Beneficial effects of adrenomedullin on left ventricular remodeling after myocardial infarction in rats

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Received 12 February 2002; accepted 4 July 2002

Abstract

Objective: We previously reported that plasma adrenomedullin (AM) levels increase in patients with acute myocardial infarction (MI) and AM inhibits growth of rat cardiac myocytes and fibroblasts. The aim of this study was to examine the effects of long-term administration of AM on left ventricular (LV) remodeling, hemodynamic and hormonal parameters in a rat model of MI.

Methods: Rats with MI induced by left coronary ligation were intravenously infused with 1.0 μg/h of recombinant human AM or saline by osmotic mini-pump. After infusion for 4 weeks, hemodynamic and hormonal studies were performed, and the myocyte size and collagen volume in non-infarct LV area were quantified morphometrically.

Results: When compared with the MI rats infused with saline, continuous infusion of AM reduced the heart weight/body weight (4.4 ± 0.2 vs. 3.6 ± 0.1 g/kg, P < 0.01), myocyte size (922 ± 23 vs. 868 ± 10 μm², P < 0.05) and collagen volume fraction of non-infarct LV area (7.6 ± 0.8 vs. 4.8 ± 0.5%, P < 0.05), without affecting the infarct size. The AM infusion had no significant effect on the arterial pressure, but decreased the LV end-diastolic pressure (8.8 ± 1.8 vs. 4.4 ± 0.5 mmHg, P < 0.05) in the MI rats. The plasma level of endogenous rat AM in the MI rats infused with human AM was reduced by 27% (P < 0.05), with a slight reduction of plasma atrial natriuretic peptide, compared with the control.

Conclusions: Continuous administration of AM had beneficial effects on LV remodeling and hemodynamics in MI rats, suggesting the possibility that this peptide could be a useful therapeutic tool for acute MI.

Keywords: Fibrosis; Heart failure; Infarction; Remodeling; Vasoactive agents

This article is referred to in the Editorial by I.M.C. Dixon (pages 347–349) in this issue.

1. Introduction

Adrenomedullin (AM) is a potent vasodilator peptide that was originally isolated from human pheochromocytoma [1]. Accumulating research data show that AM has a wide spectrum of biological actions, among which are natriuresis, diuresis, inhibition of aldosterone secretion [2] and growth inhibition of cardiac myocytes and fibroblasts [3,4]. Both AM peptide and its precursor mRNA were detected at substantial levels in various tissues and organs including cardiac ventricles, and AM is also present in the circulating blood of humans and rats [5,6]. Plasma AM levels were found to progressively increase in patients with heart failure and acute myocardial infarction (MI) in relation to the severity of the diseases [7–11]. Similarly, AM levels were higher in the left ventricle (LV) of patients with heart failure [12] or in the infarct and non-infarct area of the LV in a rat model of MI [13], compared with respective controls. Considering the beneficial biological actions of AM on heart failure, these findings suggest that...
AM participates in counteracting mechanisms of deterioration of the disease. In patients with acute MI, cardiac function becomes impaired depending on the magnitude of LV remodeling. LV remodeling is characterized by thinning of the infarct area and by hypertrophy of non-infarct LV, where hypertrophy of cardiomyocytes, proliferation of cardiac fibroblasts and deposition of extracellular matrix are also involved in the process [14]. Given the growth-inhibitory effects of AM on cardiac myocytes and fibroblasts [3,4], it may also be possible that AM functions systemically or locally to act against the progression of LV remodeling. However, there has currently been little in vivo evidence to directly prove these hypotheses on the role of AM, not only in acute MI but also in heart failure. Recently, Nagaya et al. reported the beneficial hemodynamic effects of AM infusion for 30 min in patients with heart failure [15], but no data are available regarding the effects of AM infused continuously and chronically for a longer period of time in heart failure or MI. This study was performed in order to examine the effects of long-term infusion of AM on LV remodeling, hemodynamic and hormonal parameters in rats with MI.

