Role of AT₂ Receptors in the Cardioprotective Effect of AT₁ Antagonists in Mice

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

Abstract—Angiotensin II (Ang II) acts mainly on two receptor subtypes: AT₁ and AT₂. Most of the known biological actions of Ang II are mediated by AT₁ receptors; however, the role of AT₂ receptors remains unclear. We tested the hypothesis that the cardioprotective effects of AT₁ receptor antagonists (AT₁-ant) after myocardial infarction (MI) are partially mediated by activation of AT₂ receptors; thus in AT₁ receptor gene knockout mice (AT₁−/−Y), the effect of AT₁-ant will be diminished or absent. MI was induced by ligating the left anterior descending coronary artery. Four weeks later, AT₁−/−Y and their wild-type littermates (AT₁+/−Y) were started on vehicle, AT₁-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 AT₁+/−Y and AT₁−/−Y. AT₁-ant increased ejection fraction and cardiac output and decreased left ventricular diastolic dimension, myocyte cross-sectional area, and interstitial collagen deposition in AT₁+/−Y, and these effects were significantly diminished in AT₁−/−Y. ACE inhibitors improved cardiac function and remodeling similarly in both strains. We concluded that (1) activation of AT₂ during AT₁ blockade plays an important role in the therapeutic effect of AT₁-ant and (2) the AT₂ 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 || 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, AT₁ and AT₂. Most well-known actions of Ang II in the cardiovascular system are mediated by the AT₁ receptor; whereas little is known about the function of the AT₂ receptor. It has been proposed that blockade of the AT₁ receptor increases Ang II, which activates the AT₂ 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), AT₁-ant had an antiprotectic effect similar to an ACE inhibitor (ACEi), which was partially blocked by an AT₂-ant or B₂ kinin receptor antagonist (B₂-ant). We also found that in B₂ or endothelial NO synthase (eNOS) knockout mice, the effect of AT₁-ant was diminished compared with their wild-type controls, suggesting a kinin/NO-mediated mechanism. Other studies have shown that activation of the AT₂ receptor counterbalances the effect of the AT₁ receptor, thereby inhibiting cell growth, proliferation, and hypertrophy. In cultured bovine aortic endothelial cells or aortas from mice overexpressing the AT₂ receptor gene, Ang II was found to increase the release of cGMP; this effect was further enhanced by an AT₁-ant but markedly blocked by an AT₂-ant, B₂-ant or NO synthesis inhibitor, suggesting that Ang II–stimulated release of cGMP is mediated by kinins and NO through activation of the AT₂ receptor.

The role of the AT₂ 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 AT₂ receptor in mice (AT₂−/−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 AT₁-ant diminished in AT₂ receptor knockout mice. On the other hand, Senbonmatsu et al and Mifune et al reported that targeted deletion of AT₂ receptors prevented left ventricular hypertrophy induced by pressure overload, whereas stimulation of AT₁ increased collagen synthesis.

To clarify the role of AT₂ receptors in the therapeutic effect of AT₁-ant and in regulation of cardiac function and remodeling...
eling after MI, we used AT_2^-/- mice to study whether lack of AT_2 receptors (1) diminishes the cardioprotective effects of AT_1-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**

AT_2^-/- mice were originally developed on a hybrid genetic background, then back-crossed to inbred background C57BL/6j by our Mutant Mouse Core until congenic status was reached (10+ back-cross generations). Since the AT_2 receptor gene is X-linked, male knockouts were obtained as homozygous offspring (−/−). Wild-type littermates (AT_2+/Y) 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.7,9 MI was confirmed at 4 weeks by cardiac geometry. Systolic BP (SBP) was measured with a noninvasive computerized tail-cuff system (BP-2000, Visitech Systems).22 Cardiac geometry was determined by Skinner et al Heart Failure in AT_2 Receptor Knockout Mice

**Experimental Protocols**

Doses of AT_1-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). AT_1-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+AT_1-ant; and (4) MI+ACEi, with treatment continuing for 20 weeks.

**Histopathological Study**

Hearts were stopped at diastole by intraventricular injection of 15% KCl. The LV was sectioned transversely into 3 slices and rapidly frozen in isopentane precooled in liquid nitrogen, then stored at −70°C. Infarct size was determined by Gomori trichrome staining and expressed as the ratio of the infarcted portion to total LV circumference.7 For myocardocyte 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 MSCA 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.

**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.

**Effect of AT_1-ant and ACEi on Heart, Lung, and Liver Weight and PRC in AT_2+/Y and AT_2^-/- Mice**

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<th>AT_2-/-</th>
<th>MI</th>
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<td>IS, %</td>
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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

AT$_2$−/Y appeared to have higher mortality rates during surgery than AT$_2$+/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 AT$_1$-ant group, 2 mice died during treatment (16.7%), whereas none of the AT$_2$+/Y mice died. In vehicle- and ACEi-treated mice, mortality rates were not statistically different between AT$_2$−/Y and AT$_2$+/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. AT$_1$-ant and ACEi reduced heart weight significantly in AT$_2$+/Y but not AT$_2$−/Y. There was no significant change in lung or liver weight after MI. PRC was significantly increased after MI in AT$_2$+/Y and tended to increase in AT$_2$−/Y, but this was not statistically significant. ACEi increased PRC 10-fold in AT$_2$+/Y and 7-fold in AT$_2$−/Y. AT$_1$-ant significantly increased PRC in AT$_2$+/Y but not AT$_2$−/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 AT$_1$-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 AT$_2$+/Y and AT$_2$−/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). AT$_1$-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 AT$_2$+/Y, and these effects were diminished in AT$_2$−/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. AT$_1$-ant significantly decreased MCSA and ICF in AT$_2$+/Y, and this effect was attenuated in AT$_2$−/Y. The effect of ACEi on these parameters was similar in both strains (Figures 6 and 7).

