Angiotensin II Type 2 Receptor Deficiency Exacerbates Heart Failure and Reduces Survival After Acute Myocardial Infarction in Mice

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Background—Angiotensin II plays a prominent role in the progression of heart failure after acute myocardial infarction (AMI). Although both angiotensin type 1 (AT₁) and type 2 (AT₂) receptors are known to be present in the heart, comparatively little is known about the latter. We therefore examined the role played by AT₂ receptors in post-AMI heart failure.

Methods and Results—In wild-type mice subjected to AMI by coronary artery ligation, AT₂ receptor immunoreactivity is upregulated in the infarct and border areas. Among AT₂ receptor-null (−/−) mice, the 7-day survival rate after AMI was significantly lower than among wild-type mice (43% versus 67%; P<0.05). All sham-operated animals of both genotypes survived through the study. Ventricular mRNA levels for brain natriuretic peptide were elevated in both genotypes 24 hours after coronary occlusion, with levels in AT₂ −/− significantly higher than in wild-type mice, as were their lung weights, and histological examination revealed marked pulmonary congestion in the AT₂ −/− mice. Cardiac function was significantly decreased in AT₂ −/− mice 2 days after AMI.

Conclusions—AT₂ receptor deficiency exacerbates short-term death rates and heart failure after experimental AMI in mice. The AT₂ receptor may thus exert a protective effect on the heart after AMI. (Circulation. 2003;107:2406-2408.)

Key Words: angiotensin ■ myocardial infarction ■ heart failure
mRNA levels and histological examination of the lung at 24 hours after coronary occlusion in some mice.

mRNA Quantification, Immunohistochemistry, and Echocardiographic Study

To quantify BNP, AT1a, and AT2 receptor mRNA, TaqMan RT-PCR was performed according to the manufacturer’s instructions. Levels of target gene mRNA were normalized to those of GAPDH. Immunohistochemical detection of AT2 receptors was performed as described previously. Transthoracic echocardiography was performed in pre- and 2 days post-AMI mice with the use of a 15-MHz liner probe (Power-Vision 8000, Toshiba).

Statistical Analysis

Data are expressed as mean ± SE. Statistical analyses were performed using StatView software (v. 5.0 for Windows). Two-way ANOVA followed by Tukey-Kramer tests was used to evaluate the effects of AMI and genotype. The Log-Rank test was used to evaluate survival rate. Values of P < 0.05 were considered to be significant.

Results

Expression of AT2 and AT1a Receptor After AMI

Seven days after coronary occlusion, levels of AT2 receptor mRNA were significantly increased in wild-type mice (Figure 1A; P < 0.01), whereas levels of AT1a receptor mRNA were significantly diminished in both genotypes (Figure 1B; P < 0.01). Immunohistochemical labeling, performed 7 days after coronary occlusion in wild-type mice, revealed the site of post-AMI AT2 receptor expression to be within the infarct and border areas (Figure 1C). Images obtained at a higher magnification enable one to see that most of the immunoreactivity was present in the interstitium. There was no detectable AT2 receptor immunoreactivity in the myocardium or on coronary vessels.

Survival

All sham-operated wild-type and AT2 knockout mice survived through the study (Figure 2A). Of the 42 wild-type and 75 AT2 knockout mice that underwent coronary artery occlusion, infarct sizes were similar in wild-type and AT2 knockout mice (34 ± 5% and 36 ± 4%, respectively); nevertheless, the 7-day mortality rate was significantly higher among AT2 knockout mice (57%) than among the wild-type mice (35%) (P < 0.05, Figure 2A). Almost all of the deaths were the result of acute heart failure, which was diagnosed on the basis of the presence of pleural

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**Figure 1.** Cardiac expression of AT2 (A) and AT1a (B) receptors in mouse heart 7 days after coronary artery occlusion. Values are mean ± SE; **P < 0.01. C, Left and right panels (original magnification, 100× and 400×, respectively), show heart sections from wild-type mice in which AT2 receptors are immunohistochemically labeled. Positive red staining is seen in the interstitium.