2. Methods

2.1. Animals

Male Wistar/ST rats (Nihon SLC, Hamamatsu, Japan) weighing 280–320 g were used in this study. All the rats were housed in a temperature- and humidity-controlled room with free access to standard rat chow and water. The experiments were performed under the regulations of the Animal Research Committee of Miyazaki Medical College (1998-037). This investigation conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, 1996).

2.2. Induction of MI

The animals were anesthetized by intraperitoneal injection of a dose of 50 mg/kg of pentobarbital sodium, intubated with a 16-gauge intravenous catheter and mechanically ventilated with room air with a small rodent ventilator at a rate of 80 cycles per minute with a tidal volume of 1.0 ml/100 g body weight. A left thoracotomy was performed in the fourth or fifth intercostal space, and the left coronary artery was ligated near the origin of the pulmonary artery. The chest was closed and the rats were allowed to recover. Sham-operated animals underwent the identical surgical procedure as described above without the actual coronary artery ligation.

2.3. Experimental protocols

Immediately after the surgery, the rats receiving the coronary ligation were randomly divided into two groups: one infused with recombinant human AM (n=15) and the other with saline (n=16). Similarly, the sham-operated rats were divided into AM (n=8) and saline (n=8) groups. The rats were then subcutaneously implanted with osmotic mini-pumps (model 2004, Alza, Palo Alto, CA, USA) filled with recombinant human AM dissolved in 0.9% saline to release 1.0 μg/h of the peptide for 4 weeks. The pumps were connected to the left jugular vein by a polyethylene catheter (PE-50, Becton Dickinson, Sparks, MD, USA) and positioned in a pocket constructed in the subcutaneous tissue just below the sub-scapular region. For control, 0.9% saline was infused in a similar manner to the rats receiving the coronary ligation or sham operation. The recombinant human AM used in this study was provided by Shionogi (Osaka, Japan). The purity of this recombinant peptide was tested by reverse phase high-performance liquid chromatography (HPLC) and mass spectrometry, both of which showed that the recombinant human AM was completely identical to the chemically synthesized human AM widely used in the literature [15–18].

2.4. Hemodynamic studies

The animals were anesthetized by intraperitoneal injection of 50 mg/kg pentobarbital sodium 4 weeks after the coronary ligation or sham operation. The right carotid artery was cannulated with a micromanometer-tipped catheter (SPC-320, Millar Instruments, Houston, TX, USA) inserted into the aorta for the recording of heart rate and arterial pressure, and the catheter was then advanced into the LV to measure LV end-diastolic pressure. For the measurement of central venous pressure, another catheter was placed in the thoracic vena cava through the right jugular vein.

2.5. Blood sampling and measurement of AM and atrial natriuretic peptide (ANP)

A PE-50 catheter was inserted into the right carotid artery after the hemodynamic measurements, and 6.0 ml blood was collected from the catheter into ice-cooled tubes with 70 μg/ml of aprotinin and 1.5 mg/ml of ethylenediaminetetraacetic acid (EDTA)-2Na. The blood was centrifuged immediately at 4 °C and the plasma was stored at −30 °C until the assays of AM and ANP. The plasma levels of human and endogenous rat AM were separately measured with respective immunoradiometric assay (IRMA) kits (AM RIA Shionogi, Shionogi, Osaka, Japan). Since these assay kits are specific for human or rat AM peptide, they have no cross-reactivity with AM of other species [19]. The plasma levels of rat ANP were measured by radioimmunoassay, after extraction of the plasma with a Sep-Pak C₁₈ cartridge (Waters, Milford, MA, USA), as previously described [20].
2.6. Infarct size measurement

After collecting the blood samples, 30 mM potassium chloride was injected from the catheter to arrest the hearts in diastole. The hearts were excised, submersed in saline and divided into two transverse sections at the papillary muscle level. The hearts were then weighed, fixed in 10% formalin and embedded in paraffin. Paraffin sections (2 μm) were stained with hematoxylin and eosin, and scanned by microscopic digital camera (DP11, Olympus Optical, Tokyo, Japan) at a magnification of approximately ×10. The images were projected onto a color imaging analysis system (Mac SCORP Ver. 2.3.2, Mitani, Fukui, Japan). The lengths of the epicardium and endocardium in the infarct segment were measured, and the infarct size was expressed as the percentage of epicardium and endocardium circumferences of the LV in the section [21].

