Angiotensin II Type 2 Receptor Overexpression Preserves Left Ventricular Function After Myocardial Infarction

Zequan Yang, MD, PhD*; Christina M. Bove, MD*; Brent A. French, PhD; Frederick H. Epstein, PhD; Stuart S. Berr, PhD; Joseph M. DiMaria, BA; Jennifer J. Gibson, MS; Robert M. Carey, MD; Christopher M. Kramer, MD

Background—The role of the angiotensin II type 2 receptor (AT2-R) in left ventricular (LV) remodeling may depend on the underlying stimulus. We hypothesized that cardiac AT2-R overexpression in transgenic (TG) mice would attenuate remodeling after myocardial infarction (MI).

Methods and Results—Ten wild-type (WT) C57BL/6 mice and 12 TG mice that overexpress the AT2-R in the heart were studied by cardiac MRI at baseline and days 1, 7, and 28 post-MI induced by 1 hour of occlusion of the LAD followed by reperfusion. Short-axis imaging from apex to base was used to determine LV mass index, end-diastolic and end-systolic volume indices (EDVI, ESVI), regional wall thickness and thickening, and ejection fraction (EF). Gadolinium-DTPA was infused 20 minutes before day 1 imaging to assess infarct size. At baseline, heart rate, blood pressure, LV mass index, and EDVI were similar between groups. Baseline ESVI was lower (0.20±0.07 versus 0.45±0.15 μL/g, P<0.001) and EF higher (82.3±4.9% versus 67.7±5.3%, P<0.001) in TG than WT. Infarct size was similar (36.6±7.2% in WT, 34.0±7.8% in TG, P=NS). When controlled for baseline differences, ESVI was significantly less and EF significantly higher at all time points in TG versus WT. At day 28, ESVI was 1.05±0.32 μL/g in TG and 1.63±0.41 μL/g in WT, P<0.03, and EF was 47.3±5.8% versus 34.1±9.2%, P<0.003, respectively. Regional wall thickness and thickening were greater in TG both at baseline and at day 28. At day 28, blood pressure and LV dP/dt were higher in TG.

Conclusions—Cardiac AT2-R overexpression improves LV systolic function at baseline and preserves function during post-MI remodeling. (Circulation. 2002;106:106-111.)

Key Words: remodeling ■ myocardial infarction ■ magnetic resonance imaging ■ angiotensin ■ receptors

Left ventricular (LV) remodeling is common after large, especially anterior myocardial infarction (MI).1-3 LV end-systolic volume (ESV) is the most powerful predictor of mortality after MI.4 Remodeling is associated with elongation of noninfarcted myocardial segments5,6 and cellular hypertrophy.7,8 Hypertrophy in noninfarcted myocytes is in part initiated by wall stress, local stretch, and activation of the local renin-angiotensin system, resulting in increased levels of angiotensin II (Ang II).9-11 Cardiovascular effects of Ang II include vasodilation,12 and appears to be expressed at low levels in normal cardiac myocytes13 and upregulated in pathological states.11,14,15 Conflicting data regarding its antigrowth effects have emerged from studies of mice lacking the AT2-R in models of pressure-overload hypertrophy.16,17

We hypothesized that AT2-R cardiac overexpression would attenuate remodeling in the intact post-MI heart. Cardiac MRI is an excellent method for assessing cardiac structure and global function in both infarcted and transgenic (TG) mice.18 To test this hypothesis, we used cardiac MRI to image LV size and global function serially after reperfused MI in a TG mouse that overexpresses the AT2-R and in wild-type (WT) controls.

Methods

Mouse Model
Animal protocols were performed in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication No.
The mouse laboratory of H. Matsubara, MD (Kansai Medical University, Osaka, Japan). The mouse AT2 cDNA were subcloned into pBsKS(−) plasmid. The recombinant plasmid pMHC-AT2 was digested to an 8.6-kb DNA fragment, which was then microinjected into the pronuclei of single-cell fertilized mouse embryos to generate TG mice (C57BL/6 strain). Transgene expression was assessed by Northern blot analysis confirmed with sense and antisense polymerase chain reaction primers.