Discussion

We found that although SBP, LV performance, and cardiac morphology/histology were similar between AT$_2$+/Y and
AT$_2^{-/-}$, both under normal conditions and during the development of CHF, the beneficial cardiac effects of AT$_1$-ant were significantly diminished in AT$_2^{-/-}$ mice, suggesting that the cardioprotective effect of AT$_1$-ant is mediated at least in part via the AT$_2$ receptor. The response to ACEi was similar between AT$_2^{-/-}$ and AT$_2^{-/-}$.

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, AT$_1$ and AT$_2$. Most known biological actions of Ang II, such as vasoconstriction, cellular proliferation, and matrix deposition, are attributable to the AT$_1$ receptor, whereas the physiological and pathophysiological functions of the AT$_2$ receptor remain controversial. Studies have shown that activation of AT$_2$ inhibits cell growth and proliferation, promotes cell differentiation and counterbalances the effect of AT$_1$. Overex-
expression 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 AT$_2$−/−Y mice were similar to their wild-type controls, we found that AT$_2$−/−Y mice with MI exhibited a diminished response to the therapeutic effect of AT$_1$-ant, suggesting that the AT$_2$ receptor is an important component in the cardioprotective effect of AT$_1$-ant. This agrees with our previous finding that in rats with MI, AT$_1$-ant had a cardioprotective effect similar to ACEi, while part of the effect of AT$_1$-ant was blocked by an AT$_2$ receptor antagonist (AT$_2$-ant), which by itself had no effect on LV function or remodeling. Since lack of AT$_2$ receptors in mice did not aggravate cardiac dysfunction and remodeling, our data may suggest that the AT$_2$ receptor only exerts a cardioprotective action when the AT$_1$ receptor is blocked. Inhibition of AT$_1$ may stimulate renin release, in turn increasing circulating Ang II levels; increased Ang II binds to the AT$_2$ receptor and thereby activates AT$_2$-mediated action, such as inhibiting myocyte hypertrophy and/or function or remodeling post-MI was diminished in AT$_2$−/−Y mice. AT$_2$ during blockade of AT$_1$ may also stimulate the release of autacoids such as PGE$_2$ and NO, either directly and/or via stimulation of kinins. Using cultured bovine aortic endothelial cells, Seyedi et al. 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 AT$_2$-ant, but only marginally inhibited by an AT$_1$-ant. In aortas from mice with overexpression of the AT$_2$ receptor gene, Ang II caused a significant increase in cGMP, which was further enhanced by an AT$_1$-ant but blocked by an AT$_2$-ant. B$_2$ kinin antagonist, or NOS inhibitor. These observations may suggest that both kinins and NO are involved in the AT$_2$ signaling cascade, which mediates the action of the AT$_2$ receptor. Tsutsumi et al. also reported that mice overexpressing the AT$_2$ receptor had increased kininogenase activity, which may be responsible for Ang II-stimulated kinin release. Furthermore, we recently demonstrated that the therapeutic effect of AT$_2$-ant on cardiac function and remodeling post-MI was diminished in B$_2$ kinin receptor knockout mice (B$_2$−/−) or endothelial NOS knockout mice, which may provide further evidence that kinins and endothelium-derived NO play an important role in the beneficial cardiac effect of AT$_1$-ant. The increase in kinin release produced by activation of AT$_2$ may also be partially due to inhibition of ACE activity. It has been shown that AT$_2$ receptors may have an inhibitory effect on ACE activity, since AT$_2$−/−/Y mice had increased ACE activity and exhibited a decreased vasodepressor response to bradykinin. Taken together, these results strongly suggest that production of kinins and NO by activation of AT$_2$ should be considered a potential complementary or mediator pathway during AT$_1$ receptor blockade.

Inhibition of ACE decreases formation of Ang II and degradation of BK and secondarily stimulates release of NO and PGs. 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). 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-AT$_1$ and non-AT$_2$ receptors. Therefore, if part of the effect of ACEi is mediated by increased Ang 1–7, lack of the AT$_2$ receptor may have no impact on the effect of ACEi. Indeed, we found that the beneficial cardiac effect of ACEi was preserved in AT$_2$−/−Y mice, suggesting that the AT$_2$ 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 AT$_1$ should also increase renin release due to a feedback mechanism. However, we only saw a slight increase in PRC after AT$_1$-ant treatment in both AT$_2$+Y and AT$_2$−/−Y mice. Since we have confirmed that the dose of AT$_1$-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 AT$_1$ did not increase PRC. It is possible that renin release is mediated by mechanisms beyond the AT$_1$ 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 AT$_2$+Y and AT$_2$−/−Y mice; (2) after MI, progression of cardiac dysfunction and remodeling is also similar between the two strains; and (3) blockade of the AT$_1$ receptor improves cardiac function and regresses remodeling after MI, and this effect of AT$_1$-ant is attenuated in AT$_2$−/−Y mice, whereas the effect of ACEi is preserved. Our data suggest that the AT$_2$ 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 AT$_2$ plays a significant role in the therapeutic effect of AT$_1$-ant.

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**References**


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