2.7. Determination of myocyte size and collagen volume fraction

Paraffin sections of the papillary muscle level of each ventricle were stained with hematoxylin and eosin and projected onto the above-mentioned image-analyzing system at a magnification of ×400. Cardiac myocytes in the non-infarct LV segment were traced, and the cross-sectional area of the cell was determined by the software. A total of 30 myocytes sectioned transversely at the level of nucleus were selected from each segment for the measurement, omitting oblique-sectioned cells, with resulting mean values subjected to statistical analysis. To measure collagen volume fraction, the paraffin sections were stained with Sirius red, a collagen-specific dye, the staining by which is shown to be directly correlated with the hydroxyproline contents of the tissue [22–25]. In each section, 16 separate parts of high power fields were scanned under polarized light, and the images were analyzed. The collagen volume was estimated, while omitting fibrosis of the perivascular, epicardial and endocardial areas. The collagen volume fraction was obtained by calculating the mean ratio of connective tissue to the total tissue area of all the measurements of the section.

2.8. Statistical analysis

All data are expressed as means±S.E.M. Differences among the groups were evaluated by one-way analysis of variance (ANOVA) followed by Scheffe’s F-test, and correlation between two variables by a simple regression analysis. Differences were considered significant at P<0.05.

3. Results

3.1. Basal measurements

Among the MI rats induced by coronary ligation, four of the AM-infused rats and eight of those infused with saline died during the infusion period over 4 weeks. The survival rate of the AM group (73%) was higher than that of the control (50%), but this difference was not statistically significant by a Kaplan-Meier survival analysis. Accordingly, the data shown below are from the MI rats infused with AM (n=11) or saline (n=8) that survived during the infusion period, while no rats died in the sham groups.

Table 1 shows the body weight, infarct size and heart weight of the four groups examined. Neither body weight nor body weight gain of the two MI groups was different from the sham-operated rats infused with saline. There were no differences in infarct size between the MI groups infused with saline or with AM. Both heart weight and heart weight/body weight in the MI group infused with saline were higher (P<0.01) than those in the sham-operated groups. When compared with the MI group infused with saline, the heart weight and heart weight/body weight were significantly reduced in the MI rats infused with AM by 15% (P<0.05) and by 18% (P<0.01), respectively.

3.2. Hemodynamic parameters

As shown in Table 2, no differences were noted in the heart rate and mean arterial pressure among the four groups. The central venous pressure in the saline-infused MI group was higher than in the other groups, but the

<table>
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<td>Effects of recombinant human AM on body weight, infarct size and heart weight in sham-operated and MI rats</td>
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<td>Body weight gain (g/day)</td>
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<td>Heart weight (mg)</td>
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<td>Heart weight/body weight (mg/g)</td>
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Values are means±S.E.M.

**P<0.01, compared with sham-operated rats infused with saline.

†P<0.05, †P<0.01, compared with MI rats infused with saline.
differences were not significant. The LV end-diastolic pressure in the MI group infused with saline was significantly ($P<0.01$) elevated compared with the sham group infused with saline. When compared with the MI group infused with saline, the elevation was significantly ($P<0.05$) reduced by the AM infusion. The AM infusion failed to completely normalize the LV end-diastolic pressure, but the level became statistically insignificant compared with the saline-infused sham.

