TaqMan probes were as follows: brain natriuretic peptide (BNP), forward primer: 5'-GATCCAGGAAGAGCTCGAAA-3'; reverse primer: 5'-CGGTCTATCTTCTGCCCAA-3'; TaqMan probe: 5'-TCCAAGATGGGACATAGTTCAAGCTG-3'; transforming growth factor β1 (TGF-β1), forward primer: 5'-GGCGAGTGGG-GAACCAAG-3'; reverse primer: 5'-CTGTCACAAGGAGCTGAGGCA-3'; TaqMan probe: 5'-AGTGCAAAAAGATGACAAGCGGAAGT-3'. GAPDH level in each sample.

Statistical Analysis
All data are expressed as the means ± SEM. Hemodynamic, echocardiographic, and cardiac catheterization data were analyzed by unpaired Student t test. ACE activity and mRNA data were evaluated by unpaired t test. ACE activity and mRNA data were evaluated by unpaired t test. ACE activity and mRNA data were evaluated by unpaired t test. ACE activity and mRNA data were evaluated by unpaired t test. ACE activity and mRNA data were evaluated by unpaired t test. ACE activity and mRNA data were evaluated by unpaired t test.

Results
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crographs of the transverse LV slices in the 2 groups are shown in Figure 3. Severe fibrosis around the Teflon felt developed in the LVR group, whereas there was only a little fibrosis seen in the LVR group. In remote areas away from the LVR region, myocardial fibrosis was also less in LVR+1 group compared with LVR group. Percentage of myocardial fibrosis based on computer analysis was significantly lower in LVR+1 group (Figure 4).

### Tissue ACE Activity

The levels of ACE activity in the remote myocardium were shown in Figure 5. The ACE activity in LVR group was significantly higher \((P<0.05)\) than in normal group (LVR group: 0.93\(\pm\)0.49 mU/mg protein, normal group: 0.17\(\pm\)0.07). However, ANP infusion significantly inhibited the upregulation of ACE activity (LVR+1 group: 0.47\(\pm\)0.32 mU/mg protein).

### Ventricular Expression of mRNAs Encoding BNP, TGF-\(\beta\)1, and SERCA2a

As shown in Figure 6, the expressions of BNP and TGF-\(\beta\)1 mRNA significantly increased in the remote myocardium 4 weeks after LVR. However, in LVR+1 group, BNP and TGF-\(\beta\)1 mRNA were significantly lower than in LVR group.

The expressions of SERCA2a mRNA significantly decreased after LVR surgery, and there was no difference between LVR+1 and LVR group.

### Discussion

#### ANP Infusion Dose and Duration

ANP is a circulating hormone with 28 amino acids of cardiac origin, with diuretic activity and vasodilating effects by increasing intracellular cyclic GMP (cGMP) through the guanylyl cyclase-A (GC-A). In clinical practice, the recombinant ANP and BNP are commonly used for the therapy of the patients with heart diseases. Moreover, a recent study suggests that ANP infusion may have a beneficial effect on preventing the LV remodeling after acute MI. However, effective dose and duration of ANP infusion to prevent LV remodeling is unknown. In general, LV remodeling slowly progresses. Therefore, long-term administration of drugs such as ACE-I is needed to prevent LV remodeling. We had previously reported that long-term administration of ACE-I was effective in preventing LV remodeling after LVR in rats. Thus, we speculated that long-term infusion of ANP immediately after LVR might have beneficial effects on preventing LV remodeling. However, no experimental data of long-term infusion of ANP had been available. In this study, we chose ANP doses of 10 \(\mu\)g/h for 4 weeks. ANP has vasodilating effects by increasing intracellular cyclic GMP (cGMP) through the guanylyl cyclase-A (GC-A).