**Figure 2.** A, Kaplan-Meier analysis of survival after experimental AMI. B, Quantitative analysis of ventricular BNP mRNA expression. C, Lung weight/body weight ratios. D, Representative hematoxylin-eosin-stained histological sections of lung obtained in wild-type (+/+) and AT2 receptor-deficient (−/−) mice. Bottom panels show high magnification (400×) of the corresponding top panel (100×). Comparisons were made 24 hours after coronary occlusion. Values are shown mean ± SE; *P < 0.05, **P < 0.01.
effusion and lung congestion, although some died of cardiac rupture (5% \( n=2 \)) of wild-type and 4% \( n=3 \) of AT2\(^{-/-}\) mice).

**Characteristics of the Acute Heart Failure After AMI**

 Ventricular BNP mRNA levels were found to be significantly elevated in both genotypes 24 hours after coronary artery occlusion (Figure 2B; \( P<0.01 \)), with levels in AT2\(^{-/-}\) mice significantly higher than in wild-type mice (\( P<0.05 \)). At the same time, lung weight/body weight ratios were significantly increased in AT2\(^{-/-}\) mice (Figure 2C; \( P<0.01 \)), and histological examination showed obvious congestion (Figure 2D). By contrast, lung weight/body weight ratios and alveolar structure were unchanged in wild-type mice. The LV weight/body weight ratios in AT2\(^{-/-}\) mice were significantly greater than those of wild-type mice (3.38±0.11 and 3.24±0.11, respectively; \( P<0.01 \)). There were no differences in baseline values of the LV end diastolic dimension (LVDD) and fractional shortening (FS) between both genotypes of mice (Table). LVDD was not changed at 2 days after AMI in both genotypes. FS was reduced only in AT2\(^{-/-}\) mice.

**Discussion**

The key findings of present study are as follows: (1) AT2 receptor expression is increased in cardiac interstitial cells after AMI; (2) AT2\(^{-/-}\) mice exhibit acute cardiac failure after AMI and have a lower survival rate; and (3) cardiac function was significantly decreased in AT2\(^{-/-}\) mice 2 days after AMI. Earlier studies have shown that expression of AT2 receptors is elevated in the pathological heart.\(^5\) Consistent with those studies, we found AT2 receptor mRNA expression to be elevated in hearts of wild-type mice 7 days after AMI, which was in contrast to the decreased expression of AT1a receptor mRNA. Busche et al\(^6\) reported that AT1 receptor mRNA was found in rat cardiomyocyte. However, our immunohistochemical findings confirm that AT2 receptors are expressed at interstitial cells. Indeed, AT2 receptors were presented in parallel to the \( \alpha \)-smooth muscle actin, which was the marker of the myofibroblasts (data not shown).\(^7\) No positive signal was found in cardiomyocyte.

We found for the first time that the absence of AT2 receptors exacerbated acute heart failure, as evidenced by elevated ventricular BNP levels and lung congestion, reduced cardiac function, and diminished the post-AMI survival rate. In addition, Cardiac function measured by echocardiography was significantly reduced in AT2\(^{-/-}\) mice at 2 days after coronary occlusion. To confirm the result of genetic blockade of AT2 receptor, we examined the effects of AT2 receptor antagonist, PD123319, on the mortality and heart failure in this AMI-model. PD123319 (100 \( \mu \)g/day) was subcutaneously infused using osmotic pump from 7 days before AMI to the follow-up period, totally for 14 days. However, we could not obtain aggravated effects of PD123319 similar to those obtained from AT2\(^{-/-}\) mice. Seven out of 24 PD123319-treated mice and 6 out of 22 vehicle-treated mice survived following a 7-day period after AMI. Nowadays, the effects of PD123319 are not evident, because Levy et al\(^8\) showed that PD123319 reduced arterial hypertrophy and fibrosis. Akishita et al\(^9\) reported that PD123319 exaggerated cuff-induced vascular remodeling in mice. Genetic blockade is the suitable method to investigate direct roles of AT2 receptor at present. However, careful thinking is required to interpret the data from AT2\(^{-/-}\) mice, because some biological systems, which compensate AT2 receptor actions, may affect the heart failure after AMI.

The present results suggest that AT2 receptor serves as an endogenous protective mechanism for congestive heart failure after AMI. In that regard, it is notable that angiotensin II stimulates progression of heart failure and cardiac remodeling via AT1 receptors.\(^10\) Further studies are necessary to elucidate what kinds of signals are responsible for aggravated effect of heart failure.

**References**

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