### 3.3. Plasma levels of AM and ANP

Table 3 shows the plasma levels of human AM, endogenous rat AM and ANP in the sham-operated and MI groups infused with saline or recombinant human AM. Exogenous human AM was detected in the plasma of the AM-infused sham and MI groups, and the levels were within the physiological range. The plasma level of rat endogenous AM was significantly ($P<0.01$) increased in the saline-infused MI group compared with the sham-operated rats infused with saline. In a comparison between two of the MI groups infused with saline and AM, the human AM infusion significantly ($P<0.05$) reduced the plasma level of endogenous rat AM. Similarly, the plasma levels of ANP were increased in two MI groups compared with the sham-operated group infused with saline, and the increased ANP level became lower with the AM infusion, but the differences were not significant. Simple regression analyses of the data from the four groups showed that the plasma rat AM levels had significant positive correlations ($P<0.01$) with the heart weight/body weight ($r=0.57$), LV-end diastolic pressure ($r=0.35$) and plasma ANP ($r=0.57$).

#### 3.4. Myocyte size and collagen volume fraction

Fig. 1 shows the result of morphometric analysis of

![Cross-sectional area of myocytes in non-infarct LV area. Values are means±S.E.M. **P<0.01, compared with sham-operated group infused with saline; †P<0.05, compared with MI group infused with saline.](image)

#### Table 3

<table>
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<th>Sham</th>
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<td></td>
<td>Saline</td>
<td>AM</td>
<td>Saline</td>
<td>AM</td>
</tr>
<tr>
<td>Human AM (fmol/ml)</td>
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<td>1.7±0.3</td>
<td>ND</td>
<td>1.8±0.5</td>
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<tr>
<td>Rat endogenous AM (mol/ml)</td>
<td>5.1±0.3</td>
<td>5.3±0.4</td>
<td>8.3±1.0**</td>
<td>6.1±0.4†</td>
</tr>
<tr>
<td>Rat ANP (fmol/ml)</td>
<td>13±1</td>
<td>16±2</td>
<td>104±29</td>
<td>72±25</td>
</tr>
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</table>

Plasma level of human AM, rat AM and ANP were measured in sham-operated and MI rats infused with saline or recombinant human AM for 4 weeks, as described under Methods. Values are means±S.E.M. AM, adrenomedullin; ANP, atrial natriuretic peptide.

$**P<0.01$, compared with sham-operated group infused with saline.

$†P<0.05$, compared with MI group infused with saline.
myocyte size of the non-infarct LV area in the sham-operated and MI groups infused with saline or recombinant human AM. Cross-sectional area of the myocyte significantly ($P<0.01$) increased in the saline-infused MI group (922±23 μm$^2$) compared with the saline-infused sham group (764±6 μm$^2$), and hypertrophy of the myocytes was partially, but significantly ($P<0.05$) inhibited by the AM infusion (868±10 μm$^2$). To observe the effect of chronically infused AM on interstitial fibrosis of the myocardium, we examined the collagen volume of the non-infarct LV area histologically by Sirius red staining. Photomicrographs shown in Fig. 2 are representative findings of non-infarct LV segments from the MI rats infused with saline (A) or human AM (B) and those from the sham-operated rat infused with saline (C) and human AM (D). The collagen volume increased in the MI rat (A and B) compared with the sham-operated rats (C and D), but it seemed to be less in the AM-infused MI rat (B) than in the saline-infused MI rat (A). Fig. 3 shows the result of the quantitative analysis of collagen volume. The collagen volume fraction significantly ($P<0.01$) increased in the

Fig. 2. Collagen volume of non-infarct LV segment stained by Sirius red. Photomicrographs of the non-infarct segments of the MI rat infused with saline (A) or human AM (B) and those of the sham-operated rat infused with saline (C) or human AM (D) for 4 weeks, taken under polarized light. The bar in the photomicrograph indicates 50 μm.

