Role of AT_2 Receptors in the Cardioprotective Effect of AT_1 Antagonists in Mice

Jiang Xu, Oscar A. Carretero, Yun-He Liu, Edward G. Shesely, Fang Yang, Alissa Kapke, Xiao-Ping Yang

Abstract—Angiotensin II (Ang II) acts mainly on two receptor subtypes: AT_1 and AT_2. Most of the known biological actions of Ang II are mediated by AT_1 receptors; however, the role of AT_2 receptors remains unclear. We tested the hypothesis that the cardioprotective effects of AT_1 receptor antagonists (AT_1-ant) after myocardial infarction (MI) are partially mediated by activation of AT_2 receptors; thus in AT_2 receptor gene knockout mice (AT_2−/Y), the effect of AT_1-ant will be diminished or absent. MI was induced by ligating the left anterior descending coronary artery. Four weeks later, AT_2−/Y and their wild-type littermates (AT_2+/Y) were started on vehicle, AT_1-ant (valsartan, 50 mg/kg per day), or ACE inhibitor (enalapril, 20 mg/kg per day) for 20 weeks. Basal blood pressure and cardiac function as well as remodeling after MI did not differ between AT_2+/Y and AT_2−/Y. AT_1-ant increased ejection fraction and cardiac output and decreased left ventricular diastolic dimension, myocyte cross-sectional area, and interstitial collagen deposition in AT_2+/Y, and these effects were significantly diminished in AT_2−/Y. ACE inhibitors improved cardiac function and remodeling similarly in both strains. We concluded that (1) activation of AT_2 during AT_1 blockade plays an important role in the therapeutic effect of AT_1-ant and (2) the AT_2 receptor may not play an important role in regulation of cardiac function, either under basal conditions after MI remodeling or in the therapeutic effect of ACE inhibitors. (Hypertension. 2002;40:244-250.)

Key Words: receptors, angiotensin II angiotensin antagonist myocardial infarction heart failure mice

Angiotensin II (Ang II), the principal effector peptide in the renin-angiotensin system (RAS), binds to two distinct receptor subtypes, AT_1 and AT_2. Most well-known actions of Ang II in the cardiovascular system are mediated by the AT_1 receptor, whereas little is known about the function of the AT_2 receptor. It has been proposed that blockade of the AT_1 receptor increases Ang II, which activates the AT_2 receptor and releases kinins and nitric oxide (NO), leading to cardioprotection. We previously showed that in a rat model of heart failure (HF) induced by myocardial infarction (MI), AT_1-ant had a cardioprotective effect similar to an ACE inhibitor (ACEI), which was partially blocked by an AT_2-ant or B_2 kinin receptor antagonist (B_2-ant). We also found that in B_2 or endothelial NO synthase (eNOS) knockout mice, the effect of AT_1-ant was diminished compared with their wild-type controls, suggesting a kinin/NO-mediated mechanism. Other studies have shown that activation of the AT_2 receptor counterbalances the effect of the AT_1 receptor, thereby inhibiting cell growth, proliferation, and hypertrophy. In cultured bovine aortic endothelial cells or aortas from mice overexpressing the AT_2 receptor gene, Ang II was found to increase the release of cGMP; this effect was further enhanced by an AT_1-ant but markedly blocked by an AT_2-ant, B_2-ant or NO synthesis inhibitor, suggesting that Ang II–stimulated release of cGMP is mediated by kinins and NO through activation of the AT_2 receptor.

The role of the AT_2 receptor in regulation of blood pressure (BP) and cardiac function under physiological conditions, or in the pathophysiology of cardiac and vascular remodeling, is not fully understood. It has been reported that disruption of the AT_2 receptor in mice (AT_2−/Y) increases systolic BP and leads to hypersensitivity to Ang II or susceptibility to DOCA-salt hypertension. Wu et al recently showed that coronary arterial thickening and perivascular fibrosis induced by aortic banding or cuff-induced neointima formation and inflammation were exaggerated and the response to AT_1-ant diminished in AT_2 receptor knockout mice. On the other hand, Senbonmatsu et al and Mifune et al reported that targeted deletion of AT_2 receptors prevented left ventricular hypertrophy induced by pressure overload, whereas stimulation of AT_2 increased collagen synthesis.

To clarify the role of AT_2 receptors in the therapeutic effect of AT_1-ant and in regulation of cardiac function and remod-
eling after MI, we used AT2−/− mice to study whether lack of AT2 receptors (1) diminishes the cardioprotective effects of AT1-ant and ACEi and (2) affects cardiac hemodynamics and function as well as morphology and histology, either under basal conditions or after MI.

