Adrenomedullin Administration Immediately After Myocardial Infarction Ameliorates Progression of Heart Failure in Rats

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Background—Adrenomedullin (AM) is expressed in cardiac tissue, and plasma AM levels increase in patients with acute myocardial infarction (MI). This study was performed to determine whether AM administration immediately after acute MI inhibits progression of heart failure in rats.

Methods and Results—Rats were infused with 1.0 μg/h IP AM or saline over 7 days immediately after MI induced by left coronary ligation and were examined 9 weeks after MI. Compared with the saline infusion, AM infusion significantly improved survival (59% versus 81%; P<0.05) and body weight gain (32%; P<0.01) and reduced heart weight (−28%; P<0.01), lung weight (−26%; P<0.01), left ventricular (LV) end-diastolic pressure (11.4±2.0 versus 4.0±0.6 mm Hg, mean±SEM; P<0.01), collagen volume fraction of noninfarcted LV (−39%; P<0.05), and plasma levels of endogenous rat AM (−38%; P<0.05) without affecting infarct size. To investigate the mechanism of AM actions, another series of MI rats infused with AM were killed on day 7. AM infusion had no effect on organ weights and hemodynamic parameters on day 7 of MI but significantly reduced urinary excretion of isoprostane (−61%; P<0.01) and noninfarcted LV mRNA levels of ACE (−31%; P<0.05) and p22-phox (−30%; P<0.05).

Conclusions—AM administration during the early period of MI improved the survival and ameliorated progression of LV remodeling and heart failure. This beneficial effect was accompanied by reductions in oxidative stress and ACE mRNA expression in noninfarcted LV in the AM infusion period. (Circulation. 2004;110:426-431.)

Key Words: adrenomedullin ■ heart failure ■ myocardial infarction ■ remodeling

Myocardial infarction (MI) frequently produces left ventricular (LV) dilatation with hypertrophy and collagen deposition in the noninfarct myocardium, changes referred to as LV remodeling, which leads to depressed cardiac performance.1 Previous studies have demonstrated that LV remodeling was an important factor in determining not only development of heart failure but also long-term survival after acute MI.2 It is therefore essential to inhibit LV remodeling for better clinical outcomes of patients with MI. The underlying mechanisms responsible for LV remodeling have been shown to be hemodynamic stress to the heart and activation of neurohumoral factors, including the renin-angiotensin-aldosterone (RAA) system.3,4 Meanwhile, according to recent studies, oxidative stress appears to be another important factor participating in the progression of heart failure after MI.5 Adrenomedullin (AM), originally isolated from human pheochromocytoma,6 is known as a peptide having a wide spectrum of biological actions such as vasodilatation, natriuresis, and diuresis.7 Plasma AM levels were found to increase immediately after the onset of acute MI and to return to the basal level within 1 week.8 Previous studies have suggested that AM counteracts the systemic or local RAA system in vitro and in vivo.9,10 Recently, Shimosawa et al,11 using mice lacking 1 copy of the AM gene, reported an antioxidative action of AM. Thus, it is possible that AM plays an important role as a cardioprotective factor in acute MI by counteracting excessive vasoconstrictors or oxidative stress. We have already shown that continuous administration of AM has beneficial effects on LV remodeling and hemodynamics in MI rats12; however, it remained to be explored whether AM administered in the early period of MI improves long-term outcome. If AM has a cardioprotective effect in acute MI, an AM supplement in the early period would be beneficial in inhibiting the progression of LV remodeling and

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heart failure. The aim of this study was to examine the effect of AM administered during the early period of MI on the progression of heart failure in rats.

**Methods**

**Animals and Peptide**

Male Wistar rats (Charles River Inc) weighing 220 to 280 g were used in this study. All rats were housed in a temperature- and humidity-controlled room with free access to standard rat chow and tap water. The recombinant human AM used in this study was provided by Shionogi & Co, Ltd.12

**Experimental Protocols**

MI was induced in rats by ligation of the left coronary artery as described previously,12 and sham-operated animals underwent an identical surgical procedure without the actual coronary artery ligation. Experiments were performed under the regulations of the Animal Research Committee of Miyazaki Medical College (2002–013).