Fig. 3. Quantitative evaluation of collagen volume of non-infarct LV area. The collagen volume fraction in the Sirius red-stained section was determined as described in Methods. Values are means±S.E.M. **$P<0.01$, compared with sham-operated group infused with saline; †$P<0.05$, compared with MI group infused with saline.
saline-infused MI group (7.6±0.8%) compared with the saline-infused sham group (3.6±0.1%), and the increased collagen volume was significantly (P<0.05) reduced by the AM infusion in the MI rats (4.8±0.5%), while in sham-operated rats, AM infusion had no significant effect on the collagen volume fraction (3.4±0.1%).

4. Discussion

In the present study, we showed, using a rat model of MI, that (i) chronically infused, recombinant human AM reduced the heart weight, the myocyte size and collagen volume fraction of non-infarct LV area without affecting the infarct size, and (ii) this AM infusion decreased the LV end-diastolic pressure, reducing the plasma level of endogenous AM. To the best of our knowledge, this is the first report on the long-term effects of AM infused continuously in MI.

It has been recognized that LV remodeling, which results in an impairment of LV function, is an important determinant in the prognosis of patients with acute MI [14]. LV remodeling after acute MI is characterized by thinning of the infarct segment and by hypertrophy of the non-infarct myocardium. In addition to the hypertrophy of cardiac myocytes, proliferation of cardiac fibroblasts and deposition of extracellular matrix, both of which stiffen the surviving myocardium, are also involved in the remodeling process. Mechanical stress to the infarct or non-infarct LV area is an important factor, while neurohumoral factors acting systemically or locally are also thought to participate in the progression of LV remodeling [26].

The renin–angiotensin system has been shown to play an important role in LV remodeling after acute MI [27]. Either circulating or locally-produced angiotensin II acts on the myocardium to induce hypertrophy of cardiomyocytes and promote fibrosis of the interstitium [28,29]. In fact, an angiotensin-converting enzyme inhibitor has been found to effectively inhibit cardiac remodeling, preserving cardiac function, when started at the early phase in patients with acute MI [30]. The direct effect of AM on juxtaglomerular cells secreting renin remains unclear, and its action on the cardiac tissue renin–angiotensin system has yet to be explored. Meanwhile, Yamaguchi et al. found that AM acts on the adrenal cortex to reduce the secretion of aldosterone, a steroid hormone which is thought to be involved in interstitial fibrosis of the myocardium [31]. Shimosawa et al. reported that endogenous AM antagonized angiotensin II increasing oxidative stress, by using heterozygote of AM gene-knockout mice [32]. We have previously reported that AM suppressed the plasma renin activity, reducing the plasma aldosterone level, when infused chronically at the same dose as used in this study, into two-kidney, one-clip renovascular hypertensive rats [18]. It seems, therefore, possible that chronically infused AM partly inhibited cardiac remodeling by inhibiting the renin–angiotensin–aldosterone (RAA) system in the present study. In addition to the RAA system, other humoral factors, such as endothelin-1 or transforming growth factor-β (TGF-β) are known to be involved in cardiac hypertrophy and fibrosis [33,34]. The effects of AM on these factors acting systemically or locally seem important issues that should be also addressed by future experiments.

Other important mechanisms in the inhibited cardiac remodeling by AM may be a reduced after-load or pre-load to the heart. AM has been shown to have a potent vasodilator action by ex vivo experiments [35]. However, in the present study, no significant difference in blood pressure was noted between the groups infused with AM and saline. Additionally, in another series of our experiments, where an identical dose of AM was infused to MI rats induced by coronary ligation, AM had no effect on blood pressure throughout the experiment for 4 weeks (data not shown). Thus, the reduced after-load is unlikely to be the mechanism. Meanwhile, since AM possesses natriuretic and diuretic actions, the inhibitory effect of AM on cardiac remodeling may have been partially secondary to reduced pre-load. Indeed, LV end-diastolic pressure was significantly reduced by the AM infusion in MI rats in the present study, and although not significant, both central venous pressure and plasma ANP, sensitive markers of volume retention, in the AM-infused MI rats were lower than those in controls. These findings appear comparable with the report by Lainchbury et al. [36], where AM showed significant effects on a number of endocrine and renal parameters despite a modest change only in diastolic pressure when infused intravenously to healthy men.