Twelve male TG mice and 10 age-matched male WT C57BL/6 mice 10 to 14 weeks old at the start of the study were studied before and serially after MI over a period of 28 days.

Surgery

Surgical procedures for infarct creation and reperfusion were the same as reported previously. The coronary artery was occluded for 60 minutes by tying down silk suture over a short length of PE-20 tubing. Successful occlusion was verified by color change of the LV distal to the ligation site and expected ECG changes. After 60 minutes, reperfusion was achieved by cutting the suture.

Magnetic Resonance Imaging

Mice were heavily sedated with 10 to 15 mg/kg diazepam and imaged at baseline before MI (day 0) and at days 1 (Figure 1A), 7, and 28 (Figure 1B) post-MI. Imaging was performed on a Varian 200/400 Inova 4.7-T MRI system with Magnex gradients (80 G/cm maximum strength) and a custom-built 2.5-cm birdcage receiver coil (RF Design Consulting). Before imaging on day 1 post-MI studies, 0.3 mmol/L per kg Gd-DTPA was infused intraperitoneally 20 minutes before imaging. The ECG signal was amplified (CP-302, Sable Systems), and a trigger pulse was generated from the QRS complex (Tachometer CFT-1D, Sable Systems). A 2D fast, low-angle shot (FLASH) sequence was used to obtain orthogonal long-axis images. For simultaneous assessment of LV size, global and regional LV function, and infarct size (the latter only on day 1 imaging), short-axis cine MRI was performed with an ECG-triggered 2D cine FLASH sequence (Figure 1). Short-axis slices were planned from the second orthogonal long-axis image. The echo time was 3.9 ms, and the TR value was adjusted continuously (8.0 to 14.0 ms) to obtain 16 equally spaced phases during each cardiac cycle. A 20° flip angle was used except for the post-Gd-DTPA images. For the contrast-enhanced images, a 60° flip angle was used to increase the amount of T1 weighting. A 2.56×2.56-cm field of view was acquired with a matrix of 128×128 zero-filled to 256×256, yielding a final resolution of 100×100×1000 μm3. Three signal averages were used, resulting in an acquisition time for each slice of ≈4 minutes. Six to 8 short-axis slices 1 mm thick were obtained to cover the entire heart, resulting in a total scan time of 30 to 45 minutes for each mouse.

Statistical Analysis

Changes from baseline in global parameters over time, including heart rate, LVMI, EDVI, ESVI, and EF of the TG mice were compared with those of WT mice by repeated-measures ANCOVA, including baseline values as a covariant. LVMI, ESVI, and EDVI were log-harmonically transformed before analysis. Comparisons between groups and within groups between time points were adjusted with the Bonferroni correction for multiple comparisons. Between-group changes in wall thickness and thickening from day 0 to 28 post-MI were compared by 2-way ANOVA. Between-group differences in baseline global parameters, noninvasive and invasive hemodynamics, infarct size, and signal intensity ratios were compared by unpaired t test. All values are presented as mean ± SEM, with a value of P<0.05 defined as significant.

Baseline Hemodynamics

The mean blood pressure and heart rate in conscious animals at baseline measured by a noninvasive tail-cuff apparatus.
were 104±9 mm Hg and 578±79 bpm in WT mice (n=6) and 111±11 mm Hg and 632±63 bpm in TG mice (n=12) (P=NS).

**Baseline LV Size and Function**

Body weight, LVMI, and EDVI were similar between groups at baseline (Table 1). TG mice demonstrated significantly smaller ESVI (0.20±0.07 versus 0.45±0.15, P<0.001) and higher EF (82.3±4.9% versus 67.7±5.3%, P<0.0001) than WT mice but no difference in SVI (Table 1). End-diastolic wall thickness was higher in TG than WT mice in the apex and mid-LV (Table 2), and wall thickening was higher in the base and mid-LV (Table 2).

