ortic regurgitation (AR) is a valvular disease that causes left ventricular (LV) volume overload, leading to progressive LV dilatation and dysfunction. In the present study it was examined whether blockade of angiotensin II type 1 receptor (AT1) could improve survival in cases of chronic severe AR.

Methods and Results  AR was induced by puncturing the aortic valves of wild-type (WT) and AT1a knockout (KO) mice. Mice that survived for 4 weeks after the operation were deemed to be a model of chronic severe AR and were followed up for 50 weeks (WT, n=29; KO, n=31). Baseline measurements made 4 weeks after surgery showed similar LV cavity and function in both genotypes. These conditions progressively worsened in both genotypes, but 16 weeks after baseline, KO mice showed significantly less LV dilatation, hypertrophy and interstitial fibrosis than WT mice. Cardiac mRNA expression of B-type natriuretic peptide and type I collagen was lower in KO than WT mice. The 50-week mortality rate was significantly lower among KO (45.2%) than WT (86.2%) mice, and postmortem findings indicated that the lower mortality was attributable to a lower incidence of congestive heart failure.

Conclusions  In cases of chronic severe AR, blockade of AT1 attenuates the progression of LV dilatation, hypertrophy and fibrosis, thereby mitigating heart failure and improving long-term survival. (Circ J 2007; 71: 1310–1316)

Key Words: Angiotensin; Fibrosis; Heart failure; Hypertrophy; Valvular disease

Aortic regurgitation (AR) is a valvular disease that causes left ventricular (LV) volume overload, leading to progressive LV dilatation and eventual LV dysfunction. Aortic valve replacement is considered for patients with chronic severe AR who are at high risk under conservative management. Surgical correction improves LV function and reduces cardiovascular mortality among these patients, but it is not appropriate for all patients because of the risks associated with the procedure. Vasodilator therapy has been shown to reduce LV volume and mass and to improve hemodynamics and LV function in patients with severe AR but controversy exists as to the utility of vasodilators in patients with volume overload cardiomyopathy. Indeed, 2 long-term randomized trials to determine whether vasodilators reduce or delay the need for valve surgery in patients with severe AR produced conflicting results.

The results of several clinical trials clearly demonstrate that interruption of the renin–angiotensin system (RAS) activity with angiotensin-converting enzyme (ACE) inhibitors or angiotensin II type 1 receptor (AT1) blockers improves long-term survival in patients with heart failure. On the other hand, those trials included few, if any, patients with uncorrected primary valvular disease. There has not been and will never be any prospective large-scale randomized controlled trial to determine whether vasodilator therapy improves long-term survival among patients with severe AR: the consensus is that surgical intervention is life-prolonging, making it unethical to deny suitable patients such treatment for the purposes of a clinical trial.

Our aim in the present study was to test the hypothesis that inhibition of the RAS will improve long-term survival in chronic severe AR. The pharmacological effects on the RAS are often incomplete and highly dose dependent. Therefore, to obtain more precise information on the effects of AT1 signaling, we used genetically engineered mice with targeted deletion of AT1 and evaluated changes in LV structure, LV function and cardiac gene expression, and 50-week survival.

Methods

Experimental Model

All experimental procedures were carried out according to the Kyoto University standards of animal care. AT1a KO
mice (C57BL/6 background) were originally generated at the Institute of Applied Biochemistry, University of Tsukuba, Ibaraki, Japan. Homozygous KO mice and their WT littermates were produced from crossing heterozygous mice as described previously, after which male mice were used for experimentation at 13–15 weeks of age. Genotypes were determined before and verified after experimentation using PCR.

**Experimental Chronic AR**

Mice were initially anesthetized, intubated and artificially ventilated with a small animal respirator (Shinano Co, Tokyo, Japan); the tidal volume was set at 0.6 ml and the rate at 110 breaths/min. Experimental AR was induced by puncturing the aortic valve with a 1.4-French Millar pressure catheter inserted via the right carotid artery. Sham-operated mice underwent the same operation without the aortic valve puncture. When we measured blood pressure (BP) before and after puncturing the aortic valve, we found that puncture caused a significant increase in pulse pressure in both WT and KO mice, and that there was no difference in the relative increases in pulse pressure between the 2 strains. This was taken to indicate that the degree of AR was similar in both genotypes. The degree of regurgitation was also confirmed by Doppler echocardiography after the puncture. Within 2 weeks of inducing AR, more than half the mice of both genotypes had died (WT, 57.5%; KO, 68.3%), but none died during the subsequent 2 weeks. AT1, angiotensin II type 1 receptor; AR, aortic regurgitation; KO, knockout.

