Nitric Oxide Mediates Benefits of Angiotensin II Type 2 Receptor Overexpression During Post-Infarct Remodeling

Christina M. Bove, Zequan Yang, Wesley D. Gilson, Frederick H. Epstein, Brent A. French, Stuart S. Berr, Sanford P. Bishop, Hiroaki Matsubara, Robert M. Carey, Christopher M. Kramer

Abstract—We hypothesized that nitric oxide (NO) mediates the benefits of cardiac angiotensin II type 2 (AT2-R) overexpression during postmyocardial infarction (post-MI) remodeling. Eleven wild-type (WT) C57BL/6 mice and 28 transgenic (TG) mice with AT2-R overexpression were studied by cardiac magnetic resonance imaging (CMR) at baseline and days 1 and 28 post-MI induced by left anterior descending artery occlusion and reperfusion. Sixteen TG mice were treated from day 1 through 28 post-MI with the NO synthase inhibitor N\(^{\text{O}}\)-nitro-L-arginine methyl ester in drinking water at 1 mg/mL (TG-Rx). Left ventricular mass index (LVMI), end-diastolic volume index (EDVI) and end-systolic volume index (ESVI), wall thickness, percent thickening, and ejection fraction (EF) were measured. Infarct size on day 1 was assessed by post-contrast CMR. Interstitial collagen was quantified in noninfarcted regions. At baseline, heart rate (HR), blood pressure (BP), LVMI, EDVI, and ESVI were similar between groups, as were infarct size and weekly post-MI HR and systolic BP. By day 28 post-MI, EDVI and ESVI were similar in WT and TG-Rx, but significantly lower in TG (ESVI: 1.41±0.18 μL/g versus 2.53±0.14 μL/g in WT; 2.17±0.14 μL/g in TG-Rx; P<0.008 for both). At day 28, EF was higher in TG (46.3%±2.9%) compared with WT and TG-Rx (32.7±2.3% and 33.7±2.3, respectively; P<0.003 for both). Wall thickening at day 28 post-MI was greater in the base and mid-LV in TG than WT and TG-Rx. Noninfarcted region interstitial collagen was similar between groups. Thus, the NO pathway may mediate much of the benefits of cardiac AT2-R overexpression during post-MI remodeling. (Hypertension. 2004;43:1-6.)

Key Words: angiotensin ■ MRI ■ myocardial infarction ■ remodeling ■ nitric oxide ■ receptors ■ imaging

Most of the physiological effects of angiotensin (Ang) II are mediated through the angiotensin II type 1 receptor (AT1-R).\(^1\) However, the role of the angiotensin II type 2 receptor (AT2-R) in left ventricular (LV) remodeling in disease states is an area of active investigation.\(^2\) In fact, many of the beneficial effects of AT2-R blockade postmyocardial infarction (post-MI) or of angiotensin-converting enzyme (ACE) inhibitors may be mediated by Ang II stimulation of the AT2-R.\(^7\)\(^8\)

We have previously demonstrated that AT2-R overexpression in the mouse heart preserves LV size and function during post-MI remodeling.\(^9\) The transgenic mice (TG) demonstrated preserved LV cavity size, wall thickness within the infarct zone, and regional and global LV function during post-MI remodeling compared with wild-type controls (WT). Others have shown that depletion of the AT2-R is detrimental post-MI, leading to myocardial rupture,\(^10\) heart failure, and increased mortality.\(^10\)\(^11\)

Studies in vascular smooth muscle and the kidney suggest that the benefits of AT2-R stimulation may be through signaling pathways involving the kinin and nitric oxide (NO) system.\(^12\) AT2-R–mediated increases in cGMP caused by bradykinin and NO have been demonstrated in studies of the aorta and kidney.\(^13\)\(^14\) and blood pressure-lowering effects of AT2-R blockade involve AT2-R–mediated release of renal bradykinin and NO production.\(^15\) Recently, Ang II stimulation of the AT2-R in the TG model of cardiac overexpression was shown to attenuate perivascular fibrosis, an effect mediated through the kinin/NO system, without a change in cardiomyocyte hypertrophy.\(^15\) We hypothesized that the post-MI preservation of LV size and function seen with AT2-R overexpression in the mouse heart may be mediated by NO and involve changes in interstitial fibrosis.