**Infarct Size**

Signal intensity ratios for hyperenhanced to remote regions were similar between groups (3.83±1.39 versus 3.67±1.15, P=NS). Infarct size by contrast-enhanced MRI was similar in WT and TG mice, 36.6±7.2% and 34.0±7.8% of LVM, respectively (P=NS) (Figure 1A).

**MRI Parameters of Post-MI Remodeling**

Heart rates and body weights did not change during the study period in either group. In both groups, LV dysfunction on day 1 post-MI was characterized by an increase in ESVI and LVM and a decrease in EF and SVI (Table 1). LV size and function were better preserved in TG mice than in WT mice.

### TABLE 1. Global Cardiac Parameters by MRI

<table>
<thead>
<tr>
<th></th>
<th>Strain</th>
<th>Baseline</th>
<th>Day 1</th>
<th>Day 7</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BWT, g</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>WT</td>
<td>27.8±1.2</td>
<td>25.6±1.9†</td>
<td>27.0±1.5‡</td>
<td>28.2±1.3§</td>
<td></td>
</tr>
<tr>
<td>TG</td>
<td>27.3±2.3</td>
<td>25.5±2.2‡</td>
<td>27.6±1.9‡</td>
<td>28.0±1.8‡</td>
<td></td>
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<tr>
<td><strong>HR, bpm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>383±52</td>
<td>401±77</td>
<td>442±114</td>
<td>406±76</td>
<td></td>
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<tr>
<td>TG</td>
<td>431±103</td>
<td>457±43</td>
<td>425±92</td>
<td>392±92</td>
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</tr>
<tr>
<td><strong>LVMI, mg/g</strong></td>
<td></td>
<td></td>
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<tr>
<td>WT</td>
<td>2.82±0.36</td>
<td>3.44±0.32†</td>
<td>3.50±0.47†</td>
<td>3.65±0.49†</td>
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<tr>
<td>TG</td>
<td>2.98±0.28</td>
<td>3.70±0.40†</td>
<td>3.26±0.30‡</td>
<td>3.42±0.50‡</td>
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<tr>
<td><strong>EDVI, µL/g</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>WT</td>
<td>1.38±0.34</td>
<td>1.58±0.18†</td>
<td>2.31±0.45†</td>
<td>2.54±0.61†‡</td>
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<tr>
<td>TG</td>
<td>1.15±0.17</td>
<td>1.16±0.16*</td>
<td>1.32±0.40*</td>
<td>1.99±0.50†‡§</td>
<td></td>
</tr>
<tr>
<td><strong>ESVI, µL/g</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>0.45±0.15</td>
<td>0.85±0.23†</td>
<td>1.33±0.32†</td>
<td>1.63±0.41†‡</td>
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<tr>
<td>TG</td>
<td>0.20±0.07*</td>
<td>0.41±0.10†</td>
<td>0.59±0.32†</td>
<td>1.05±0.32†‡§</td>
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<tr>
<td><strong>SVI, µL/g</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>0.93±0.22</td>
<td>0.73±0.18†</td>
<td>0.98±0.24</td>
<td>0.85±0.25</td>
<td></td>
</tr>
<tr>
<td>TG</td>
<td>0.94±0.13</td>
<td>0.75±0.12†</td>
<td>0.74±0.13†</td>
<td>0.93±0.50</td>
<td></td>
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<tr>
<td><strong>EF, %</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>67.7±5.3</td>
<td>46.6±10.6†</td>
<td>42.7±6.7†</td>
<td>34.1±9.2†</td>
<td></td>
</tr>
<tr>
<td>TG</td>
<td>82.3±4.9*</td>
<td>64.7±6.3†‡</td>
<td>58.5±13.6†</td>
<td>47.3±5.8†‡§</td>
<td></td>
</tr>
</tbody>
</table>

TG mice were compared to WT using repeated measures ANCOVA adjusted for baseline differences.

BWT indicates body weight.