Methods

Animals

AT2−/− mice were originally developed on a hybrid genetic background, then back-crossed to inbred strain C57BL/6J by our Mutant Mouse Core until congenic status was reached (10+ back-cross generations). Since the AT2 receptor gene is X-linked, male knockouts were obtained as homozygous offspring (−/−). Wild-type littermates (AT2+/+) were used as controls. Animals were housed in an air-conditioned room with a 12-hour light/dark cycle and given standard chow with free access to tap water. This study was approved by the Henry Ford Hospital Institutional Animal Care and Use Committee (IACUC).

Induction of Myocardial Infarction

Male mice 10 to 12 weeks old (22 to 25 g) were anesthetized with sodium pentobarbital (50 mg/kg IP), intubated, and ventilated with room air by a positive-pressure respirator (Harvard 680). MI was created as described previously.25

Systolic Blood Pressure and Echocardiography

Systolic BP (SBP) was measured with a noninvasive computerized tail-cuff system (BP-2000, Visitech Systems).22 Cardiac geometry and function were evaluated in awake mice, using a Doppler device.24 Cardiogenic exercise and function were calculated using the echocardiograph, (LVDd), were traced manually and digitized by goal-directed, diagnostically driven software installed within the echocardiograph, and expressed as the ratio of the infarcted portion to total LV circumference.7 For myocyte cross-sectional area and interstitial collagen fraction, 6-μm sections from each slice were double-stained with (a) fluorescein-labeled peanut agglutinin to delineate the myocyte cross-sectional area (MCSA) and interstitial space and (b) rhodamine-labeled Griffonia simplicifolia lectin I to show the capillaries.2 MCSA was measured by computer-based planimetry (Jandel). ICF was calculated as percentage of total surface area occupied by the interstitial space minus percentage of total surface area occupied by the capillaries.

Experimental Protocols

Doses of AT1-ant (valsartan, 10, 20, 40, and 60 mg/kg per day; Novartis) and ACEi (enalapril, 5, 10, and 20 mg/kg per day; Merck) were tested for their inhibitory effect on mean blood pressure (MBP) response to Ang II or Ang I (12.5, 25, 50, and 100 μg/mouse). AT1-ant and ACEi were administered in drinking water for 4 weeks. We found that valsartan at 40 mg/kg per day blocked 70% of the vasopressor effect of exogenous Ang II, and enalapril at 20 mg/kg per day blocked 67% of the vasopressor effect of Ang I. Therefore, 50 mg valsartan and 20 mg enalapril were chosen for the present study. Four weeks after MI or sham MI, each strain was divided into (1) sham MI; (2) MI+vehicle (tap water); (3) MI+AT1-ant; and (4) MI+ACEi, with treatment continuing for 20 weeks.

Data Analysis

Data are expressed as mean±SEM. Mortality rates were compared by means of a Cox proportional hazards model. ANOVA with repeated measures was used to compare changes (after treatment) from week 4 (before treatment) within and between strains in SBP and echocardiographic parameters. When significant group or strain interactions were observed over time, Student’s t test was used to compare the prespecified group (or strain) at all time points. A paired t test was used to compare the difference between the fourth week and the average from 8 through 24 weeks between strains and within treatment groups. For heart and lung weight, infarct size, PRC, and histopathological data, Wilcoxon’s 2-sample test was used. When multiple comparisons were performed, Hochberg’s method was used to adjust the alpha level of significance.25

Effect of AT1-ant and ACEi on Heart, Lung, and Liver Weight and PRC in AT2+/+ and AT2−/− Mice