**Long-Term Study**

To examine the effects of AM on the progression of heart failure and LV remodeling after MI, rats receiving the coronary ligation were randomly divided into 3 groups: 2 groups infused with AM at a low (0.3 \(\mu\)g/h; \(n=12\)) or a high (1.0 \(\mu\)g/h; \(n=11\)) dose and 1 group infused with saline that served as controls (\(n=16\)). The rats were intraperitoneally implanted with osmotic minipumps (model 2001, DURECT Co) filled with recombinant human AM dissolved in 0.9% saline to release 0.3 or 1.0 \(\mu\)g/h peptide. Similarly, both the control group and the sham-operated rats (\(n=7\)) were infused with saline by an identical method. The infusion was discontinued on day 7 after the surgery by removing the pump from the rats. After an observational period of 8 weeks, rats were examined for the hemodynamic and hormonal parameters, organ weights, and histological evaluation of the heart.

**Short-Term Study**

To investigate the mechanisms of AM actions on LV remodeling and heart failure, we performed a separate series of experiments in which sham-operated (\(n=18\)) or MI rats infused with 1.0 \(\mu\)g/h AM (\(n=31\)) or saline (\(n=45\)) were examined during or at the end of the 7-day AM infusion period. To evaluate the systemic and local RAA system, 3 groups of sham-operated or MI rats infused with AM or saline were killed by decapitation on day 7 of MI. For measurements of plasma renin activity and aldosterone concentration, blood samples were collected into chilled tubes with 1.5 mg/mL of EDTA-2Na and centrifuged at 2000 \(\times g\) for 15 minutes at 4°C. Plasma renin activity and aldosterone concentration were measured with radioimmunoassay kits as described previously.13 After collection of blood samples, hearts were resected to measure the mRNA levels of ACE, angiotensin II type 1 (AT1) receptor, and p22-phox, a critical component of NADPH oxidase.14 The other 3 groups of sham-operated and MI rats infused with AM or saline were placed in individual metabolic cages to collect urine samples every 24 hours for measurement of urinary 8-iso-prostaglandin F2\(_\alpha\) (isoprostane), a marker of oxidative stress,15 during the AM infusion period of 7 days. Urinary isoprostane excretion was measured by an enzyme immunoassay according to the manufacturer’s instructions (Assay Design, Inc). On day 7 of the AM infusion, rats were subjected to hemodynamic, hormonal, and histological studies.

**Hemodynamic Studies and Peptide Measurements**

The animals were anesthetized by injection of 50 mg/kg IP pentobarbital sodium on day 7 or 9 weeks after the MI induction. Hemodynamic parameters were measured with a micromanometer-tipped catheter (SPC-320, Millar Instruments, Inc) as described previously.12 After the hemodynamic measurements, blood samples were collected and plasma levels of endogenous rat AM and ANP were measured with an immunoradiometric assay12 and a radioimmunoassay,13 respectively.

**Determination of Infarct Size and Collagen Volume Fraction**

After collection of the blood samples, 30 mmol/L potassium chloride was injected from the catheter to arrest the hearts in diastole. Then, the hearts were weighed, fixed in 10% formalin, and embedded in paraffin. Infarct size was measured by hematoxylin and eosin staining, and the collagen volume fraction of the noninfarcted LV area was determined by staining with Sirius red, a collagen-specific dye, as described previously.12

**Measurement of ACE, AT1 Receptor, and p22-phox mRNA in Noninfarcted LV**

Total RNA was extracted from the noninfarcted LV with TRIzol (Invitrogen, Inc) according to the manufacturer’s protocol and then subjected to reverse transcription by means of SuperScript reverse transcriptase (Gibco-BRL, Life Technologies, Inc) into cDNA. To measure rat ACE, AT1 receptor, p22-phox, and GAPDH mRNA levels, we used the quantitative reverse transcription–polymerase chain reaction method, real-time–quantitative polymerase chain reaction (Prism 7700 Sequence Detector; Applied Biosystemics).16 Oligonucleotides used as probes and primers for the ACE, AT1 receptor, and GAPDH measurements were previously described by Nakamura et al and Naito et al, and those used for p22-phox were as follows: probe, 5’-TGTCTCCCACTTACTGCTGCCATTGC-3’; forward primer, 5’-TGTCCTCCACTTACTGCTGCGTC-3’; and reverse primer, 5’-GCTCATCTGTCTGGAGTA-3’. The mRNA levels were compared after they had been normalized relative to those of GAPDH.

**Statistical Analysis**

All data are expressed as mean±SEM. Multiple comparisons were evaluated by 1-way ANOVA, followed by the Scheffe F test, and differences were considered significant at \(P<0.05\).