*P<0.05 versus WT. Within group: †P<0.05 vs baseline; ‡P<0.05 vs day 1; §P<0.05 vs day 7.

### TABLE 2. End-Diastolic Wall Thickness and End-Systolic Wall Thickening at Baseline and Day 28 Post-MI

<table>
<thead>
<tr>
<th></th>
<th>End-Diastolic Wall Thickness, mm</th>
<th>Wall Thickening, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td>Base</td>
<td>Mid</td>
</tr>
<tr>
<td>WT</td>
<td>0.95±0.17</td>
<td>0.85±0.09</td>
</tr>
<tr>
<td>TG</td>
<td>0.97±0.46</td>
<td>0.96±0.09*</td>
</tr>
</tbody>
</table>

**Day 28**

<table>
<thead>
<tr>
<th></th>
<th>End-Diastolic Wall Thickness, mm</th>
<th>Wall Thickening, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>0.88±0.09</td>
<td>0.84±0.13</td>
</tr>
<tr>
<td>TG</td>
<td>0.97±0.09*</td>
<td>0.92±0.07</td>
</tr>
</tbody>
</table>

*P<0.05 vs WT; †P<0.001 vs baseline.
as evidenced by lower EDVI (1.16 ± 0.16 versus 1.58 ± 0.18 μL/g, \( P<0.001 \)) and ESVI (0.41 ± 0.10 versus 0.85 ± 0.23 μL/g, \( P<0.001 \)) and higher EF (64.7 ± 6.3% versus 46.6 ± 10.6%, \( P<0.002 \)) (Table 1, Figures 2, 3, and 4). No significant changes in LV size or function occurred in either group between days 1 and 7 post-MI, other than a decrease in LVMI in TG from 3.70 ± 0.40 to 3.26 ± 0.30 mg/g (\( P<0.05 \)).

By day 28 post-MI, there was a stepwise increase in EDVI and ESVI and a decrease in EF in both groups (Table 1). When corrected for baseline differences, however, ESVI was lower and EF was higher at each time point in TG mice (Figure 2, 3, and 4). At day 28, ESVI was 1.05 ± 0.32 μL/g in TG and 1.63 ± 0.41 μL/g in WT, \( P<0.03 \), and EF was 47.3 ± 5.8% versus 34.1 ± 9.2%, \( P=0.002 \), respectively. LVMI was similar between groups at each time point (Table 1), and SVI transiently decreased in both groups at day 1 but returned to baseline values by day 28.

**Figure 2.** Change in EDVI over time, with correction for baseline differences. Data are shown as mean±95% CI. \(+P<0.05\) vs WT, \(+P<0.05\) vs baseline, \(\dagger P<0.05\) vs day 1, \(\# P<0.05\) vs day 7.

**Figure 3.** Change in ESVI over time, with correction for baseline differences. Data are shown as mean±95% CI. \(+P<0.05\) vs WT, \(+P<0.05\) vs baseline, \(\dagger P<0.05\) vs day 1, \(\# P<0.05\) vs day 7.

**Regional LV Function After MI**
At day 28 post-MI, end-diastolic wall thickness was greater at the base and apex in TG mice than in WT controls (Table 2) and tended to be higher in the midventricle (Figure 1B). Systolic wall thickening remained greater in TG than WT in both the base and midventricle (Table 2).

**Hemodynamics at 28 Days After MI**
There was no difference in heart rates between the baseline and post-MI follow-up or between the 2 groups. Systolic, diastolic, and mean blood pressures were greater in TG than WT (Table 3). LV end-diastolic pressure was lower in TG than WT mice (4.1 ± 0.7 versus 7.5 ± 0.8 mm Hg, \( P<0.01 \)). LV systolic and diastolic function was preserved in TG mice compared with WT (Table 3).