Methods

Mouse Model

Animal protocols were performed in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication no. 85-23, revised 1996) and were approved by the University of Virginia Animal Care and Use Committee. The transgenic mouse strain with cardiac overexpression of the AT2 receptor in mice on a C57Bl/6 background\(^2\) yields approximately 22% to 37% AT2-R relative to AT1-R levels and were generously supplied by the laboratory of H.
Matsubara, MD, PhD. Transgene expression was assessed by Northern blot analysis of tail RNA and confirmed with sense and antisense PCR primers.

Twenty eight male TG mice and 11 age-matched male WT C57Bl/6 mice (Jackson Labs, Bar Harbor, ME) aged 10 to 14 weeks were studied before and at days 1 and 28 post-MI. Surgical procedures for infarct creation and reperfusion were reported previously.17

CMR

Anesthesia was induced with 3.0% inhaled isoflurane and maintained with 1.0% isoflurane administered via nosecone during imaging at baseline before MI (day 0) and days 1 (Figure 1) and 28 (Figure 2) post-MI. Details of CMR were previously published.9

Treated Group

Sixteen of the TG mice (TG-Rx) were treated with the NO synthase inhibitor Nω-nitro- L -arginine methyl ester ( L -NAME) administered in the drinking water at 1 mg/mL from day 1 to day 28 post-MI. This dose has previously been shown to inhibit NO synthesis chronically while causing no significant increase in systemic blood pressure.12

Noninvasive Hemodynamics

Weekly noninvasive mean systolic blood pressures (SBPs) and heart rates (HRs) were measured in conscious mice using a tail-cuff apparatus (Visitech BP-2000 Analysis Systems).

Histopathology

After euthanizing the mice, the heart was removed and fixed in formaldehyde, embedded in paraffin, sectioned at 6 μm, and stained with picric acid Sirius red. Quantitative morphometry was performed on infarct border (adjacent) and remote myocardium in 5 TG, 5 TG-Rx, and 6 WT mice using an Olympus microscope with a green 540-nm filter and a CCD72 videocamera interfaced to a computer with a Universal Imaging Image 1/AT morphometry system (West Chester, Pa). A minimum of 35 fields approximately 575×750 μm each was measured from 3 or 4 sections from each region, and volume percent collagen calculated as the mean from all fields in each region for each animal.

Results

Noninvasive Hemodynamics

The mean SBP in conscious animals measured by a noninvasive tail-cuff apparatus were similar at baseline and there were no significant changes in the 3 groups between time points, although SBP tended to be higher in TG-Rx mice (101±5 at baseline to 112±4 mm Hg by day 28 post-MI) (Table 1). Mean HR in conscious mice was also similar between all 3 groups at baseline and each later time point (Table 1). Likewise, mean HR in sedated animals during CMR sessions was similar for all 3 groups (Table 2).

Infarct Size

Infarct size by contrast-enhanced CMR was similar in WT, TG-Rx, and TG mice: 41.1±1.4%, 40.5±1.7%, and 42.2±2.5% of LV mass, respectively (P=NS) (Figure 1).
Regional LV Size and Function Post-MI
At baseline, regional wall thickness was similar between groups (Table 3). Wall thickening was greater at baseline than WT in the base in both TG groups, as shown previously.9 At day 28 post-MI, there was no significant change in regional EDWT in any of the 3 groups. However, percent thickening decreased significantly in the mid-ventricle and apex in all 3 groups and at the base in the TG-Rx group (Table 3). At day 28, percent thickening at the base was greater in TG (45.8% ± 5.7%) than the other 2 groups (23.1% ± 4.3% in WT and 23.2% ± 4.5% in TG-Rx; P < 0.008 for both). Similarly, in the mid-LV, percent thickening at the base was greater in TG (30.7% ± 4.6%) than WT (12.6% ± 3.5%; P < 0.009) and TG-Rx (11.9% ± 3.8%; P < 0.008). All groups demonstrated similarly reduced percent thickening within the apex (Table 3).