**Results**

**Survival Rate**

To assess the AM effect on mortality, we compared the survival rates between the 2 MI groups infused with saline or 1.0 \(\mu\)g/h AM (Figure 1). Eight rats in the AM-infused and 25 in the saline-infused MI group died during the long- and short-term experimental periods, whereas no rats in the sham group died. As shown in Figure 1, the survival rate of the AM infusion group (81%) was significantly (\(P<0.05\)) higher than that of the control group (59%) by Kaplan-Meier survival analysis, whereas the effect of 0.3 \(\mu\)g/h AM did not reach statistical significance (data not shown).
Infarct Size, Body Weight, Organ Weights, and Collagen Volume Fraction

As shown in Table 1, no significant differences were noted in the infarct sizes among the 3 MI groups. Both the body weight and body weight gain of the saline-infused MI rats were lower (P<0.01) than those of the sham group, although the impaired weight gain was significantly (P<0.01) improved by the 1.0 µg/h AM infusion. The heart, lung, and body weights in the MI groups infused with saline were heavier (P<0.01) than in the sham group but were significantly (P<0.01) reduced by the 1.0 µg/h AM infusion. A similar tendency was observed in plasma ANP, although the differences were not statistically significant.

Hemodynamic and Hormonal Parameters

No significant differences were noted in heart rate and mean arterial and central venous pressures among the 4 groups at 9 weeks (Table 2). The LV end-diastolic pressure in the saline-infused MI group was raised (P<0.01) compared with the sham group but was significantly (P<0.01) lowered by 1.0 µg/h AM infusion. The plasma level of rat endogenous AM was increased (P<0.05) in the saline-infused MI group compared with the sham, and the increased collagen volume was significantly (P<0.05) reduced by the 1.0 µg/h AM infusion.

Results of the Short-Term Experiment

To investigate the mechanism(s) responsible for the beneficial effect of AM on the late outcome described above, we examined the sham-operated or MI rats infused with saline or 1.0 µg/h AM during or at the end of the 7-day infusion period. There were no differences in infarct size, heart rate, and mean and central venous pressures among the groups (Table 3). The LV end-diastolic pressure was elevated in 2 MI groups compared with the sham group, but at this time point, the AM infusion had no significant effect on LV end-diastolic pressure. In addition, no differences were observed in body, heart, and lung weights on day 7 (data not shown). As shown in Table 4, no differences were noted in the plasma renin activity and aldosterone concentration of the sham-operated and MI groups. Meanwhile, the urinary excretion of isoprostane was increased (P<0.01) in the saline-infused MI rats compared with the sham. Interestingly, the AM infusion significantly (P<0.01) reduced the urinary isoprostane excretion to the control level during the 7-day period. To detect any change in the local RAA system and oxidative stress, we measured AT1 receptor, ACE, and p22-phox mRNA levels in the noninfarcted LV (Figure 2). No significant differences were noted in the AT1 receptor expressions in the noninfarcted LV (Figure 2A), but as shown in Figure 2B, ACE expression in the saline-infused MI rats was increased by 121% (P<0.01) compared with the sham rats. This augmentation was significantly (P<0.05) reduced in the AM-infused MI group by 31%. Similarly, p22-phox

TABLE 1. Infarct Size, Body and Organ Weights, and Collagen Volume Fraction of the Noninfarcted LV Area at 9 Weeks

<table>
<thead>
<tr>
<th>MI</th>
<th>Sham</th>
<th>Saline</th>
<th>0.3 µg/h AM</th>
<th>1.0 µg/h AM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rats, n</td>
<td>7</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Infarct size, %</td>
<td></td>
<td>0.01</td>
<td>43.3±2.5</td>
<td>43.8±1.8</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>460±6</td>
<td>411±10†</td>
<td>441±11</td>
<td>458±7‡</td>
</tr>
<tr>
<td>Body weight gain, g/d</td>
<td>3.7±0.1</td>
<td>2.8±0.2†</td>
<td>3.3±0.2</td>
<td>3.7±0.1§</td>
</tr>
<tr>
<td>Heart weight/body weight, mg/g</td>
<td>3.0±0.1</td>
<td>4.7±0.2†</td>
<td>4.5±0.4‡</td>
<td>3.4±0.1§</td>
</tr>
<tr>
<td>Lung weight/body weight, mg/g</td>
<td>3.2±0.1</td>
<td>6.5±0.6†</td>
<td>4.8±0.5*</td>
<td>3.7±0.1§</td>
</tr>
<tr>
<td>Collagen volume fraction, %</td>
<td>3.4±0.2</td>
<td>8.3±0.7†</td>
<td>7.0±1.1*</td>
<td>5.1±0.6‡</td>
</tr>
</tbody>
</table>

Values are mean±SEM. *P<0.05, †P<0.01 vs sham rats; ‡P<0.05, §P<0.01 vs MI rats infused with saline.

