Angiotensin II Type 1A Receptor Knockout Mice Display Less Left Ventricular Remodeling and Improved Survival After Myocardial Infarction

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Background—Angiotensin II (Ang II) has been implicated in ventricular remodeling after myocardial infarction (MI), which is an important determinant for prognosis after MI. The aim of this study was to determine whether Ang II type 1A receptor (AT1A)–mediated Ang II signals are critically involved in the mortality and LV remodeling after MI.

Methods and Results—We examined survival, cardiac geometry and function, cardiac fibrosis, and gene expression of AT1A knockout (KO) mice and wild-type (WT) mice at 1 and 4 weeks after large MI. The survival rate was higher in KO mice than in WT mice at 4 weeks after MI. All WT survivors showed severe heart failure, detected by marked increases in both RV weight and lung weight. LV remodeling, such as the development of LV dilatation, LV dysfunction, and cardiac fibrosis at the noninfarcted area, were comparable in both kinds of mice at 1 week after MI. At 4 weeks after MI, however, WT mice showed more marked remodeling than KO mice. mRNA levels of AT1 at the noninfarcted area were increased from 1 to 4 weeks after MI only in WT mice, whereas levels of AT2 were not changed by MI in either kind of mouse. Accompanied by the development of geometric and structural remodeling, expression of fetal-type genes, collagen, and transforming growth factor-β1 genes were upregulated and sustained in the noninfarcted area of WT hearts. In contrast, they were rapidly downregulated to basal levels at 4 weeks after MI in that of KO hearts.

Conclusions—These results indicate that AT1A signals play a pivotal role in the progression of LV remodeling after MI, resulting in overt heart failure. (Circulation. 1999;100:2093-2099.)

Key Words: angiotensin ■ myocardial infarction ■ mortality ■ remodeling

Myocardial infarction (MI) induces global changes of ventricular architecture, called post-MI ventricular remodeling. Early changes in the left ventricular (LV) architecture after MI are adaptive responses of the heart to acute loss of functional myocardium and initially preserve cardiac performance. However, once these processes develop after large MI, the infarcted heart progressively dilates and accelerates the deterioration of ventricular dysfunction that eventually results in heart failure.1–3

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Accumulating evidence has suggested that the cardiac renin-angiotensin system (RAS) is activated during the remodeling process after MI.4 Local concentration and generation of Ang II and the number of Ang II receptors have been reported to be increased in infarcted hearts.5–8 In addition, many studies have demonstrated that inhibition of the cardiac RAS with ACE inhibitors improves LV function and prevents geometric remodeling, which leads to increased survival of patients5,9 as well as in animal models10,11 after MI, suggesting that Ang II plays an important role in postinfarct remodeling. However, because ACE inhibitors inhibit not only synthesis of Ang II but also breakdown of bradykinin, increased kinins may play an important role in the prevention of LV remodeling. Actually, it has been reported that bradykinin activation resulting from ACE inhibition attenuates structural remodeling in infarcted heart.12 Therefore, it remains to be determined whether Ang II is critical in LV remodeling.

The biological effects of Ang II are exerted through specific 7-transmembrane Ang II receptors.13 At present, Ang II receptors are divided into 2 major subtypes, Ang II type 1 (AT1) and type 2 (AT2) receptors, and AT1 is further subdivided into AT1A and AT1B. It is generally accepted that most of the well-known Ang II functions in the cardiovascular system are mediated through AT1.13 In vitro studies have shown that Ang II directly stimulates proliferation of cardiac fibroblasts and production of extracellular matrix proteins,
such as collagen, through AT₁. It has also been reported that transforming growth factor-β₁ (TGF-β₁) is involved in Ang II–induced synthesis of collagen. In vivo studies have demonstrated that chronic infusion of a subpressor dose of Ang II causes proliferation of cardiac fibroblasts and an increase in collagen deposition, which contributes to an increase in cardiac muscle stiffness and the development of diastolic dysfunction. In addition, chronic administration of ACE inhibitors or AT₁ antagonists significantly attenuates cardiac fibrosis after MI. Together, these results suggest that AT₁-mediated Ang II signals play a pivotal role in the process of remodeling after MI. To test this hypothesis, we made large MI and examined survival, cardiac geometry, function, fibrosis, and gene expression in hearts of AT₁A knockout (KO) mice.