In addition to the systemic hemodynamic or hormonal effects of AM, we need to discuss the direct action on the myocardium. Substantial levels of AM peptide and AM gene expression were found in the myocardium of rats and humans [5,6]. Comparable with this, we found that AM is produced and secreted from cultured rat cardiac myocytes and fibroblasts into the media [3,4], and that the AM production is augmented by mechanical stretching in the myocytes [37]. Nagaya et al. reported an increased AM level in the non-infarct LV area following MI in rats, with a significant correlation between the AM level and LV end-diastolic pressure [13]. These observations suggest an important role of AM in the myocardium exposed to excessive mechanical stress. Using cell culture experiments, we found that AM inhibits the hypertrophy of cardiomyocytes and proliferation of cardiac fibroblasts in an autocrine or paracrine fashion [3,4]. Taken together, we may be able to raise the possibility that exogenously infused AM acts together with endogenous AM to inhibit LV remodeling following MI partly by directly inhibiting the growth of cardiac myocytes and fibroblasts in the non-infarct LV area.

In the present study, the AM infusion partially inhibited hypertrophy of myocytes in the non-infarct LV area in the
MI group but not in the sham, a result consistent with our previous in vitro data and that of others [3,4,38–40] showing the anti-hypertrophic effect of AM only in the myocytes incubated with the growth factors. Meanwhile, according to those studies, AM suppressed proliferation of the cardiac fibroblasts even in the basal condition. In the present study, the AM infusion reduced collagen volume fraction in the MI group, but not in the sham. One explanation for this discrepancy may simply be a difference between the experimental conditions in vitro and in vivo. Additionally, we need to mention the different concentration and duration of the AM exposure. Our preliminary study shows that the plasma level of human AM increases to a level of 10 fmol/ml at 24 h, and then gradually decreases to 1–2 fmol/ml in 7 days when infused to control rats with an identical experimental protocol to that of the present study. Currently, the underlying mechanism for this time course of the plasma AM level is unknown, though it is clear that the myocardium had been exposed to exogenous AM at the lower concentration for a longer period of time in the present study, compared with those in vitro studies.

We previously showed increased plasma AM levels in patients with acute MI [8]. The plasma level of AM was also found to be progressively elevated in patients with heart failure in relation to the severity of the diseases including ischemic heart disease [7–11]. In accordance with this, in the present study, the plasma endogenous rat AM levels were closely correlated with heart weight, plasma ANP and LV end-diastolic pressure. Given the beneficial biological actions of AM such as vasodilation, natriuresis and inhibition of the renin–angiotensin system, AM has been assumed to have a role in acting against the deterioration of heart failure. This hypothesis appears to be further supported by the present study. In the meantime, the above-mentioned time course of the plasma level of exogenous human AM level may lead us to the speculation that the increased AM level in the initial phase of MI might have been more important for the beneficial effect than that in the late phase in the present study. Indeed, the total AM levels, recombinant human AM plus endogenous rat AM levels, at 4 weeks of the experiment were similar between two groups infused with saline and human AM in the present study. It should be of interest to compare these results with the increased plasma AM level during the early phase of acute MI patients [8–10]. As discussed, a number of possibilities could be raised as to the mechanisms for the beneficial actions of AM, and these issues should be explored by future experiments.

In summary, long-term infusion of recombinant human AM partially inhibited the progression of LV remodeling in a rat model of MI, reducing the LV end-diastolic pressure. The present study provides in vivo evidence of the AM roles in counteracting the progression of heart failure and in modulating LV remodeling in MI of rats, suggesting the possibility of utilizing this peptide as a therapeutic tool.

References