Quantitative Collagen Analysis
In day 28 post-MI hearts, there was no significant difference in mean collagen content within the adjacent (P = 0.33) or remote regions (P = 0.85) between the three groups. However, adjacent collagen content was greater than remote within each group (10.8% ± 2.1% versus 1.2% ± 0.4% in WT, P < 0.003; 13.9% ± 1.4% versus 1.5% ± 0.3% in TG, P < 0.001; and 13.7% ± 1.0% versus 1.3% ± 0.3% in TG-Rx, P < 0.001).

Discussion
This study confirms our previous findings that AT2-R overexpression in the heart offers protection against post-infarct LV remodeling. This benefit is largely abrogated by the use of the nitric oxide synthase antagonist, t-NAME. AT2-R overexpression was associated with lower EDVI, lower ESVI, and higher EF at day 28 post-MI than both WT controls and t-NAME–treated TG mice despite equivalent infarct sizes in the 3 groups. These parameters indicative of LV remodeling were similar between WT and t-NAME treated TG mice. Wall thickening at day 28 post-MI in the base and mid-LV was greater in TG mice than WT and the

---

**TABLE 2. Global Parameters by CMR on Day 0 and Day 28 Post-MI**

<table>
<thead>
<tr>
<th></th>
<th>HR (bpm)</th>
<th>LVMI (mg/g)</th>
<th>EDVI (μL/g)</th>
<th>ESVI (μL/g)</th>
<th>EF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day 0</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>443 ± 22</td>
<td>2.68 ± 0.14</td>
<td>1.76 ± 0.16</td>
<td>0.69 ± 0.14</td>
<td>61.4 ± 2.3</td>
</tr>
<tr>
<td>TG</td>
<td>456 ± 21</td>
<td>2.92 ± 0.14</td>
<td>1.46 ± 0.15</td>
<td>0.39 ± 0.13</td>
<td>74.7 ± 2.2†</td>
</tr>
<tr>
<td>TG-Rx</td>
<td>448 ± 18</td>
<td>2.79 ± 0.12</td>
<td>1.83 ± 0.13</td>
<td>0.60 ± 0.12</td>
<td>69.9 ± 2.2†</td>
</tr>
<tr>
<td><strong>Day 28</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>425 ± 22</td>
<td>3.79 ± 0.14*</td>
<td>3.72 ± 0.16*</td>
<td>2.53 ± 0.14*</td>
<td>32.7 ± 2.3*</td>
</tr>
<tr>
<td>TG</td>
<td>441 ± 27</td>
<td>3.66 ± 0.18*</td>
<td>2.56 ± 0.019†</td>
<td>1.41 ± 0.17†</td>
<td>46.3 ± 2.9†</td>
</tr>
<tr>
<td>TG-Rx</td>
<td>443 ± 22</td>
<td>3.99 ± 0.14*</td>
<td>3.23 ± 0.16‡</td>
<td>2.17 ± 0.14‡</td>
<td>33.7 ± 2.3‡</td>
</tr>
</tbody>
</table>

HR indicates heart rate; LVMI, left ventricular mass index; EDVI, end-diastolic volume index; ESVI, end-systolic volume index; EF, ejection fraction; WT, wild-type; TG, transgenic; TG-Rx, transgenic treated with t-NAME.

