New Angiotensin II Type 1 Receptor Blocker, Azilsartan, Attenuates Cardiac Remodeling after Myocardial Infarction

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After an acute myocardial infarction (MI), neurohumoral systems including renin-angiotensin-aldosterone system (RAAS) are activated which in turn aggravate cardiac remodeling. Angiotensin receptor blockers (ARBs) are useful drugs for suppression of RAAS. The purpose of this study was to evaluate a new ARB, azilsartan, for suppressing cardiac remodeling and progression to heart failure after MI. We created MI by left anterior descending coronary artery ligation in male mice, and these mice were orally administered saline (0.2 mL) in the control group (Group C), 0.1 mg/kg/d of azilsartan in the low dose group (Group L), and 1.0 mg/kg/d in the high dose group (Group H) everyday. Blood pressure was decreased in Group H, but not in Group L, compared to Group C. At 2 weeks after MI creation, infarct size and fibrotic change at the site remote to the myocardial infarcted area were attenuated in Group L and Group H compared to Group C. Echocardiography revealed that cardiac remodeling was suppressed in Group L and Group H compared to Group C. Increases of mRNA expression levels related to fibrotic change were attenuated in Group L and Group H compared to Group C. The new ARB, azilsartan, had a cardiac remodeling suppression effect after MI, and this effect was observed without blood pressure lowering.

Key words myocardial infarction; cardiac remodeling; heart failure; angiotensin receptor blocker; azilsartan

Hypertension, diabetes, and dyslipidemia are increasing in modern society, and myocardial infarction (MI), which is a consequence of these diseases, is the leading cause of progression to heart failure (HF) and cardiac death. Acute phase mortality by acute MI has decreased in recent decades because of the development of percutaneous coronary intervention (PCI) as the acute phase therapy. However, immediately after coronary artery occlusion, irreversible cell necrosis can occur within minutes. Even though the treatment in acute phase has been evolved, cardiac systolic function is reduced in the infarcted heart. To compensate for the lack of systolic function, the infarcted heart induce morphological change called remodeling. The post-infarct prevention and treatment of HF have emerged as a growing challenge to improve quality of life, and reduce healthcare costs.

As one of the post-MI left ventricular (LV) remodeling, neurohumoral activation including renin-angiotensin-aldosterone system (RAAS) is a very important factor, because activated RAAS causes the perpetual vasoconstriction, LV hypertrophy, sympathetic nervous activation, and endothelial dysfunction. These RAAS activations lead to a vicious cycle for the progression of cardiac remodeling, therefore suppression of RAAS is an important target for HF after MI. Angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) are recommended for use in the management of patients with HF post-MI, because some large-scale clinical trials have documented the benefits of pharmacological therapies in the post-MI period aimed at limiting LV remodeling and progressive HF. ACE inhibitors suppress angiotensin II production because the enzymatic activity of ACE catalyzes the conversion of angiotensin I to angiotensin II, leading to not only angiotensin II type 1 (AT1) receptor but also AT2 receptor blockade effect. Stimulation of the AT2 receptor has also been shown to induce vasodilation and natriuresis. Moreover, the ACE is also responsible for the degradation of bradykinin, which is a potent vasodilator. Unlike ACE inhibitors, ARBs selectively block AT1 receptor activity without blocking effect on the AT2 receptor, and the blockade of AT1 receptor results in enhanced stimulation of the AT2 receptor. Further cardiovascular protection may be afforded from the enhanced AT2 receptor activity via vasodilation and fibrinolytic mechanisms.

The purpose of this study was to examine the beneficial effect of new ARB, azilsartan, on cardiac remodeling and progression of HF after MI.

MATERIALS AND METHODS

Animal Model We used C57BL/6 wild type mice (CLEA Japan, Inc. Tokyo, Japan) and housed them in a facility with a 12/12-h light/dark cycle and they were given free access to water and standard rodent chow. The room was kept specific pathogen-free. All animal experiments were performed in accordance with the guidelines of Fukushima Medical University Animal Research Committee. Research protocol was approved by the institutional review board. The investigation conformed to the Guide for the Care and Use of Laboratory Animals 8th edition, published by the U.S. National Research Council.

Experimental Protocols Induction of MI was performed as described previously. Briefly, ten to twelve week old male mice (20–25 g of body weight) were anesthetized by intraperitoneal injection with a tribromoethanol (0.25 mg/g of body weight). The animals were intubated with a 20-gauge polyethylene catheter and were ventilated with a rodent ventilator. An incision was performed along the left sternal border, and the fourth rib was cut proximal to the sternum. The left anterior descending coronary artery was identified, and an 8–0 proline suture was passed around the artery and subsequently tied off. Successful ligation of the coronary artery was...
verified visually by the discoloration of the left ventricular myocardium. In sham-operated animals, the same procedure was performed except for the coronary artery ligation. Finally, the heart was repositioned in the chest, and the chest wall was closed. The animals remained in a supervised setting until becoming fully conscious.

