Cardioprotective Role of AT2 Receptor in Postinfarction Left Ventricular Remodeling

Yoshihiko Oishi, Ryoji Ozono, Yoko Yano, Yasuhiro Teranishi, Masahiro Akishita, Masatsugu Horiuchi, Tetsuya Oshima, Masayuki Kambe

Abstract—The aim of this study was to determine the role of the AT2 receptor (AT2R) in left ventricular (LV) remodeling after myocardial infarction (MI). The left anterior descending arteries were ligated in AT2R gene knockout (Agtr2-) and wild-type (Agtr2+) mice. The LV remodeling was evaluated by echocardiography and histology over a period of 2 weeks after MI. The infarct sizes in hearts excised from Agtr2+ and Agtr2- mice on day 1 were similar. The mortality rate of Agtr2- mice (62.9%) on day 14 after MI was significantly (P<0.05) higher than that of Agtr2+ mice (39.7%). Accordingly, LV/body weight ratios (3.7±0.2 versus 3.0±0.1 on day 14) and LV end-diastolic (4.8±0.3 versus 3.9±0.4 mm on day 7) and end-systolic (4.4±0.3 versus 3.2±0.6 mm on day 7) dimensions evaluated by echocardiography were significantly greater in Agtr2- than in Agtr2+ mice. The rates of ventricular arrhythmias, rates of cardiac rupture, and blood pressures in the 2 strains were similar after MI. Myocyte cross-sectional areas were increased after MI, but the magnitudes were similar in Agtr2+ and Agtr2- mice, indicating the greater increases in LV dimensions and weight in Agtr2- mice are due to elongation of myocyte length and/or an increase in the interstitial weight (including vasculatures, infiltrated cells, and interstitial fluid). Interstitial fibrosis in remote myocardium was not evident in either strain. These results indicate AT2R plays a significant role in the protection against early development of LV dilation, thereby reducing the early mortality rate after MI. (Hypertension. 2003;41[part 2]:814-818.)

Key Words: mice ● myocardial infarction ● receptors, angiotensin ● remodeling

Myocardial infarction induces global changes in ventricular architecture, called post-myocardial infarction (MI) left ventricular (LV) remodeling, which involves LV dilation, myocyte hypertrophy, and interstitial fibrosis, leading to a deterioration in LV function that is associated with increased rate of mortality.1,2 A substantial body of evidence3-5 has suggested that angiotensin II (Ang II) plays a critical role in the development of post-MI LV remodeling. Ang II has 2 major receptor subtypes, AT1 receptor (AT1R) and AT2R, both of which are expressed in the heart.6 It has been suggested that AT1R signaling mediates vasoconstriction, aldosterone secretion, cardiomyocyte hypertrophy, proliferation of fibroblasts, interstitial collagen deposition, and catecholamine release, all of which are implicated in progression of LV remodeling.7,8 Harada et al7 recently reported that AT1R gene-knockout mice had less LV remodeling and improved survival after MI. A deletion of AT1R or a blockade of AT1R with specific antagonists may result in selective binding of Ang II to the AT2R, indicating that the effect of AT1R blockade could be in part mediated by the effect of AT2R.7 However, little is known about the role played by AT2R in post-MI LV remodeling. The AT2R is thought to have opposite effect to AT1R and has been shown to suppress myocardial hypertrophy,9-11 fibroblast proliferation,10,12 and vascular cell hyperplasia.13,14 We have recently demonstrated in gene-engineered mice that interstitial fibrosis associated with LV hypertrophy was enhanced by a deletion of AT2R15 and inhibited by cardiac-specific overexpression of AT2R.16 Therefore, it is hypothesized that the AT2R plays a cardioprotective role in LV remodeling after MI. In the present study, to test this hypothesis, we induced MI on Agtr2- and Agtr2+ mice and compared the survival rates, cardiac geometries and functions, and histological findings in the 2 strains of mice over a period of 2 weeks after MI. We present a finding that the AT2R plays an essential role in protection against LV dilation and in improving survival rate in the early phase after MI.

