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

Yuichiro Adachi, MS; Yoshihiko Saito, MD; Ichiro Kishimoto, MD; Masaki Harada, MD; Koichiro Kuwahara, MD; Nobuki Takahashi, MD; Rika Kawakami, MD; Michio Nakanishi, MD; Yasuaki Nakagawa, MD; Keiji Tanimoto, MD; Yoshitomo Saitoh, MD; Shintaro Yoshi, MD; Masahiko Sato, MD; Masakazu Horiuchi, MD; Kazuwa Nakao, MD

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−/− mice 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

The cardiac renin-angiotensin system is activated immediately after acute myocardial infarction (AMI).1 Evidence now suggests that inhibiting this system using an angiotensin-converting enzyme (ACE) inhibitor or an AT1 receptor antagonist improves left ventricular (LV) function and prevents geometric remodeling after AMI, thereby increasing survival.2 Of the two major angiotensin II (Ang II) receptor subtypes, type 1 (AT1) and type 2 (AT2), the role of the former in post-AMI heart failure has been extensively studied, whereas the role of the latter remains controversial. In the present study, therefore, we used a mouse model in which the AT2 receptor was genetically disrupted to investigate its role in post-AMI heart failure.

Methods
All experimental procedures were approved by the Institutional Review Committee for the Care and Use of Animals at Kyoto University and were performed in accordance with their guidelines.

Animals
We used female homozygous AT1 receptor-null (−/−) and wild-type mice (aged 8 to 12 weeks; bred in our laboratory) because of the high incidence of fatal cardiac rupture in male mice within 3 to 4 days after AMI.3 The background of this mouse model was described previously.4 The cardiac histology and weight were not different in both genotypes (data not shown).

Surgical Procedure
Mice were anesthetized with 1.0% to 1.5% isoflurane, and open-chest coronary artery ligation was performed. The left coronary artery about 2 mm under the left auricle was occluded using an 8-0 nylon suture. After ligation, the chests were closed, and the mice were allowed to recover. The same procedure without coronary ligation was performed in sham operations. We followed up 7 days after AMI, because most of mice were dead 2 to 3 days after AMI. There was no difference in survival rate during 7 to 42 days after AMI between wild-type and AT2−/− mice (1 out of 15 and 2 out of 14 mice died, respectively). Therefore, to investigate the cause of death in AT2−/− mice, we examined pathological changes 24 hours after AMI, such as ventricular brain natriuretic peptide (BNP)
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 Stat-View 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 in the marginal zone. There was no detectable AT2 receptor immunoreactivity in the myocardium or on coronary vessels.

Survival

All sham-operated wild-type and AT2−/− mice survived through the study (Figure 2A). Of the 42 wild-type and 75 AT2−/− mice that underwent coronary artery occlusion, infarct sizes were similar in wild-type and AT2−/− mice (34±5% and 36±4%, respectively); nevertheless, the 7-day mortality rate was significantly higher among AT2−/− 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 effusion.
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 (4.39±0.13 and 3.89±0.12, 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. 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. Busch et al. reported that AT2 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 α-smooth muscle actin, which was the marker of the myofibroblasts (data not shown). 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 μg/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. showed that PD123319 reduced arterial hypertrophy and fibrosis. Akishita et al. 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. Further studies are necessary to elucidate what kinds of signals are responsible for aggravated effect of heart failure.

References

Angiotensin II Type 2 Receptor Deficiency Exacerbates Heart Failure and Reduces Survival After Acute Myocardial Infarction in Mice
Yuichiro Adachi, Yoshihiko Saito, Ichiro Kishimoto, Masaki Harada, Koichiro Kuwahara, Nobuki Takahashi, Rika Kawakami, Michio Nakanishi, Yasuaki Nakagawa, Keiji Tanimoto, Yoshitomo Saitoh, Shinji Yasuno, Satoru Usami, Masaru Iwai, Masatsugu Horiuchi and Kazuwa Nakao

_Circulation_. 2003;107:2406-2408; originally published online May 5, 2003; doi: 10.1161/01.CIR.0000072763.98069.B4

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/107/19/2406

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ 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 _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/