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AT2+/+</th>
<th></th>
<th>AT2−/−</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham MI (n=8)</td>
<td>Vehicle (n=11)</td>
<td>AT1-ant (n=11)</td>
<td>ACEi (n=10)</td>
</tr>
<tr>
<td>BW, g</td>
<td>30.8±0.9</td>
<td>31.2±0.7</td>
<td>31.3±0.5</td>
<td>30.8±0.6</td>
</tr>
<tr>
<td>Atria, mg</td>
<td>9.7±1</td>
<td>22.1±1.8**</td>
<td>16.8±1.7†</td>
<td>17.1±1.8†</td>
</tr>
<tr>
<td>RV, mg</td>
<td>23.4±1.8</td>
<td>40.2±4.4***</td>
<td>31.2±1.8</td>
<td>32.5±3.6</td>
</tr>
<tr>
<td>LV, g/10 g</td>
<td>31.6±1.9</td>
<td>49.5±1.9***</td>
<td>40.2±1.6†</td>
<td>42.3±1.9†</td>
</tr>
<tr>
<td>Lungs, g/10 g</td>
<td>57.2±3</td>
<td>58.7±3.8</td>
<td>51.8±1.7</td>
<td>55.8±3.7</td>
</tr>
<tr>
<td>Liver, g/10 g</td>
<td>470±8</td>
<td>440±14</td>
<td>454±12</td>
<td>467±15</td>
</tr>
<tr>
<td>PRC, μg Al/mL/h</td>
<td>0.8±0.3</td>
<td>1.3±0.2*</td>
<td>5.9±1.3†</td>
<td>14.9±8.3‡</td>
</tr>
<tr>
<td>IS, %</td>
<td>...</td>
<td>41.5±2.2</td>
<td>39.5±1.7</td>
<td>37.4±2.8</td>
</tr>
</tbody>
</table>

Values are mean±SE. BW indicates body weight; RV, right ventricular weight; LV, left ventricular weight corrected for BW; PRC, plasma renin concentration; IS, infarct size.

*p<0.05 vs sham; **p<0.01 vs sham; ***p<0.001 vs sham; †p<0.05 vs vehicle; ‡p<0.01 vs vehicle.
Results

Early and Late Mortality
AT2−/Y appeared to have higher mortality rates during surgery than AT2+/Y; however, it did not reach statistical significance (27.9% versus 14.1%). During the first 4 weeks of MI, mortality rates were similar between strains. In the AT1-ant group, 2 mice died during treatment (16.7%), whereas none of the AT2+/Y mice died. In vehicle- and ACEi-treated mice, mortality rates were not statistically different between AT2−/Y and AT2+/Y (28.6% versus 26.7% with vehicle; 0 versus 9.1% with ACEi).

Body, Heart, Lung and Liver Weight, Plasma Renin Concentration, and Infarct Size
There was no significant difference in any of these parameters between strains in the sham-ligated groups. In the MI+vehicle groups, heart weight increased similarly in both strains. AT1-ant and ACEi reduced heart weight significantly in AT2+/Y but not AT2−/Y. There was no significant change in lung or liver weight after MI. PRC was significantly increased after MI in AT2+/Y and tended to increase in AT2−/Y, but this was not statistically significant. ACEi increased PRC 10-fold in AT2+/Y and 7-fold in AT2−/Y. AT1-ant significantly increased PRC in AT2+/Y but not AT2−/Y. Infarct size was similar in all groups (Table).

Systolic Blood Pressure and Heart Rate
Basal SBP and HR were similar between strains in all groups. After MI, SBP in the MI+vehicle group was lower than sham and tended to decrease further with ACEi or AT1-ant in both strains (Figure 1, top). There was a slight increase in HR after MI, but it was not statistically significant. Drug treatment had no effect on HR (Figure 1, bottom).

Cardiac Function and Remodeling
There was no difference between sham-ligated AT2+/Y and AT2−/Y with regard to EF, CO, LVDd, or mass. After MI, EF and CO decreased, whereas LVDd and mass increased significantly by 1 month and progressed similarly over time in both strains (Figure 2). AT1-ant (20 weeks’ treatment) significantly increased EF by 69±14% and CO by 37±11% and reduced LVDd by 14±3% and mass by 16±6% in AT2+/Y, and these effects were diminished in AT2−/Y (EF: +6±7%; CO: −9±6%; LVDd: −0.6±2 and mass: +5±5%) (Figures 3 through 5). ACEi increased EF and CO and decreased LVDd and mass similarly in both strains.

Myocyte Size and Interstitial Collagen Fraction
MCSA and ICF were similar among sham-ligated mice. After MI, MCSA and ICF increased similarly in vehicle-treated groups from both strains. AT1-ant significantly decreased MCSA and ICF in AT2+/Y (28.6% versus 26.7% with vehicle; 0 versus 9.1% with ACEi).

Discussion
We found that although SBP, LV performance, and cardiac morphology/histology were similar between AT2+/Y and
AT₂⁻/⁻, both under normal conditions and during the development of CHF, the beneficial cardiac effects of AT₁-ant were significantly diminished in AT₂⁻/⁻ mice, suggesting that the cardioprotective effect of AT₁-ant is mediated at least in part via the AT₂ receptor. The response to ACEi was similar between AT₂⁻/⁻ and AT₂⁻/⁻.