*P < 0.001 vs day 0; †P < 0.02 vs WT; ‡P < 0.03 vs TG.
Studies in models of targeted deletion of the AT2-R suggest that differences in the extent of fibrosis in the infarct region may account for increased rates of myocardial rupture in the knockout mice. Myocardial rupture is uncommon in the model used in the present study and may reflect differences in the genetic background of the mice. In the present study, no between-group differences in wall thickness or in wall thickening were noted in the apical infarct zone. All of the differences in these parameters were seen in noninfarcted regions in the mid-LV and base, without differences in interstitial collagen, arguing against the primary effect of the AT2-R on fibrosis.

AT2-R-mediated increases in cGMP and hypotensive effects caused by bradykinin and NO have been shown in the aorta of the spontaneously hypertensive rat. The effects of AT1-R blockade on blood pressure are affected by the AT2-R through release of renal bradykinin, which then mediates NO production.

Recent evidence suggests that the same pathways apply to myocardial AT2-R signaling. A specific AT2-R agonist, CPG 42112, increased eNOS expression in myocytes by 2.4-fold. eNOS expression was increased by direct Ang II stimulation and antagonized by the AT2-R antagonist PD 123319. In vivo, in AT2-R knockout mice, eNOS protein expression was significantly reduced. The upregulation of eNOS was mediated through a pathway involving calcineurin and nuclear factor of activated T cells. These studies suggest that Ang II stimulation of the AT2-R is responsible for increases in eNOS and resultant beneficial cardiac effects.

In rats with MI, NO inhibition with L-NAME led to a 22% decrease in LV dP/dt, without a change in mean arterial pressure or LV end-diastolic pressure, suggesting direct cardioinhibitory effects of NOS blockade. Specific iNOS antagonism with aminoguanidine in this model increased LV dP/dt, suggesting that iNOS activation is detrimental and eNOS activation is protective post-MI. Further evidence of the former is that iNOS knockouts demonstrated higher dP/dt, less apoptosis, and improved survival at 4 months post-MI compared with WT. Conversely, eNOS knockouts demonstrated lower EF, higher EDVs, and LV mass at 4 weeks post-MI than WT controls. eNOS may contribute to the

TABLE 3. End-Diastolic Wall Thickness and Percent Wall Thickening at Baseline and Day 28 Post-MI

<table>
<thead>
<tr>
<th></th>
<th>Base</th>
<th>Mid</th>
<th>Apex</th>
<th>Base</th>
<th>Mid</th>
<th>Apex</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day 0</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>0.93±0.04</td>
<td>0.98±0.03</td>
<td>0.85±0.04</td>
<td>30.7±4.8</td>
<td>34.2±3.8</td>
<td>22.1±5.0</td>
</tr>
<tr>
<td>TG</td>
<td>0.85±0.04</td>
<td>0.90±0.03</td>
<td>0.84±0.04</td>
<td>50.8±4.1†</td>
<td>58.7±3.5†</td>
<td>56.5±4.8†</td>
</tr>
<tr>
<td>TG-Rx</td>
<td>0.84±0.03</td>
<td>0.81±0.02</td>
<td>0.78±0.03</td>
<td>51.4±3.9†</td>
<td>48.3±3.2†</td>
<td>32.8±4.1†</td>
</tr>
<tr>
<td><strong>Day 28</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>0.93±0.04</td>
<td>0.90±0.03</td>
<td>0.74±0.04</td>
<td>23.1±4.3</td>
<td>12.6±3.5*</td>
<td>7.8±4.8*</td>
</tr>
<tr>
<td>TG</td>
<td>0.88±0.05</td>
<td>0.84±0.03</td>
<td>0.84±0.05</td>
<td>45.8±5.7†</td>
<td>30.7±4.6†</td>
<td>12.6±6.0*</td>
</tr>
<tr>
<td>TG-Rx</td>
<td>0.88±0.04</td>
<td>0.82±0.03</td>
<td>0.82±0.04</td>
<td>23.2±4.5†</td>
<td>11.9±3.8†</td>
<td>−0.8±5.1†</td>
</tr>
</tbody>
</table>

WT indicates wild-type; TG, transgenic; TG-Rx, transgenic treated with L-NAME.