TABLE 2. Hemodynamic and Hormonal Parameters at 9 Weeks

<table>
<thead>
<tr>
<th>MI</th>
<th>Sham</th>
<th>Saline</th>
<th>0.3 µg/h AM</th>
<th>1.0 µg/h AM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, bpm</td>
<td>415±10</td>
<td>394±17</td>
<td>405±18</td>
<td>422±6</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>85±6</td>
<td>91±4</td>
<td>82±8</td>
<td>90±6</td>
</tr>
<tr>
<td>Central venous pressure, mm Hg</td>
<td>0.2±0.1</td>
<td>2.9±1.8</td>
<td>0.5±0.3</td>
<td>0.3±0.3</td>
</tr>
<tr>
<td>LV end-diastolic pressure, mm Hg</td>
<td>2.3±0.3</td>
<td>11.4±2.0†</td>
<td>6.5±1.8</td>
<td>4.0±0.6§</td>
</tr>
<tr>
<td>Rat endogenous AM, fmol/mL</td>
<td>3.5±0.3</td>
<td>5.3±0.8*</td>
<td>3.7±0.6</td>
<td>3.3±0.3‡</td>
</tr>
<tr>
<td>Rat ANP, fmol/mL</td>
<td>11±3</td>
<td>61±29</td>
<td>53±16</td>
<td>19±3</td>
</tr>
</tbody>
</table>

*Values are mean±SEM. The number of rats examined is given in Table 1. *P<0.05, †P<0.01 vs sham rats; ‡P<0.05, §P<0.01 vs MI rats infused with saline.
expression in the MI group infused with saline was increased by 76% (P<0.01), and the increased expression was significantly (P<0.05) decreased in the AM infusion group by 30% (Figure 2C).

Discussion
In the present study, we showed that intraperitoneal AM infusion over 7 days immediately after MI induction reduced the LV end-diastolic pressure, collagen volume fraction of the noninfarcted LV, and heart and lung weights, which were determined at 9 weeks of MI, in rats. A dose setting of 1.0 μg/h of human recombinant AM was chosen through reference to our previous study10,12; in this study, we also used a lower dose of 0.3 μg/h AM and found milder effects that did not reach statistically significant levels. We previously reported that plasma AM levels in patients with heart failure progressively increased in relation to disease severity and that the elevated levels were gradually reduced by successful treatment.18 Accordant with this, the increased endogenous rat AM levels in the MI rats were significantly reduced by human AM infusion in the present study. Thus, AM administration during the early period of MI ameliorated chronic progression of LV remodeling and heart failure in rats.

Although AM has been shown to possess a wide spectrum of biological actions,7 we may first need to discuss whether the vasodilator and natriuretic actions of AM contributed to the beneficial effects observed in the present study. Long-term infusion of AM has been found to lower blood pressure in a rat model of renovascular hypertension.10 However, in the present study, the mean arterial pressure in the AM-infused MI group remained unchanged at day 7 of the infusion, a finding consistent with our previous observation.12 To examine the natriuretic and diuretic effects in the AM infusion period, we measured the LV end-diastolic pressures on day 7 of MI. As shown, the elevated LV end-diastolic pressure was lowered by AM infusion, but at this time point, the difference was not statistically significant. To further examine the natriuretic and diuretic actions, we measured urine volume and urinary sodium excretion during the AM infusion period using individual metabolic cages but again failed to detect a significant increase in urinary output and sodium excretion (data not shown). Thus, either an afterload or preload reduction during the AM infusion period may be unlikely as the major mechanism alleviating chronic progression of LV remodeling and heart failure, although we should

<table>
<thead>
<tr>
<th>TABLE 3. Infarct Size and Hemodynamics at Day 7</th>
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<tr>
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<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Rats, n</td>
</tr>
<tr>
<td>Infarct size, %</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
</tr>
<tr>
<td>Central venous pressure, mm Hg</td>
</tr>
<tr>
<td>LV end-diastolic pressure, mm Hg</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

*P<0.05, †P<0.01 vs sham group.