The RAS plays an important role in cardiovascular, electrolyte, and fluid homeostasis through its effector Ang II. Ang II can also be a pathological factor in cardiac hypertrophy, fibrosis, and CHF. The biological effects of Ang II are mediated by at least two known subtypes, AT₁ and AT₂. Most known biological actions of Ang II, such as vasoconstriction, cellular proliferation, and matrix deposition, are attributable to the AT₁ receptor, whereas the physiological and pathophysiological functions of the AT₂ receptor remain controversial. Studies have shown that activation of AT₂ inhibits cell growth and proliferation, promotes cell differentiation and counterbalances the effect of AT₁. Overex-

Figure 3. Representative 2D M-mode echocardiograms showing LV dilatation (DD, diastolic dimension) and reduced interventricular septum (IS) and posterior wall (PW) motion in mice with HF as well as diminished response to AT₁-ant in AT₂⁻/⁻ mice. Sham indicates sham coronary ligation.

Figure 4. Effect of AT₁-ant and ACEi on EF in AT₂⁺/⁺ and AT₂⁻/⁻ with MI. Curved graphs show time and group effect; bar graph shows average percentage increase from before treatment (week 4) to after treatment (8 to 24 weeks). B, Before MI.
pression of AT2 attenuated the vasopressor response to exogenous Ang II, whereas deletion of the AT2 receptor (AT2−/Y) increased SBP and led to hypersensitivity to Ang II or susceptibility to DOCA-salt hypertension. However, Hein et al showed that basal SBP was no different between AT2+/Y and AT2−/Y mice, although AT2−/Y had increased sensitivity to Ang II. More recently, Senbonmatsu et al and Mifune et al showed that lack of AT2 receptors prevented the development of LV hypertrophy, whereas stimulation of AT2 increased collagen synthesis. In the present study, we observed no difference between AT2+/Y and AT2−/Y with regard to SBP, cardiac function, chamber dimensions, collagen deposition or myocyte size, either under basal conditions or after MI, suggesting that the AT2 receptor is not important for regulation of cardiac hemodynamics and function or the pathophysiology of cardiac remodeling after MI. However, we cannot exclude the possibility that we did not observe a difference in cardiac remodeling between AT2+/Y and AT2−/Y due to the fact that the infarcts in our study were too large (35% to 45% of the LV) and injury too severe, so that the compensatory capacity of the residual noninfarcted myocardium reached a maximum and no further functional and/or histopathological difference could be detected between strains.

Figure 5. Effect of AT1-ant and ACEi on LV diastolic dimension (LVDd) in AT2+/Y and AT2−/Y with MI. Curved graphs show time and group effect; bar graph shows average percentage decrease from before treatment (week 4) to after treatment (8 to 24 weeks). B, Before MI.

Figure 6. Representative slides showing increased MCSA and interstitial collagen deposition (green staining) in mice with heart failure induced by MI. AT1-ant and ACEi reduced MCSA and collagen deposition; effect of AT1-ant was diminished in AT2−/Y mice.