**Statistical Analysis**

All data are expressed as means ± SEM. Analysis of survival after AR was carried out using the Kaplan-Meier method with the log-rank test. Data were analyzed using 1-factor ANOVA. If a statistically significant effect was found, a posthoc Newman-Kleus test was performed to iso-
late the differences between groups. Values of p<0.05 were considered significant.

Table 1 Baseline Characteristics and Echocardiographic Measurements of Sham-Operated and AR-Operated Mice

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>AR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WT (n=40)</td>
<td>AT1a KO (n=43)</td>
</tr>
<tr>
<td></td>
<td>AT1a KO (n=54)</td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>characteristics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (weeks)</td>
<td>18.2±0.3</td>
<td>18.3±0.4</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>30.1±0.6</td>
<td>29.4±0.5</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>110.6±2.3</td>
<td>92.2±3.3*</td>
</tr>
<tr>
<td>PR (beats/min)</td>
<td>684.9±12.3</td>
<td>695.3±22.0</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>echocardiographic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>measurements</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVDd (mm)</td>
<td>4.63±0.08</td>
<td>4.72±0.15</td>
</tr>
<tr>
<td>LVDs (mm)</td>
<td>3.41±0.07</td>
<td>3.41±0.07</td>
</tr>
<tr>
<td>%FS</td>
<td>27.2±0.59</td>
<td>27.6±0.60</td>
</tr>
</tbody>
</table>

Values are mean±SEM; *p<0.05 vs the corresponding WT mice; #p<0.05 vs sham in each strain.

AR, aortic regurgitation; WT, wild-type; AT1, angiotensin II type 1 receptor; KO, knockout; BP, blood pressure; PR, pulse rate; LVDd, left ventricular diastolic diameter; LVDs, left ventricular systolic diameter; FS, fractional shortening.

Fig 2. Time-dependent changes in echocardiographic parameters during chronic aortic regurgitation (AR). Left ventricular (LV) diastolic diameter (A) and % fractional shortening (B) were evaluated every 4 weeks for 16 weeks beginning at baseline (4 weeks after induction of AR). Values are means±SEM (n=11 to 13); *p<0.05 between wild-type and knockout (KO) mice at each week. AT1, angiotensin II type 1 receptor.

Fig 3. Changes in left ventricular (LV) weight-to-body weight ratios in sham-operated mice 16 weeks after baseline and in aortic regurgitation (AR)-operated mice 8 and 16 weeks after baseline. Values are means±SEM (n=8 to 11); *p<0.05 vs wild-type mice on each week. KO, knockout; AT1, angiotensin II type 1 receptor.

Results

Baseline Characteristics and Echocardiographic Measurements

The baseline characteristics and echocardiographic measurements of sham-operated and AR-operated mice from both genotypes (4 weeks after operation) are shown in Table 1. There were no differences between the 2 genotypes with respect to age, body weight or pulse rate, although systolic BP was significantly lower in KO mice, as described previously. In both genotypes, the LVDd and LVDs of AR mice were significantly higher and the %FS significantly lower than for the sham-operated mice. In AR mice, there were no significant differences between the genotypes with respect to LVDd, LVDs or %FS, indicating that WT and KO mice had similar LV cavity size and LV systolic function.
Changes in Echocardiographic Findings

We carried out echocardiography every 4 weeks for 16 weeks beginning with the baseline measurements. In AR mice, we found that LVDd and LVDs continued to increase, whereas %FS continued to decline in both WT and KO mice, indicating a progressive worsening of LV dilatation and systolic dysfunction after baseline in both genotypes (Fig 2). However, after 8 weeks, the LVDd was significantly smaller in the KO than in the WT mice (WT 7.01±0.08 mm vs KO 6.80±0.06 mm; p<0.05), and this difference persisted through the final measurements made 16 weeks after baseline (WT 7.30±0.12 mm vs KO 6.94±0.09 mm; p<0.05) (Fig 2A). Similar results were obtained for the measurements of LVDs. On the other hand, there was no significant difference between the 2 genotypes with respect to %FS at any time up to 16 weeks (WT 16.5±0.56% vs KO 17.2±0.36%; p=0.3) (Fig 2B). In the sham-operated mice, no significant changes were observed up to 16 weeks in either WT (LVDd 4.67±0.07 mm, %FS 26.8±0.46%) or KO mice (LVDd 4.73±0.12 mm, %FS 27.2±1.02%).

LV Hypertrophy and Fibrosis

To evaluate LV hypertrophy and myocardial fibrosis, we harvested sham-operated hearts 16 weeks after baseline (20 weeks after operation) and AR-operated hearts at 8 weeks and 16 weeks after baseline from both genotypes and determined the LV weight-to-body weight ratios (LVW/BW) and stained histological sections with Sirius red. We found that LV hypertrophy and the severity of interstitial fibrosis were similar in sham-operated hearts from WT and KO mice (Figs 3,4). In AR-operated hearts at 8 weeks and 16 weeks after baseline from both genotypes, the LVW/BW was markedly larger than in sham-operated hearts, but significantly smaller in KO than WT mice at both time points (Fig 3). Moreover, Sirius red staining of AR hearts revealed progressive interstitial fibrosis in the WT mice that was virtually absent in KO mice (Fig 4).