Just after MI was created, we randomly divided the mice into three groups, i) control, ii) low dose azilsartan, and iii) high dose azilsartan groups. The mice were orally administered saline (0.2 mL) in the control group (Group C), 0.1 mg/kg/d in the low dose azilsartan group (Group L), and 1.0 mg/kg/d in the high dose azilsartan group (Group H) everyday. Azilsartan was kindly provided by Takeda Pharmaceutical Co., Ltd. (Osaka, Japan).

**Histological Examination** To assess morphological changes, the mice were weighed and their hearts were excised then weighed at 2 weeks after MI creation. The heart was embedded in paraffin and sliced serially at the papillary muscle level of the mitral valve. These sections were stained with hematoxylin-eosin or Masson trichrome stain. In each muscle level of the mitral valve. These sections were stained with Masson trichrome-stained connective tissue area (stained blue) to total myocardial area obtained by calculating the ratio of Masson trichrome-stained areas were acquired. With the use of M-mode images from the parasternal short-axis view at papillary muscle level, intraventricular septal thickness (IVS), left ventricular posterior wall thickness (LVPW), left ventricular end-diastolic dimension (LVEDd), and left ventricular end-systolic dimension (LVESd) were measured and averaged for 3 cardiac cycles. Left ventricular fractional shortening (FS) was calculated as 100× ((LVEDd–LVESd)/LVEDd).

**Extraction of Total RNA and Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)** To assess the fibrosis marker genes, mRNA expression levels of collagen type I and transforming growth factor-beta (TGF-β) in mouse hearts were determined by RT-PCR technique. Total RNA was extracted from mouse hearts using RNeasy Mini Kit (Qiagen, Venlo, the Netherlands) according to the manufacturer’s instruction. The concentrations of all RNA samples were determined spectrophotometrically. The cDNA was produced from total RNA using ReverTra Ace qPCR RT Master Mix (TOYOBO Co., Ltd., Osaka, Japan) according to the manufacturer’s instruction and RT-PCR was performed using GeneAmp PCR System 9700 (Applied Biosystems, Santa Clara, CA, U.S.A.). The expression level of collagen type I and TGF-β was normalized to that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and expressed as a ratio relative to GAPDH. Primers were designed on the basis of GenBank sequences (collagen type I, NM007742; TGF-β, NM011577 and GAPDH, NM001001303).

**Statistical Analysis** All results are expressed as mean± standard error (S.E.). Effects of MI on heart weight, blood pressure, histological data, and echocardiographic data, which were compared between sham operated mice and azilsartan administered mice, were analyzed by one way analysis of variance (ANOVA) followed by multiple comparisons by using Scheffe’s test. A p value less than 0.05 was considered statistically significant. Statistical analysis was performed with a standard statistical program package (Stat View, version 5.0, SAS Institute Inc., Cary, NC, U.S.A).

**RESULTS**

**Gravimetric and Hemodynamic Data after Myocardial Infarction** The body weight (BW) did not change among each group after MI (Table 1). Although the ratios of heart weight (HW) to BW were increased at 2 weeks after MI operation compared to sham operated mice, these increases were attenuated in Group L and Group H compared to Group C (p<0.05, respectively). Similarly, lung wet weight (LW) to

| Table 1. Gravimetric and Hemodynamic Data at Two Weeks after MI Operation |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
|                                 | Sham            | Group C         | Group L         | Group H         |
| Gravimetric data                |                 |                 |                 |                 |
| BW (g)                          | 25.4±0.81       | 24.8±0.63       | 25.8±0.52       | 25.7±0.31       |
| HW/BW ratio (mg/g)              | 5.06±0.07       | 7.21±0.20**     | 6.34±0.15**     | 6.17±0.16**     |
| LW/BW ratio (mg/g)              | 5.14±0.10       | 7.92±0.34**     | 6.73±0.21**     | 6.24±0.34**     |
| Hemodynamic data                |                 |                 |                 |                 |
| HR (beats/min)                  | 472.3±24.1      | 487.8±45.6      | 476.7±14.0      | 497.6±42.1      |
| sBP (mmHg)                      | 112.0±5.29      | 104.0±5.76      | 100.0±4.88      | 95.4±3.96**     |
| mBP (mmHg)                      | 74.6±5.17       | 72.8±4.97       | 71.0±2.57       | 68.0±3.16*      |
| dBP (mmHg)                      | 62.1±2.1        | 60.5±2.0        | 59.3±2.6        | 59.0±2.6        |

**MI, myocardial infarction; BW, body weight; HW, heart weight; LW, lung wet weight; HR, heart rate; sBP, systolic blood pressure; mBP, mean blood pressure; dBP, diastolic blood pressure. All data are shown as mean±S.E. (n=5 per group). *p<0.05 and **p<0.01 vs. sham-operated mice; †p<0.05 vs. Group C, respectively.**
BW ratios, which demonstrate the degree of congestive heart failure due to MI, were significantly increased after MI operation, and these increases were attenuated in Group L and Group H compared to Group C ($p<0.05$, respectively).