<table>
<thead>
<tr>
<th>TABLE 4. Plasma Renin Activity and Aldosterone Concentration at Day 7 and Urinary 8-Iso-Prostaglandin F2α Excretion During 7-Day AM Infusion Period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Plasma renin activity, ng · mL⁻¹ · h⁻¹ (n)</td>
</tr>
<tr>
<td>Plasma aldosterone concentration, pg/mL (n)</td>
</tr>
<tr>
<td>8-Iso-prostaglandin F2α, ng/d (n)</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

*P<0.01 vs sham group; †P<0.01 vs MI rats infused with saline.
not totally exclude the possibility that the slightly lower LV end-diastolic pressure contributed to the beneficial effects.

A number of neurohumoral factors acting systemically or locally are thought to be involved in the progression of LV remodeling or heart failure after MI.19 Among them, the RAA system has an important role,3,4 and blockade of the RAA system with ACE inhibitors or AT1 receptor blockers improves the cardiac function or prognosis of patients with MI.20,21 We reported that AM continuously infused for 2 weeks reduced plasma renin activity and aldosterone concentration in rats with renovascular hypertension.10 In the present study, plasma renin activity was not reduced in the MI group at day 7 of the AM infusion, with a slightly lower concentration of plasma aldosterone. We are unable to attribute the beneficial actions of AM largely to the inhibition of the systemic RAA system, but considering the role of aldosterone in LV remodeling,22 even a slight reduction should be raised as a possibility. In an effort to see the local RAA system, we measured ACE and AT1 mRNA levels in the noninfarcted LV on day 7 of the infusion. The ACE mRNA level increased in the noninfarcted LV of the saline-infused MI group, and the elevated level was significantly reduced at this time point, without a significant change in AT1 mRNA. Although the role of the local RAA system in LV remodeling or heart failure remains unclear, these findings suggest the possible modulation of the cardiac RAA system by AM.

An important finding is the reduced oxidative stress. Urinary excretion of 8-isoprostanone, a marker of systemic oxidative stress,15 was decreased in the MI rats during the AM infusion period. This finding is comparable to that of Shimosawa et al,11 who recently showed an antioxidative effect of AM using mice lacking the AM gene. In addition, we found that the AM infusion reduced expression of p22-phox, an essential component of NADH/NADPH oxidase,14 in noninfarcted LV. Because the NADH/NADPH oxidase system is known to be a major source of superoxide anion production in cardiac myocytes,23 the AM infusion might have reduced not only systemic but also local oxidative stress in the MI rats. Both animal and human studies suggest that an increase in free radical formation or oxidative stress is associated with the progression of heart failure.5,24 In fact, antioxidant therapies have been found to have beneficial effects on heart failure and LV remodeling after MI.25,26 Although the precise mechanism in the AM-induced reduction of urinary 8-isoprostanone and p22-phox expression remains to be explored, reduced oxidative stress may have contributed in part to the beneficial effects of AM observed in this study.

Recent studies suggest that AM exerts antiapoptotic effects in a rat model of myocardial ischemia-reperfusion injury probably through an Akt-dependent mechanism.27,28 We currently have no data on gene expression related to apoptosis, although it is possible that an antiapoptotic effect of AM participates in inhibiting LV remodeling. On the other hand, AM was reported to increase endothelial nitric oxide synthase expression or increase nitric oxide production in vascular walls via a phosphatidylinositol 3-kinase/Akt-dependent pathway.29 We measured endothelial nitric oxide synthase mRNA levels in noninfarcted LV, but no differences were noted in the saline- and AM-infused groups compared with controls (data not shown).

Finally, it should be noted that the mortality rate in MI rats was reduced by the AM infusion. Of interest, this effect was observed during the AM infusion period in the present study; we could not specify the cause of death despite postmortem examination. It has been reported that overexpression of AM by adenovirus-mediated gene delivery reduced ventricular arrhythmia after reperfusion injury in rats.27 Reduced fatal arrhythmia can be raised as a possibility for improved survival by AM infusion, but this hypothesis should be carefully tested by future experiments.

In summary, AM administration during the early period of MI reduced the mortality rate and alleviated the progression of LV remodeling and heart failure in rats. These beneficial effects were accompanied by reductions in oxidative stress and ACE expression in noninfarcted LV in the AM infusion period. The present findings suggest the possibility of AM as a new therapeutic tool for the treatment of acute MI.

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References


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