Methods

Murine MI Model

All protocols were approved by local institutional guidelines. AT₁KO mice (n=27) and wild-type (WT) mice (n=32) 14 to 16 weeks old from the same genetic background were used in the present study. After mice were anesthetized and artificially ventilated with a small-animal respirator, MI was produced by permanent ligation of the left coronary artery (LCA) with a 10-0 nylon surgical suture under a dissecting microscope as previously described. Successful ligation of the LCA was verified visually by the color change of the ischemic area, and we monitored the ECG continuously during the operation. In the sham procedure, the same procedure was performed except the LCA ligation. At 1 and 4 weeks after MI, the ventricles and the lung were excised and weighed after physiological studies. Infarct size was calculated and expressed as a percentage of LV surface area as previously described. Animals with <30% of infarct size were excluded from analysis because they did not show typical LV remodeling.

Physiological Studies

Echocardiograms were recorded with an echocardiographic system (Hewlett-Packard, Ltd) equipped with a 7.5-MHz imaging transducer at 1 or 4 weeks after surgery as described previously. LV internal dimensions, such as end-diastolic dimension (EDD) and end-systolic dimension (ESD), and LV posterior wall thickness (PWT) were measured as described previously. Percent fractional shortening (%FS) and relative wall thickness were calculated as [(EDD-ESD)/EDD]×100 and 2×PWT/EDD, respectively. As for hemodynamic measurements, a catheter (stretched PE 10 tubing) was placed into the LV via the right carotid artery under constant pressure monitoring. Catheter positions were verified by registration of typical pressure waves with pressure transducers. Tracings from the carotid artery and LV catheters were recorded and used to obtain heart rate, LV systolic pressure, and LV end-diastolic pressure.

Histological Analysis

Fixed tissues with 10% formalin were prepared for routine histology. To determine the degree of collagen fiber accumulation, we selected 8 fields randomly and calculated the ratio of van Gieson–stained fibrosis area divided by total myocardial area with an image analysis software as described previously. Infarcted areas were excluded from this measurement. Immunohistochemical stainings for collagen type I and III were carried out on paraffin sections with anti-rat type I collagen (Cosmo Bio Co) and anti-mouse type III collagen (Cosmo Bio) with LSAB kit (Dako), respectively.

Competitive RT-PCR Analysis

The competitive reverse transcriptase–polymerase chain reaction (RT-PCR) analysis was performed for AT₁ and AT₂ mRNA quantification, which was established with deletion-mutated cRNA as described previously. The amplification efficiencies of target and competitor transcripts are equal under optimal concentrations of competitor transcripts. Because the primers used for the amplification of AT₁ correspond to common sequences between AT₁A and AT₁B, both AT₁A and AT₁B mRNAs were amplified. To verify that equal amounts of RNA were subjected to RT-PCR, GAPDH mRNA was also amplified with specific primers. Denaturing (94°C for 45 seconds), annealing (58°C for 1 minute), and extension (72°C for 1 minute) reactions were performed for 30 cycles. The range of concentrations of sample RNA and internal control deleted cRNA, as well as the number of amplification cycles, was selected from within the exponential phase.

Statistical Analysis

All results are expressed as mean±SEM. Analyses of survival after MI were carried out by the Kaplan-Meier method with the log-rank (Cox-Mantel) method. Multiple comparisons among ≥3 groups were carried out by 2-way ANOVA and Fisher’s exact test for post hoc analyses. Statistical significance was accepted at a value of P<0.05.