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**Table 2. Cardiac Catheterization and Tissue Weight Variables 4 Weeks After LVR**

<table>
<thead>
<tr>
<th></th>
<th>LVR</th>
<th>LVR+1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma levels of h-ANP (pg/mL)</td>
<td>ND</td>
<td>1265(\pm)431</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>18(\pm)6</td>
<td>10(\pm)4*</td>
</tr>
<tr>
<td>Ees (mm Hg/(\mu)L)</td>
<td>0.19(\pm)0.11</td>
<td>0.34(\pm)0.2*</td>
</tr>
<tr>
<td>(\tau) (ms)</td>
<td>17(\pm)2</td>
<td>13(\pm)2*</td>
</tr>
<tr>
<td>LV weight (g)</td>
<td>1.14(\pm)0.11</td>
<td>0.93(\pm)0.05*</td>
</tr>
<tr>
<td>RV weight (g)</td>
<td>0.33(\pm)0.09</td>
<td>0.23(\pm)0.04*</td>
</tr>
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h-ANP indicates Human atrial natriuretic peptide; LVEDP, LV end-diastolic pressure; Ees, end-systolic elastance; \(\tau\), the time constant of isovolumic relaxation. *P<0.05 vs LVR group.
effect and can increase the risk of hypotension when used at high doses. Several studies have shown that $10^{-9}$ mol/L to $10^{-7}$ mol/L of ANP causes arteriolar and venous dilatation. This study showed the plasma concentration of human ANP, which was infused from the osmotic pump, was $\approx 4 \times 10^{-10}$ mol/L, and significant hypotension was not observed during the infusion of ANP. Moreover, at high concentrations of ANP ($10^{-7}$ mol/L to $10^{-5}$ mol/L), downregulation of ANP receptors is caused. However, it was difficult for downregulation to occur in the low ANP concentration of the present study. We think that the long-term infusion of low-dose ANP may be a reasonable and effective therapy to prevent LV remodeling after LVR.

**Inhibition of RAS**

The mechanisms of LV remodeling after LVR are not fully understood. However, in our previous study, RAS played an important role in LV remodeling after LVR. Hayashi et al showed the clinical effects of ANP infusion on LV remodeling after acute MI (AMI). They reported that in patients with a first anterior AMI, LV function and dimension 1 month after AMI was better after treatment with ANP infusion for 2 to 3 days after AMI, and plasma angiotensin II and aldosterone levels were significantly decreased in the ANP-treated group. Moreover, a recent study demonstrates that ANP directly inhibits aldosterone production in cultured rat cardiocytes, and aldosterone induces the ACE upregulation within the local cardiac RAS. The present study showed that the tissue ACE activity was reduced in ANP-treated group after LVR. In general, ACE-I reduce ACE activities of plasma and heart tissue. These findings can demonstrate that ANP infusion can be the therapy that inhibits RAS systemically and locally, such as ACE-I.

**Myocardial Fibrosis**

One of the progression factors of LV remodeling is myocardial fibrosis. Myocardial fibrosis was implicated in increased myocardial stiffness, resulting in diastolic dysfunction. Diastolic function may be impaired by this kind of LV volume reduction surgery like the Batista procedure. Kanashiro et al and Ratchiffe et al have reported that LV aneurysm repair increases LV chamber stiffness. In fact, our LVR rat model showed severe myocardial fibrosis near the LVR plication site and deterioration of diastolic function. Myocardial fibrosis increased even in remote areas of the LVR site. However, myocardial fibrosis was inhibited in the ANP-treated group both near and away from the LVR plication site and reduced the mRNA expression of Transforming growth factor (TGF)-$\beta$ in the myocardium. Consequently, diastolic function that indicates the degree of LV end-diastolic pressure and $\tau$ were maintained in ANP-treated group. TGF-$\beta$ is a locally generated cytokine and has a major influence on extracellular matrix production and myocardial fibrosis. Recent studies have reported that GC-A knockout mice, which inhibits effects of ANP and BNP, showed severe myocardial fibrosis and increased expression of TGF-$\beta$ mRNA. Thus, ANP may exert a role in myocardial fibrosis and expression of TGF-$\beta$, and could globally reduce myocardial fibrosis after LVR.