Fig 4. Representative Sirius red-stained sections of left ventricle from sham-operated hearts 16 weeks after baseline and aortic regurgitation (AR)-operated hearts collected 8 and 16 weeks after baseline. The images show the deposition of interstitial collagen (red staining). Bars=50µm. KO, knockout; AT1, angiotensin II type 1 receptor.

Fig 5. Kaplan-Meier analysis of survival after baseline among aortic regurgitation (AR)-operated wild-type (WT) (WT/AR, n=29) and knockout (KO) mice (AT1a KO/AR, n=31) and among sham-operated WT (WT/Sham, n=28) and KO mice (AT1a KO/Sham, n=30). In AR mice, KO mice showed a significantly higher rate of survival than WT mice (p<0.0001). AT1, angiotensin II type 1 receptor.
Survival and Causes of Death

Survival among AR mice (WT, n=29; KO, n=31) was monitored for 50 weeks after baseline (Fig 5). During that period, there were 25 deaths (86.2%) among the WT mice, but only 14 (45.2%) among the KO mice (p<0.0001). This difference in mortality rate first reached statistical significance at 13 weeks (WT, 37.9% vs KO, 12.9%; p<0.05) and then increased with time. Postmortem findings revealed that almost all of the mice of both genotypes had pulmonary congestion with increased lung weight, indicating that they died of congestive heart failure. Accordingly, the lower incidence of mortality among KO mice was attributed to a lower incidence of worsening heart failure. No sham-operated mice (WT, n=28; KO, n=30) of either genotype died during the 50-week follow-up period (Fig 5).

Survival in Subgroups Assigned According to Baseline LV Function

To further assess the effect of AT1 blockade on long-term survival, we compared survival rates between subgroups of WT and KO mice defined according to their baseline LV echocardiographic values (Fig 6). WT mice with an echo-

Fig 6. Survival among aortic regurgitation-operated wild-type (WT) and knockout (KO) mice after dividing the 2 genotypes into subgroups defined according to left ventricular diastolic diameter (LVDd) at baseline. (A) Baseline LVDds after dividing the WT and KO mice into small LVDd (<6.2 mm) and large LVDd (≥6.2 mm) subgroups. The LVDd values were similar in the corresponding subgroups of WT and KO mice. Values are means±SEM. (B) Kaplan-Meier analysis of survival of the small LVDd groups (WT, n=11, KO, n=14). (C) Kaplan-Meier analysis of survival of the large LVDd groups (WT, n=18, KO, n=17). AT1, angiotensin II type 1 receptor.

Fig 7. Relative levels of B-type natriuretic peptide (BNP) (A) and type I collagen (B) mRNA in the left ventricle normalized to the corresponding GAPDH mRNA levels measured using quantitative RT-PCR in sham-operated and aortic regurgitation (AR)-operated hearts 16 weeks after baseline. Mean mRNA levels in sham-operated wild-type (WT) hearts were assigned a value of 1.0. Values are means±SEM (n=8–11); *p<0.05 vs the corresponding WT mice; #p<0.05 vs sham in each strain. KO, knockout.
cardiographic LVDd ≥6.2 mm (the median value) at baseline showed a significantly (p<0.05) lower rate of survival than WT mice with an LVDd <6.2 mm. Although the LVDd values were similar in the corresponding subgroups of WT and KO mice (Fig 6A), the survival rate was significantly higher among KO mice in both the small LVDd (p<0.05, Fig 6B) and large LVDd (p<0.0005, Fig 6C) groups. Similar results were obtained when mice were divided into subgroups according to baseline LVDs or %FS measurements. The favorable effect of AT1 blockade on long-term survival appeared to be independent of baseline LV structure and function.

Cardiac Gene Expression

Ventricular mRNA levels of both BNP and type I collagen were lower in sham-operated hearts at 16 weeks after baseline from KO mice than in those from WT mice, though the difference only reached statistical significance for type I collagen mRNA (Fig 7). In AR hearts at 16 weeks after baseline from both WT and KO mice, levels of BNP and type I collagen mRNA were significantly higher than in the corresponding sham-operated hearts, but the levels were significantly lower in KO than WT mice.