Results

Survival After MI

The survival rates of KO mice and wild-type (WT) mice were compared up to 4 weeks after MI. The survival rate was significantly higher in KO mice than in WT mice at 4 weeks (P<0.05) (Figure 1). Although the cause of death is not clear, the early death within 1 week after MI was 13.6% in WT mice and 5.9% in KO mice. Of the WT mice, 9.1% died between 1 and 4 weeks after MI, whereas no KO mice died in this period. This marked difference in survival was not due to differences in infarct size, because mean infarct size was comparable in both kinds of mice (WT, 39±4% versus KO, 37±3%).

Heart Failure After MI

At 4 weeks after MI, all WT mice showed tachypnea and lethargy and kept still in 1 corner of their cages. In contrast, KO mice with MI actively moved in their cages. It has been reported that animals were considered to have heart failure when it was detected by pathological findings, such as RV
Figure 2. Changes in cardiac geometry and function after MI. a, RVW/BW ratio and lung W/BW ratio were markedly increased in WT mice at 4 weeks after MI compared with KO mice. b, Representative charts of transthoracic M-mode echocardiograms of infarcted mice. All echocardiograms were obtained at level of papillary muscles: WT mice at 1 week after MI (top left); KO mice at 1 week after MI (top right); WT mice at 4 weeks after MI (bottom left); KO mice at 4 weeks after MI (bottom right). Note increased LV internal dimension (LVID) and akinesis and thinning of anterior wall (AW). PW indicates posterior wall. c, Echocardiographic analysis. d, Hemodynamic measurements. All data are expressed as mean±SEM of 5 to 15 mice per group. *P<0.05, **P<0.01.
hypertrophy and pleural effusion. The RV weight (RVW)/body weight (BW) ratio was significantly larger in infarcted mice than in sham-operated mice at 1 week after MI. There were no significant differences in the RVW/BW ratio and the lung weight/BW ratio between WT mice and KO mice at 1 week after MI. At 4 weeks after MI, however, both of these ratios increased more prominently in the surviving WT mice than in KO mice (Figure 2a), suggesting that WT mice with MI developed more severe heart failure at 4 weeks after MI than KO mice. The LVW/BW ratio was also increased more prominently in the surviving WT mice than in KO mice at 4 weeks after MI (WT, 11.3±1.9 versus KO, 7.1±1.0; P<0.05), whereas there was no significant difference in this ratio in either kind of mouse at 1 week after MI.

Progressive LV Dilatation and Impaired Cardiac Function After MI

We assessed changes in the LV geometry and function using M-mode echocardiography (Figure 2b). EDD was larger in all mice with MI than in sham-operated mice (Figure 2c). In WT hearts, EDD was more markedly increased from 1 week to 4 weeks after MI than in KO hearts (WT, 48% increase versus KO, 28% increase; P<0.01) (Figure 2c). In addition, relative wall thickness was markedly decreased only in WT mice at 4 weeks after MI (WT, 37% decrease versus KO, 5% decrease; P<0.01) (Figure 2c), indicating that the increase in LV cavity size is disproportionate to the increase in thickness of the surviving myocardium. As for functional changes, we assessed %FS, which is 1 of the echocardiographic indexes of LV systolic function. %FS of infarcted mice was always significantly lower than that of sham-operated mice. %FS in WT mice at 4 weeks after MI was markedly lower than that in KO mice (WT, 54% decrease versus KO, 9% decrease; P<0.05) (Figure 2c). To further analyze LV function of infarcted mice, we also measured LV systolic pressure and LV end-diastolic pressure. There were no significant differences in heart rate between the 2 animal groups. At 4 weeks after MI, LV end-diastolic pressure was higher in WT mice than in KO mice (Figure 2d). These results suggest that although MI initially induces LV dilatation and dysfunction due to loss of myocardium in KO mice as well as in WT mice, progression of LV enlargement and dysfunction was less prominent in KO mice than in WT mice.