**Myocardial Hypertrophy**

LV remodeling is also associated with myocardial hypertrophy of the viable myocardium, as indicated by increases in LV mass and right ventricular mass. Recently, the relationship between GC-A and cardiac hypertrophy was reported by Holtwick et al. Their study of GC-A knockout mice indicated that an inhibition of the local cardiac ANP effects would accelerate the progression of cardiac hypertrophy. We measured the expression of SERCA2a, the molecular marker of myocardial hypertrophy. Downregulation of SERCA2a was seen in hypertrophied or failing myocardium and was linked to systolic and diastolic dysfunction. In the present study, the expression of SERCA2a mRNA decreased after LVR, but there was no difference between the LVR and LVR+A group. An explanation for these different results is that the heart weight of LVR+A group was significantly lower than LVR group, but not significant in SERCA2a mRNA level, which may be related to the degree of myocardial hypertrophy. In rats with moderate (20% to 30%) cardiac hypertrophy, the levels of SERCA2 mRNA or protein were unaltered or unregulated. These findings support that the LVR procedure cannot prevent progression of myocardial hypertrophy, but adjuvant use of ANP infusion can reduce it, in part.

**Border-zone Extension**

Another novel aspect of the present study was that the akinetic segment of the echocardiography gradually increased in the LVR group but remained the same in the LVR+ANP groups. The border zone between the plication site and the viable myocardium may play a special role in the progression of LV remodeling. Once the LVR reduces LV volume, mechanical stress on the remote myocardium is decreased. However, stretch force near the plication site might be increased, and fibrosis could develop. Pharmacological afterload reduction may contribute to reduction of stretch force. However, in this study, ANP infusion therapy might prevent the border-zone extension even at the infusion dose level, which did not induce severe hypotension. As another probable mechanism, we should consider the myocardial ischemia in the border zone. In the myocardium near LVR, myocardial ischemia is probably induced by tightened sutures. ANP has a protective action toward ischemic myocardium through the cGMP, which is the second messenger of nitric oxide and reduces $O_2$ consumption during ischemia. In postischemic myocardium, cGMP concentration is severely reduced; there-
fore, increasing myocardial cGMP by infusion of ANP may protect the ischemic myocardium. Jackson et al23 and Ratcliffe et al24 have reported other probable reason for the border-zone extension in the process of LV remodeling. They speculate the stretch force toward the border zone may induce myocyte apoptosis and contribute to the development of border-zone extension. Recent study reveals that ANP has anti-apoptosis action through the elevation of intracellular cGMP.25 However, in the present study, we do not have any information about myocardial ischemia and apoptosis in the border zone. Therefore, future studies are needed that focus on the mechanisms of developing the border-zone extension.

Study Limitations
Our study has some limitations. First, the LVR method used in this study is similar to that of linear closure, but not identical. The surgical technique advocated by Dor et al2 called endoventricular circular patch plasty technique, has been widely accepted. However, in this study, the scar area was completely excluded by plication without excising any myocardium or scar tissue. We believe that this model simulated the clinical LVR because of the improved LV size, shape, and function after the repair. Secondly, ANP has vasodilatory and natriuretic actions; these may contribute to the inhibition of LV remodeling after LVR. In this study, the blood pressure during week 1 was significantly lower in ANP infusion group. We have to further examine whether afterload reduction during week 1 as an inhibition of remodeling is important. We did not measure urine output, but there was no significant difference in the body weight between both groups. Therefore, we believe that there is not significant difference in preload. Thirdly, the duration of ANP infusion in this study was 4 weeks, which may be too long in clinical cases. However, there are no reports of long-term infusion of ANP in clinical or experimental study. The present study can demonstrate the efficacy of long-term infusion of ANP immediately after LVR, which suggests that ANP infusion can be an alternative therapy of ACE-I after LVR and may be useful in postoperative management of patients who cannot take oral medications because of severe conditions or prolonged respiratory management.

Conclusions
Intravenous administration of ANP after LVR can inhibit RAS and partially prevents LV remodeling and myocardial fibrosis, which is associated with suppressed TGF-β1. ANP could be a useful intravenous infusion drug for postoperative management immediately after LV repair surgery.

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References
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