Methods

Animals
Pairs of adult male Agtr2-17 and Agtr2+ littermates were used in this study. Because the AT2R gene is located on the X chromosome, heterozygous females were mated with Agtr2+ males to obtain hemizygous and Agtr2+ males. These mice were back-crossed for at least 6 generations onto a FVB/N background.15 All experimental procedures were approved and carried out in accordance with the
Surgical Procedure

Male mice at ages of 10 to 12 weeks were anesthetized with pentobarbital (50 \( \mu \)g/g), intubated, and artificially ventilated with a rodent respirator. A left thoracotomy was performed, and MI was induced by permanent ligation of the left anterior descending artery (LAD) with a 7–0 nylon surgical suture, 1 to 2 mm from the tip of the left auricle. Successful ligation of the LAD was verified by color change of the ischemic area and by ST-segment elevation on the ECG. Some mice were killed 1 day after ligation and the infarct size was calculated as described previously, with some modification. Briefly, the hearts were cut into 2 pieces transversely at the middle portion of the site of ligation and apex. In cross sections, infarct size was calculated as the ratio of infarction length to the circumference of the endocardium. For the sham procedure, the same procedure was performed except for the LAD ligation.

Agtr2- and Agtr2 mice were randomly allocated to 1 of 4 study groups. In one group, the natural prognosis after myocardial infarction was examined. The mice in this group were observed over a period of 2 weeks after MI, and the causes of death were estimated by autopsy (31 Agtr2+ mice and 35 Agtr2- mice). In another group, physiological profiles were examined. Transthoracic echocardiography was performed before (day 0) and on days 3, 7, and 14 after surgery or sham operation (18 Agtr2+ mice with MI, 4 Agtr2+ mice underwent sham-operation, 15 Agtr2-mice with MI, and 6 Agtr2- mice underwent sham-operation). Blood pressure was measured on days 0 and 3 by the tail-cuff method as previously described. In another group, histologic and morphometric examinations were performed. The mice in this group were killed on days 0, 3, and 14, and the hearts and lungs were excised and weighed. The hearts were subjected to histological assessment. The numbers of animals are indicated in tables and in figure legends. In the final group, Western blot analysis was performed. Three mice of each strain were killed on day 3 after MI.

Physiological and Morphological Assessment

Cardiac geometry and function were evaluated with the use of an echocardiographic system (Toshiba SSA 550A) equipped with a 14-MHz linear transducer. All studies were performed under spontaneous respiration and light anesthesia with an intraperitoneal tribromoethanol/amylene hydrate (Avertin) 2.5% wt/vol solution (5\( \mu \)L/g of mouse). Avertin was chosen for its negligible hemodynamic effects at this dose. LV end-diastolic dimension (LVDd) and end-systolic dimension (LVDs) were measured at the distal level of the papillary muscle by using short-axis M-mode images. Three beats were averaged for each measurement. Percent fractional shortening (%FS) was calculated as \([\text{LVDd} - \text{LVDs}] / \text{LVDd} \times 100\).}

Histopathological Assessment

Mice were killed with KCL injection through the jugular vein. The hearts were fixed with 10% buffered formalin and embedded in paraffin. One to two-micrometer-thick sections were cut and stained with Masson’s trichrome for measurement of myocyte cross-sectional area and perivascular fibrosis as previously described. For measurement of cross-sectional area, 100 myocytes (per mouse) having circular profiles were chosen from a noninfarcted area and the areas were traced. Perivascular fibrosis was assessed by calculating the ratio of the collagen area to total vessel area. The rate was defined as the perivascular fibrosis index. More than 10 coronary arteries were measured per mouse. All the parameters were quantitatively analyzed with Scion Image 1.62 software (National Institutes of Health Service Branch).

Western Blotting of AT1R

The amount of AT1R was examined by Western blot analysis with the use of an anti-AT1R antibody (Santa Cruz, AT1 N10, sc1173), as previously described. Membrane fractions were prepared from the left ventricles of Agtr2+ and Agtr2- mice 3 days after MI or sham operation. The samples were subjected to SDS-PAGE and transferred to nitrocellulose membranes. The membranes were probed with the anti-AT1R antibody.