**Discussion**

Although several clinical studies have demonstrated both short-term and long-term beneficial effects of vasodilator therapy in patients with severe AR,6–9,13 others have reported no beneficial effects of therapy, or even that it is detrimental.10,14 In addition, there have been no large controlled clinical trials evaluating the effects of vasodilator therapy on long-term survival among patients with chronic severe AR. In the present study, we used an experimental AR model with genetically engineered mice to show that, in chronic severe AR, the progression of LV dilatation, hypertrophy and interstitial fibrosis is attenuated and long-term survival is improved when AT1 signaling is blocked. To our knowledge, this is the first report to suggest that AT1 blockade exerts a beneficial effect on long-term survival in chronic severe AR.

We induced AR by puncturing the aortic valve with a Millar pressure catheter. That more than half of both the WT and AT1a KO mice died within 2 weeks after the procedure indicates that the degree of regurgitation was very severe. None of the surviving mice died during the subsequent 2 weeks, and we therefore designated the values obtained 4 weeks after surgery as the baseline of the chronic AR model; at that point there were no differences in any of the echocardiographic parameters between the 2 genotypes.

The mortality rates among KO and WT mice began to differ within 13 weeks after baseline and increased over time, so that by 50 weeks the mortality rate was much lower among KO mice than WT mice (45.2% vs 86.2%, respectively). Postmortem findings showed that almost all of the mice had lung congestion, indicating that a lower incidence of worsening heart failure likely underlies the lower mortality rate among the KO mice, which is consistent with their lower level of cardiac BNP mRNA expression.

Notably, the progression of LV dilatation and hypertrophy was attenuated in KO mice, although there was no attenuation in the progressive LV systolic dysfunction. Moreover, the marked myocardial interstitial fibrosis seen in WT mice was virtually absent in KO mice, which is consistent with the observed downregulation of cardiac expression of type I collagen mRNA in KO mice.

It is well established that accumulation of interstitial collagen results in increased wall stiffness, decreased LV compliance and impaired diastolic function, which in turn impairs LV filling and increases end-diastolic pressure.25,26 In patients with hypertensive heart disease, myocardial fibrosis reportedly can be reversed by inhibition of the RAS, leading to improved LV diastolic function.27,29 Likewise, regression of hypertrophy also improves LV diastolic compliance.30,31 Accordingly, the lower incidence of congestive heart failure seen in the KO mice would be mainly attributable to attenuation of LV diastolic dysfunction, not systolic dysfunction.

Aortic valve replacement is associated with reduced cardiovascular mortality in patients with severe AR; but preoperative LV size and function are major determinants of the management strategy, as patients with marked LV enlargement or dysfunction show increased rates of operative and postoperative mortality.4,5 The subgroup analysis carried out in the present study showed that inhibition of AT1 signaling improves long-term survival, regardless of the degree of LV dilatation and dysfunction, which suggests that medical treatment with AT1 blockers may be an effective therapy in patients who are poor candidates for surgery, including those with marked LV enlargement or dysfunction and those with severe coexisting conditions.

Within the first 2 weeks after AR operation, KO mice tended to have higher mortality than WT mice, although the difference did not reach statistical significance (Fig 1). Induction of acute AR causes marked reduction of cardiac output, coronary blood flow and organ perfusion. Therefore, activation of the renin–angiotensin–aldosterone system may be required to compensate the hemodynamic change in the early stage of acute AR until cardiac output increases to some degree by LV remodeling. However, further studies are needed to assess the role of AT1 signaling in acute AR.

**Study Limitations**

The principal limitation of this study is that systolic BP was significantly lower in KO than WT mice. It is possible that the improved survival and the attenuated LV remodeling in KO mice may be at least partially caused by the lower BP. Inhibition of the RAS with ACE inhibitors exerts a more beneficial effect on LV performance and hemodynamics than other vasodilators in patients with AR, despite similar capacities to lower BP.30,31 Similarly, volume overload-induced hypertrophy in the aortocaval shunted rat was attenuated by an AT1 receptor blocker, but not by a calcium-channel blocker.32 These suggest that the beneficial effects of AT1 blockade are primarily mediated by actions other than lowering BP. However, additional studies using a control group for BP-lowering intervention will be required before drawing a conclusion as to the specific effects of AT1 signaling.

**Conclusions**

Genetic blockade of AT1 signaling in chronic severe AR reduces the risk of disease progression by attenuating LV dilatation, hypertrophy and interstitial fibrosis, thereby mitigating congestive heart failure and improving long-term survival. The reduction in congestive heart failure appears to be mainly attributable to attenuation of progressive LV diastolic dysfunction, and AT1 blockade appears
to exert a favorable effect on long-term survival irrespective of the degree of LV enlargement and dysfunction.

Acknowledgments
This work was supported by research grants from the Japanese Ministry of Education, Culture, Sports, Science and Technology and the Japanese Ministry of Health, Labour and Welfare, and a Grant-in-aid for scientific research from the Japanese Ministry of Education, Culture, Sports, Science and Technology. We thank Komaki Okazaki for her excellent secretarial work.

References