Figure 7. Effect of AT1-ant and ACEi on MCSA and ICF in AT2+/Y and AT2−/Y after MI.
Despite the fact that cardiac hemodynamics and phenotype in AT2−/+Y mice were similar to their wild-type controls, we found that AT2−/+Y mice with MI exhibited a diminished response to the therapeutic effect of AT1-ant, suggesting that the AT2 receptor is an important component in the cardioprotective effect of AT1-ant. This agrees with our previous finding that in rats with MI, AT1-ant had a cardioprotective effect similar to ACEi, while part of the effect of AT1-ant was blocked by an AT2 receptor antagonist (AT2-ant), which by itself had no effect on LV function or remodeling. Since lack of AT2 receptors in mice did not aggravate cardiac dysfunction and remodeling, our data may suggest that the AT2 receptor only exerts a cardioprotective action when the AT1 receptor is blocked. Inhibition of AT2 may stimulate renin release, in turn increasing circulating Ang II levels; increased Ang II binds to the AT2 receptor and thereby activates AT2-mediated action, such as inhibiting myocyte hypertrophy and/or fibroblast proliferation, leading to cardioprotection.2,8,10 The mechanism(s) responsible for the action of the AT2 receptor remains unclear. Masaki et al27 reported that overexpression of AT2 in mice with HF significantly inhibited Ang II–induced mitogen-activated protein kinase activation in fibroblasts, inhibiting collagen synthesis. Activation of AT2 during blockade of AT1 may also stimulate the release of autacoids such as PGE2 and NO, either directly and/or via stimulation of kinins.2,14,15,27 Using cultured bovine aortic endothelial cells, Seyedi et al14 found that Ang II induced a 6- to 7-fold increase in cGMP release; this effect was abolished by a kinin antagonist and a NO synthesis inhibitor, markedly inhibited by an AT2-ant, but only marginally inhibited by an AT1-ant. In aortas from mice with overexpression of the AT2 receptor gene, Ang II caused a significant increase in cGMP, which was further enhanced by an AT1-ant but blocked by an AT2-ant, B2 kinin antagonist, or NO inhibitor.15 These observations may suggest that both kinins and NO are involved in the AT2 signaling cascade, which mediates the action of the AT2 receptor. Tsutsumi et al15 also reported that mice overexpressing the AT2 receptor had increased kinogenase activity, which may be responsible for Ang II–stimulated kinin release. Furthermore, we recently demonstrated that the therapeutic effect of AT1-ant on cardiac function and remodeling post-MI was diminished in B2 kinin receptor knockout mice (B2−/−)8 or endothelial NOS knockout mice,13 which may provide further evidence that kinins and endothelium-derived NO play an important role in the beneficial cardiac effect of AT2-ant. The increase in kinin release produced by activation of AT2 may also be partially due to inhibition of ACE activity. It has been shown that AT2 receptors may have an inhibitory effect on ACE activity, since AT2−/+Y mice had increased ACE activity and exhibited a decreased vasodepressor response to bradykinin.8 Taken together, these results strongly suggest that production of kinins and NO by activation of AT2 should be considered a potential complementary or mediator pathway during AT2 receptor blockade.

Inhibition of ACE decreases formation of Ang II and degradation of BK and secondarily stimulates release of NO and PGs.2,28,29 Inhibition of ACE may also increase Ang 1–7 by accelerating its formation (due to increased Ang I, which is cleaved to Ang 1–7 by endopeptidases) and decreasing its degradation (Ang 1–7 is degraded to Ang 1–5 by ACE).30,31 It has been suggested that Ang 1–7 is an endogenous competitive inhibitor of Ang II and is able to stimulate release of kinins, PGs, and NO through non-AT1 and non-AT2 receptors.31,32 Therefore, if part of the effect of ACEi is mediated by increased Ang 1–7, lack of the AT2 receptor may have no impact on the effect of ACEi. Indeed, we found that the beneficial cardiac effect of ACEi was preserved in AT2−/+Y mice, suggesting that the AT2 receptor is not involved in the action of ACEi.

It is well known that ACE inhibition stimulates renin release, as we found in our study. Theoretically, blockade of AT1 should also increase renin release due to a feedback mechanism. However, we only saw a slight increase in PRC after AT2-ant treatment in both AT2−/+Y and AT2−/−Y mice. Since we have confirmed that the dose of AT2-ant we used blocked about 70% of exogenous Ang II–induced vasoconstriction, similar to the effect of ACEi on exogenous Ang I, we assume this dose is sufficient to block the action of endogenous Ang II. At the present time, we do not have a good explanation as to why antagonism of AT1 did not increase PRC. It is possible that renin release is mediated by mechanisms beyond the AT1 receptor, which need to be investigated further.

**Perspective**

The primary findings of the present study are (1) under basal conditions, cardiac hemodynamic, functional, and histological phenotypes are similar between AT2+/Y and AT2−/−Y mice; (2) after MI, progression of cardiac dysfunction and remodeling is also similar between the two strains; and (3) blockade of the AT1 receptor improves cardiac function and regresses remodeling after MI, and this effect of AT1-ant is attenuated in AT2−/−Y mice, whereas the effect of ACEi is preserved. Our data suggest that the AT1 receptor does not play an essential role in regulation of cardiac function and morphology, either under normal conditions or during the development of HF; however, activation of AT2 plays a significant role in the therapeutic effect of AT1-ant.

**Acknowledgments**

This work was supported by National Institutes of Health grant HL-28982 and a Novartis Pharmaceuticals research grant.

**References**


Role of AT$_2$ Receptors in the Cardioprotective Effect of AT$_1$ Antagonists in Mice
Jiang Xu, Oscar A. Carretero, Yun-He Liu, Edward G. Shesely, Fang Yang, Alissa Kapke and Xiao-Ping Yang

_Hypertension_. 2002;40:244-250; originally published online July 29, 2002;
doi: 10.1161/01.HYP.0000029095.23198.AD

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 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/40/3/244

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/