Statistical Analysis

All results are expressed as mean±SEM. Analyses of survival after MI were carried out by the Kaplan-Meier method with the Breslow-Gehan-Wilcoxon method. Multiple comparisons among ≥3 groups were carried out by 1-way ANOVA and the Fisher exact test for post hoc analyses. Statistical significance was accepted at a value of \( P<0.05 \).

Results

Survival Rate After Myocardial Infarction

There was no difference between the infarct sizes in Agtr2+ (62.7±6%, \( n=10 \)) and Agtr2- mice (66.3±5%, \( n=9 \)) on day 1. The results of Kaplan-Meier analysis in 31 Agtr2+ and 35 Agtr2- mice showed that the survival rate after MI over the 2-week period was significantly \( (P<0.05) \) lower in Agtr2- mice than in Agtr2+ mice (Figure 1.). Postmortem examination of the hearts showed that cardiac rupture had occurred in 11 (48%) of the 23 Agtr2- mice and 7 (50%) of the 14 Agtr2+ mice, but other causes of death were not identifiable. Electrocardiograms of randomly selected mice with MI were monitored for 2 hours, but no arrhythmia was evident in either strain of mice.

hemodynamic and Morphological Assessments

Systolic blood pressure tended to decrease after MI in both Agtr2+ and Agtr2- mice, but there was no significant difference in systolic and diastolic blood pressures between the 2 strains of mice either before or after MI (Table 1). Echocardiography was performed on days 0, 3, 7, and 14 after surgery or sham operation (Figure 2). LVDd and LVDs were progressively enlarged in both Agtr2+ and Agtr2- mice after MI. However, on days 3 and 7 after MI, both LVDd and LVDs were significantly larger in Agtr2+ mice than in Agtr2- mice, indicating that early expansion of infarct and/or remote myocardium after MI was enhanced in Agtr2- mice. There was no significant difference between %FS in Agtr2+ mice and that in Agtr2- mice with MI throughout the study period (Figure 2C). LVDd, LVDs, and %FS in Agtr2+ mice and those in Agtr2- mice before surgery were similar and were not affected by the sham operation.
Body weights, LV weights, and lung weights of mice killed on days 0, 3, and 14 after surgery are shown in Table 2. Consistent with the results of echocardiography showing exaggerated LV dilation after MI in Agtr2- mice, the LV/body weight ratio in Agtr2- mice was significantly ($P < 0.05$) larger than that in Agtr2+/+ mice on days 3 and 14. The lung/body weight ratio, an index for heart failure, tended to be larger in Agtr2- mice than in Agtr2+/+ mice on days 3 and 14, but the difference did not reach statistical significance.

### Myocyte Hypertrophy, Interstitial Fibrosis, and Histological Assessment

The myocyte cross-sectional area in the myocardium remote from infarction progressively increased after MI (from day 3 to day 14) both in Agtr2+/+ and Agtr2- mice, but the magnitude of increase in the 2 strains of mice was not different (Figure 3). On the other hand, LV weight and LV dimension were larger in Agtr2- mice during the 2 weeks after MI, indicating that cardiomyocytes were elongated to a larger extent in Agtr2- mice. Other explanations for this finding may be an increase in the weight of interstitium, including those of remodeled vasculature, infiltrated cells, and interstitial fluid, in Agtr2- mice.

MI did not increase the perivascular fibrosis index until day 14 either in Agtr2+/+ or Agtr2- mice (Figure 4). Infiltration of the infarct zone and the border zone by neutrophils and macrophages did not appear to be different in Agtr2+/+ and Agtr2- mice (data not shown).