Myocardial Fibrosis in Infarcted Hearts

We examined myocardial fibrosis at 4 weeks after MI, because reactive fibrosis at the noninfarcted area adversely alters myocardial stiffness, leading to cardiac dysfunction. A fibrotic scar was observed in the infarcted area in both kinds of mice. In the noninfarcted area, notable reactive fibrosis, such as perivascular and interstitial fibrosis, was observed in WT mice but not in KO mice (Figure 3), which is consistent with the pathophysiological responses in the present study. In addition, immunoreactivities for collagen type I and III are markedly increased at 4 weeks after MI in WT mice but not in KO mice. These results indicate that although MI caused fibrous tissue formation in infarcted myocardium in KO hearts as well as in WT hearts, reactive fibrosis in the noninfarcted area is significantly attenuated by the absence of AT1A-mediated Ang II signals.

Cardiac Gene Expression in Infarcted Hearts

mRNA levels of AT1 and AT2 in viable areas of infarcted hearts were assessed by competitive RT-PCR analysis (Figure 4a and 4b). The AT1 gene was abundantly expressed in WT hearts. Because the AT1A gene was deleted in KO mice, slight transcription (<10%) of the AT1B gene was detected in KO hearts. mRNA levels of AT1, but not AT2, were increased from 1 week to 4 weeks after MI in WT hearts. In KO mice, neither AT1 nor AT2 mRNA levels were changed by MI. These results suggest that AT1A-mediated Ang II signals could be activated in infarcted WT hearts. In the
noninfarct area, we next examined expression of fetal-type genes, such as ANP and \(\beta\)-MHC genes, and collagen type I and III genes, which are induced in the rat MI model in association with cardiac dysfunction and heart failure\(^4\) (Figure 4c). We also examined expression of TGF-\(\beta\) because TGF-\(\beta\) is known to directly induce the synthesis of extracellular matrix proteins in cardiac fibroblasts\(^{4,14-18}\). At 1 week after MI, mRNA levels of ANP, \(\beta\)-MHC, and collagen III were upregulated in both KO hearts and WT hearts. In contrast, mRNA levels of collagen type I and TGF-\(\beta\) were higher in WT hearts than in KO hearts at 1 week after MI (Figure 4d). At 4 weeks after MI, high levels of these gene expressions were still observed in WT hearts, whereas expression levels were already decreased to basal levels in KO hearts (Figure 4d). These results suggest that AT\(_{1a}\) signals are critically involved in genetic responses during LV remodeling.

**Figure 4.** Cardiac gene expressions in noninfarcted area. AT\(_1\) (a) and AT\(_2\) (b) mRNA levels in heart of sham-operated and infarcted mice. RT-PCR was performed with specific primers. Amplified DNA was electrophoresed and stained with ethidium bromide (left). Normalized values in sham-operated hearts of WT mice are arbitrarily expressed as 100% (right). All data shown are mean±SEM of 4 separate experiments with duplicate determinations in each experiment. *\(P<0.01\) vs sham-operated mice of WT mice. \(\Delta\)AT1 and \(\Delta\)AT2 indicate amplified DNA from deleted cRNA as internal control. c, Representative autoradiograms of Northern blot analysis. d, Summary of Northern blot analysis. mRNA levels in sham-operated wild-type hearts are expressed as 1.0. Data are expressed as mean±SEM of 4 independent experiments. *\(P<0.05\) vs mRNA levels in WT hearts at each time point. S indicates sham-operated mice; 1W, 1 week after MI; 4W, 4 weeks after MI; Col I, collagen type I; and Col III, collagen type III.
Discussion