*P<0.001 vs baseline; †P<0.01 vs WT; ‡P<0.008 vs TG.
benefits of pharmacologic therapy as well. The eNOS knockouts demonstrated attenuated responses to ACE inhibition and AT₁-R blockade compared with WT mice post-MI.25

In a study of Ang II-induced hypertrophy and interstitial fibrosis in the same transgenic model as in the present study, there was significantly less Ang II-induced perivascular fibrosis of intramuscular coronary arteries in TG, and this inhibition of perivascular fibrosis was abolished by concomitant treatment with L-NAME.16 No difference in the extent of interstitial fibrosis was seen in the present study between WT, TG, or TG-Rx groups. This may relate to the differences between direct Ang II-mediated fibrosis and the multifactorial fibrosis induced after MI. No difference in fibrosis in noninfarcted regions was seen between WT and AT₂-R knockout (KO) mice in a post-MI study.4 These investigators also noted similar increases in myocyte cross-sectional area in the 2 groups, suggesting that the increase in LV mass seen in the AT₂-R knockouts post-MI could be caused by myocyte elongation characteristic of volume-overload hypertrophy.

Hemodynamics alone are probably not responsible for the effects of L-NAME in this model as no significant effect between groups on BP or HR was seen over the 28 days post-MI, although there was a trend toward increasing BP in the L-NAME group. In the vascular smooth muscle model of AT₂-R overexpression, BP was only elevated by 11 ± 1 mm Hg after 14 days of therapy with the same dose of L-NAME. It may be that overexpression of AT₂-R protects against the hypertensive effect of L-NAME as a previous study demonstrated that AT₂-R null mice demonstrated significantly greater blood pressure response to L-NAME than WT controls.26

Study Limitations
If the dose of L-NAME were inadequate, NO synthase inhibition would have been incomplete, in which case remodeling would have been attenuated. However, in the vascular smooth muscle model of AT₂-R overexpression, this dose of L-NAME completely antagonized the effects of the AT₂-R without significantly altering blood pressure.13 Baseline LV function is known to be greater in TG mice than WT controls.9 However, in the TG-Rx mice, LV EF decreased to the same level as of WT controls by day 28 post-MI, suggesting that baseline differences were abolished by NO inhibition post-MI. Further work is necessary to understand the effects of AT₂-R overexpression on myocyte size and function.

Perspectives
The AT₂-R plays an increasingly well understood role in remodeling in myocardial disease states including volume and pressure overload hypertrophy. The specific mechanisms of benefit of commonly used pharmacologic agents after MI, including ACE inhibitors and AT₁-R receptor blockers, are incompletely understood and may involve the AT₂-R to a major extent. Understanding the mechanisms underlying the effects of the AT₂-R on post-infarct remodeling will support the development of specific methods of manipulating levels of the AT₂-R to limit remodeling. Future studies will use pharmacologic and genetic approaches to better understand the interplay between bradykinin and NO pathways as well as the cross-talk between the AT₁-R and AT₂-R.

Acknowledgments
We acknowledge the outstanding technical assistance of Joseph M. DiMaria. The study was supported by RO1 HL-52980 (C.M.K.), AHA Mid-Atlantic Affiliate grant-in-aid 0256343U (C.M.K.), and T32 HL07355 (C.M.B.).

References


Nitric Oxide Mediates Benefits of Angiotensin II Type 2 Receptor Overexpression During Post-Infarct Remodeling

Christina M. Bove, Zequan Yang, Wesley D. Gilson, Frederick H. Epstein, Brent A. French, Stuart S. Berr, Sanford P. Bishop, Hiroaki Matsubara, Robert M. Carey and Christopher M. Kramer

_Hypertension_, published online January 19, 2004;

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/early/2004/01/19/01.HYP.0000115924.94236.91.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Hypertension_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Hypertension_ is online at:
http://hyper.ahajournals.org//subscriptions/