### AT1R Expression

As shown in Figure 5, the amounts of AT1R determined by Western blot analysis were not different in Agtr2+/+ and Agtr2- mice on day 3, excluding the possibility that the LV dilation and increased mortality rate after MI in Agtr2- mice were a result of upregulation of the number of AT1R in the myocardium. However, we cannot exclude a possibility that AT1R activity, not the number, was increased in Agtr2- mice, based on a recent report by AbdAlla et al that AT2R binds directly to the AT1R and thereby antagonizes the function of the AT1R.19

### Discussion

The major findings in the present study are (1) the survival rate of Agtr2- mice was reduced in the first 2 weeks after MI and (2) LV dilation on days 3 and 7 after MI was greater in Agtr2- mice. Since there was no apparent difference between the rates of occurrences of cardiac rupture or fatal arrhythmia in Agtr2+/+ and Agtr2- mice, the most likely cause of the increased mortality rate of Agtr2- mice is heart failure. These observations suggest that the AT2R plays a cardioprotective role in the mechanism of preventing early development of LV dilation/LV failure after acute MI. Recently, Xu et al20 reported that post-MI survival rate was not different between AT2R-null mice and wild-type mice. The discrepancy between the results of our study and theirs may be explained by the differences in the introduced infarct size (62% to 66% of...
TABLE 2. Body Weight, LV and RV Weights, and Lung Weight Before and After Myocardial Infarction in Agtr2+ and Agtr2- Mice

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Basal</th>
<th>Day 3</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agtr2+ (n=21)</td>
<td></td>
<td>(n=7)</td>
<td>(n=5)</td>
</tr>
<tr>
<td>BW, g</td>
<td>28.4±0.3</td>
<td>27.5±0.7</td>
<td>28.0±1.0</td>
</tr>
<tr>
<td>LW, mg</td>
<td>72.2±1.7</td>
<td>85.4±4.4*</td>
<td>85.6±2.4†</td>
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<tr>
<td>LW/BW, mg/g</td>
<td>2.5±0.1</td>
<td>3.1±0.1†</td>
<td>3.0±0.1</td>
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<tr>
<td>RVW, mg</td>
<td>18.6±0.5</td>
<td>17.1±0.6</td>
<td>18.7±1.3</td>
</tr>
<tr>
<td>RVW/BW, mg/g×10</td>
<td>6.5±0.2</td>
<td>6.2±0.2</td>
<td>6.7±0.3</td>
</tr>
<tr>
<td>Lung W, mg</td>
<td>144.8±2.2</td>
<td>173.7±7.4†</td>
<td>182.8±13.8†</td>
</tr>
<tr>
<td>Lung W/BW, mg/g</td>
<td>5.1±0.1</td>
<td>6.3±0.3*</td>
<td>6.5±0.7*</td>
</tr>
<tr>
<td>Agtr2- (n=17)</td>
<td>(n=5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW, g</td>
<td>28.5±0.2</td>
<td>26.5±0.6*</td>
<td>25.9±0.8†</td>
</tr>
<tr>
<td>LW, mg</td>
<td>76.4±1.1</td>
<td>95.6±2.0†</td>
<td>95.0±1.8†</td>
</tr>
<tr>
<td>LW/BW, mg/g</td>
<td>2.7±0.1</td>
<td>3.6±0.1†</td>
<td>3.7±0.2†</td>
</tr>
<tr>
<td>RVW, mg</td>
<td>18.8±0.4</td>
<td>19.0±1.0</td>
<td>20.3±1.7</td>
</tr>
<tr>
<td>RVW/BW, mg/g×10</td>
<td>6.6±0.1</td>
<td>7.2±0.3</td>
<td>7.8±0.6†</td>
</tr>
<tr>
<td>Lung W, mg</td>
<td>146.4±1.8</td>
<td>202.2±24.0†</td>
<td>192.4±8.3†</td>
</tr>
<tr>
<td>Lung W/BW, mg/g</td>
<td>5.1±0.1</td>
<td>7.6±0.9*</td>
<td>7.4±0.5*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. BW indicates body wt; LW, left ventricular weight; RVW, right ventricular weight; and Lung W, lung weight.

*P<0.01, †P<0.001 vs basal within strains; ‡P<0.05 vs Agtr2+ mice.

the LV circumferential length in our study versus 40% in Xu’s study) and the background strains of mice on which the gene targeting was established (FVB/N versus C57/BL).