**Discussion**
This study demonstrates 2 important findings: (1) AT₂ overexpression in the murine heart is associated with improved LV function at baseline, and (2) during LV remodeling after reperfused MI, LV global and regional function are preserved in this TG model. At baseline, TG mice had smaller ESVI and increased regional and global function compared with WT controls, whereas LVMI and SVI were similar. During LV remodeling after reperfused MI, ESVI remained lower and EF higher at each time point up to day 28 post-MI. At day 28,

**TABLE 3. Invasive Hemodynamics at Day 28 Post-MI**

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>TG</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, bpm</td>
<td>427±52</td>
<td>447±56</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>97.2±5</td>
<td>107±5*</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>71±6</td>
<td>76±6*</td>
</tr>
<tr>
<td>MBP, mm Hg</td>
<td>79±5</td>
<td>87±6*</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>7.5±2.4</td>
<td>4.1±1.8*</td>
</tr>
<tr>
<td>dP/dt(+), mm Hg/s</td>
<td>6547±1653</td>
<td>8267±1165*</td>
</tr>
<tr>
<td>dP/dt(−), mm Hg/s</td>
<td>6272±1644</td>
<td>7958±1389*</td>
</tr>
</tbody>
</table>

\*\( P<0.05 \) vs WT.
LV wall thickness, filling pressures, and contractile function measured invasively and noninvasively were higher in TG than WT mice. The beneficial effects of AT2 overexpression on LV remodeling and LV function occurred in the absence of any differences in heart rate, SV, or LVM between the 2 groups. These results indicate a beneficial role for the AT2-R in volume-overload states, including post-MI remodeling.

The mechanism of increased systolic function at baseline with AT2 overexpression is unclear. No developmental abnormalities or cardiac morphological or histological differences have been observed in mice lacking the AT2-R gene.24-25 No differences were noted in cardiac morphology or ratio of heart to body weight between WT and cardiac AT2-R–overexpressed mice,19 but no in vivo measures of cardiac function were made. In an isolated heart preparation, dP/dt was similar to that in WT.19 The hypertensive and chronotropic response to Ang II, however, was markedly attenuated.19 It may be that attenuation of effects of basal Ang II levels enhances systolic function. Another potential mechanism is altered geometry and lower regional wall stress, because wall thickness is greater in TG mice with similar end-diastolic volume. Another potential mechanism is vasodilation, a known effect of AT2-R overexpression in vascular smooth muscle.26 In the present model, however, AT2-R overexpression is limited to the myocardium, and blood pressure was similar at baseline between strains.

The protective effect of AT2-R overexpression on LV systolic function post-MI is consistent with previous work suggesting that the AT2-R limits growth and fibrosis. In addition, the reduction in wall stress with greater wall thickness and smaller cavity volumes probably contributes to the attenuation of post-MI remodeling. The AT2-R is upregulated in pathological states associated with tissue remodeling or inflammation, such as cardiac hypertrophy, aortic banding models, MI models, and cardiomyopathy. In failing cardiomyopathic hamster hearts, the AT2-R is upregulated and partially blocked by icatibant.30 The benefits of AT2-R antagonists may therefore be partly a result of activation of the AT2-R, mediated in turn by bradykinin. A previous study by our group in an ovine infarction model showed that combination therapy with ACE inhibition and AT2-R antagonism was associated with an increase in the ratio of AT2-R/AT1-R, and this combination attenuated LV remodeling to a greater extent than standard doses of either therapy alone.32 This suggests that modulation of the AT2-R may mediate much of the benefit of renin-angiotensin system blockade post-MI.

Study Limitations
Baseline differences between groups complicate the analysis of changes post-MI. The statistical analysis performed, however, accounted for baseline differences. The study was extended only to 28 days post-MI. Studies extended to a later time point might have demonstrated different results. Previous studies by our group, however, demonstrate that most of the changes in ESVI and EF that take place over 6 months post-MI in the mouse model occur by day 28 post-MI.33 Further work is necessary to understand the effects of AT2-R overexpression on interstitial fibrosis and hypertrophy at the myocyte level. With the further development of MRI tagging techniques for murine imaging, the analysis of regional myocardial function in this post-MI model will become more refined.

Acknowledgments
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References


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