Although geometric remodeling after MI occurs as an adaptation, excessive change may cause detrimental effects on cardiac function and result in congestive heart failure. A growing body of evidence has suggested that cardiac RAS is activated in the infarcted heart. Many clinical and experimental studies have demonstrated that inhibition of the RAS with ACE inhibitors suppresses geometric remodeling after MI and shows beneficial effects on survival. However, it remains unknown whether a decrease in Ang II or an increase in bradykinin inhibits the initiation or progression of the post-MI remodeling process. Therefore, we created a murine MI model using AT1A KO mice to elucidate the role of AT1A-mediated Ang II actions in the process of ventricular remodeling. At 1 week after MI, geometric, functional, and structural remodeling occurred equally in both animal groups, suggesting that AT1A signals are not required for the initiation of remodeling. At 4 weeks after MI, however, WT mice consistently exhibited more marked remodeling, such as further progression of LV enlargement, ventricular dysfunction, and fibrosis, and showed higher mortality. In contrast, KO mice with MI exhibited mild LV dilatation without heart failure and a high survival rate. RT-PCR analysis revealed that mRNA levels of AT1, but not AT2, were markedly increased from 1 to 4 weeks after MI in the noninfarced area of WT hearts, whereas those of AT1 and AT2 were not changed by MI in that of KO hearts. Our findings suggest that AT1A-mediated Ang II signals play a critical role in the progression of maladaptive geometric remodeling after large MI, which was associated with the occurrence of heart failure, thereby leading to a poor prognosis.

There are several possible reasons why KO mice showed fewer remodeling events after MI and improved survival. First, recent studies have identified myofibroblasts expressing ACE and Ang II receptors at sites of fibrosis in both infarcted and noninfarcted myocardium. It has also been reported that Ang II stimulates collagen synthesis directly or through TGF-β, in cardiac fibroblasts or myofibroblasts. The chronic stimulation of the cardiac RAS, therefore, may induce excessive fibrosis in the noninfarcted area through AT1, which contributes to the occurrence of lethal heart failure. In the present study, all WT survivors showed not only overt heart failure but also excessive fibrosis (collagen accumulation) in the noninfarct area, where AT1 was increased. High survival in KO mice may be due to the attenuation of unfavorable and maladaptive fibrosis by the absence of AT1A signals. Second, Ang II may have direct negative effects on myocardial contractility that induce progression of heart failure. It has been reported that Ang II significantly reduces cardiac contractility in the failing heart through AT1. The absence of AT1A signals may prevent the impairment of mechanical behavior of failing myocardi- um. Third, recent studies have reported that the AT1 antagonist losartan significantly reduces ischemia-induced ventricular arrhythmias. Moreover, we have also found that AT1A KO mice showed fewer lethal arrhythmias after ischemia-reperfusion. Recently, clinical studies also showed that the AT1 antagonist losartan was more beneficial than the ACE inhibitor captopril by lowering sudden death (Evaluation of Losartan in the Elderly Study, ELITE). Therefore, the inhibition of arrhythmias may account for the improved survival of KO mice. Finally, a reduction in blood pressure and LV loading in KO mice may have beneficial effects leading to fewer LV remodeling events. However, this may not be the case, because previous studies have indicated that structural remodeling in the failing heart after MI occurs irrespective of hemodynamic load.

In the present study, KO mice did not show progressive heart failure with the late deterioration of LV dysfunction seen in WT survivors or the amelioration of reduced LV function. It is unknown why this amelioration was not detected in KO mice. Treatment with ACE inhibitors has been demonstrated to improve LV function in the rat MI model compared with AT1 antagonists. In addition, results of the ELITE ventricular function substudy indicated that captopril prevents geometric remodeling more effectively than losartan. These effects may be attributable to the additional cardioprotective effects of enhanced kinins, which induce release of several growth-inhibitory factors, such as nitric oxide, endothelium-derived growth factor, and prostacyclin. Future studies to clarify whether enhancing the production of these counterbalancing factors can reverse severe dysfunction in the failing heart are needed.

In conclusion, this study showed that AT1A signals are necessary for progression of postinfarct LV remodeling associated with overt heart failure. Recent clinical studies in patients with heart failure demonstrated that treatment with losartan significantly reduces mortality compared with captopril. Our findings in this study may present not only the potential of AT1 antagonism as a strategy for prevention of LV remodeling but also the molecular mechanism by which blockade of AT1 signals improves survival after MI.

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References


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