Post-MI LV remodeling has been divided into an early phase (within 72 hours) and a late phase (after 72 hours).1,21 The early phase mainly involves expansion of the infarct zone. In the present study, LV dilation and decreased survival rate persisted beyond 3 days. However, in cases of extended transmural MI, the noninfarct zone also undergoes a progressive lengthening that is consistent with secondary volume-overloaded hypertrophy and that causes persistent LV dilation associated with a reduction in survival.1 It is thought that such processes of early remodeling may have been exaggerated in Agtr2- mice for some reasons related to the absence of AT2R. On the other hand, late remodeling progresses 1 to 2 months after MI, involving LV dilation, myocyte hypertrophy, and interstitial fibrosis.3,21,22 In the present study, an interstitial fibrosis, which characterizes the late remodeling, was not evident either in Agtr2+ or Agtr2- mice, supporting that the process of the late LV remodeling had not started in these mice. In fact, the interstitial fibrosis developed in the 4th week after MI, the extent of which was significantly larger in Agtr2- mice than in Agtr2+ mice (data not shown).

The mechanisms whereby a deletion of the AT2R results in severer postinfarction heart failure is not clear. In support of our observation, Yang et al23 recently suggested that overexpression of the AT2R in cardiomyocytes improved LV contractile function in posts ischemia/reperfusion LV remodeling in transgenic mice. We could not detect a difference between the LV contractile functions in Agtr2+ and Agtr2- mice by %FS; this may have been due to the limited capability of our method for evaluating global LV function.

In the same transgenic mice as used by Yang et al,23 we have recently observed that stimulation of the AT2R in cardiomyocytes activated kinin formation from the

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**Figure 3.** Bar graph showing changes in myocyte cross-sectional areas after MI. Myocyte cross-sectional area progressively increased both in Agtr2+ and Agtr2- mice to a similar extent (n=5 to 11). *P<0.05 vs basal within strain.

**Figure 4.** Bar graph showing changes in perivascular fibrosis index after MI. Perivascular fibrosis index was calculated as fibrotic area/vessel area×100 (n=5 to 9).

**Figure 5.** Western blot analysis of the AT1R in noninfarcted myocardium 3 days after MI. Densitometric analysis indicated that there was no significant difference between amounts of AT1R.
myocytes. Results of other studies also implicate the AT2R in activation of the kinin/NO system. It is possible that the LV dilation and increased mortality rates in Agtr2- mice are mediated by the loss of kinin/NO activation after MI, although the role of kinin/NO system in post-MI remodeling remains incompletely understood. It is also possible that the exaggerated heart failure in Agtr2- mice could be a secondary phenomenon caused by a compromised systemic hemodynamics. Since AT2Rs in the kidney and systemic vasculature are involved in the mechanisms of natriuresis and a vasorelaxation, respectively, a deletion of AT2R may cause volume overload and increased systemic vascular resistance, leading to a deterioration of systemic hemodynamics after MI. More studies are needed to clarify the mechanism of cardioprotection mediated by the AT2R.

**Perspectives**

In the present study, we demonstrated that deletion of AT2R resulted in exaggerated development of LV dilation and a reduction in the survival rate after MI in mice. Although the underlying mechanism is unclear, a cardioprotective role of the AT2R unrelated to myocyte hypertrophy and interstitial fibrosis in post MI remodeling has never been described. The clinical relevance of this observation may be important since administration of an AT1-receptor antagonist causes elevation in the plasma level of Ang II, which specifically binds to AT1Rs in the heart and may serve as an AT2R agonist. It would be an intriguing topic whether or not this class of drug could benefit the early mortality rate after MI.

**Acknowledgments**

This study was supported by Grants-in Aid for Scientific Research (Nos. 08457639, 07407065, 11771511, and 14572183) from the Ministry of Education, Japan, and a grant from the Charitable Trust Clinical Pathology Research Foundation of Japan.

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Hypertension. 2003;41:814-818; originally published online December 23, 2002; doi: 10.1161/01.HYP.0000048340.53100.43
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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