Systolic, mean, and diastolic blood pressures did not change between Group C and the sham operation group (Table 1). Although systolic, mean, and diastolic blood pressures were not significantly different between Group L and Group C, systolic blood pressure was lower in Group H than in Group C ($p<0.05$). These results demonstrated that the low dose azilsartan (0.1 mg/kg/d) did not influence blood pressure.

**Left Ventricular Remodeling Evaluated by Histology after Myocardial Infarction** The percent of infarct size was estimated among three groups at 2 weeks after MI op-
eration. As shown in Fig. 1, the infarct size was significantly decreased in Group L (40.8% vs. 52.7%, \( p < 0.05 \)) and Group H (35.8% vs. 52.7%, \( p < 0.01 \)) compared to Group C, respectively.

Interstitial fibrosis is developed in the noninfarcted myocardium, particularly in the heart with large MI (Fig. 2). At the site remote to the myocardial infarcted area, which was not directly influenced by ischemia due to coronary artery ligation, fibrotic tissue accumulation was augmented after MI operation, and this increase was attenuated by azilsartan administration (Group C vs. Group L, 10.1% vs. 7.1%, \( p < 0.05 \), and Group C vs. Group H, 10.1% vs. 5.7%, \( p < 0.01 \)).

**Cardiac Function Evaluated by Echocardiography after Myocardial Infarction** We examined cardiac function by echocardiography at 2 weeks after MI operation (Fig. 3 and Table 2). In Group C, IVS was thin, LVEDd was enlarged, and FS was decreased after MI compared to sham. These structural and functional changes after MI are called as cardiac remodeling. Enlargement of LVEDd after MI was attenuated in both Group L and Group H compared to Group C as shown in Table 2 (\( p < 0.05 \), respectively). The decrease of FS after MI was improved in both Group L and Group H compared to Group C (\( p < 0.05 \) and \( p < 0.01 \), respectively). The thinning of IVS was also attenuated in Group L and Group H compared to Group C (\( p < 0.05 \), respectively). There was no significant difference between Group L and Group H, suggesting that low dose of azilsartan had enough effect for suppression of cardiac remodeling after MI.

**Expression of Fibrotic Genes after MI** We determined the mRNA expression levels of collagen type 1 and TGF-\( \beta \) by RT-PCR (Fig. 4). The expression levels of collagen type 1 and TGF-\( \beta \) were increased by MI compared to sham-operated mice (Group C vs. sham, \( p < 0.05 \), respectively), and these levels were decreased similarly in the both Group L and Group H compared to Group C (\( p < 0.01 \) and \( p < 0.05 \), respectively). There was no significant difference between Group L and Group H.

**DISCUSSION**

Excessive RAAS activation leads to hypertension which is a major risk factor for cardiovascular disease morbidity and mortality. The presence and extent of coronary artery disease accelerate the progression of heart failure, and ischemic heart failure is associated with shorter survival than non-ischemic heart failure.\(^{15}\) After acute MI, loss of functioning myocytes occurs, with ensuing myocardial fibrosis, LV dilatation and neurohumoral activation including catecholamine and RAAS. The resulting neurohumoral activation and LV remodeling

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**Table 2. Echocardiographic Data at Two Weeks after MI Operation**

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Group C</th>
<th>Group L</th>
<th>Group H</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVSd (mm)</td>
<td>0.87±0.04</td>
<td>0.55±0.04**</td>
<td>0.65±0.07**</td>
<td>0.69±0.04**</td>
</tr>
<tr>
<td>LVPWd (mm)</td>
<td>0.87±0.04</td>
<td>0.71±0.06</td>
<td>0.84±0.04</td>
<td>0.85±0.10</td>
</tr>
<tr>
<td>LVEDd (mm)</td>
<td>3.06±0.09</td>
<td>3.92±0.14**</td>
<td>3.47±0.06**</td>
<td>3.50±0.07**</td>
</tr>
<tr>
<td>FS (%)</td>
<td>50.3±2.45</td>
<td>22.5±1.79**</td>
<td>28.8±1.41**</td>
<td>36.1±3.20***</td>
</tr>
</tbody>
</table>

IVSd, intra ventricular septum diameter; LVPWd, left ventricular posterior wall diameter; LVEDd, left ventricular end-diastolic dimension; FS, fractional shortening. All data are shown as mean±S.E. (\( n=6-8 \) per group). *\( p < 0.05 \) and **\( p < 0.01 \) vs. sham-operated mice; *\( p < 0.05 \) and **\( p < 0.01 \) vs. Group C, respectively.

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**Fig. 4. Fibrotic Gene Expression Levels at 2 Weeks after MI by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)**

Each expression levels were normalized to the GAPDH levels and expressed as fold increase over the levels in the sham-operated mice. (A) collagen type 1 and (B) transforming growth factor-\( \beta \). *\( p < 0.01 \) vs. sham-operated mice. *\( p < 0.05 \), **\( p < 0.01 \) vs. Group C, respectively. Results are expressed as mean±S.E. (\( n=5-6 \)).
lead to progressive deterioration of the remaining viable myocardium. Although revascularization with thrombolytic agents or PCI has been shown to significantly decrease mortality in post-MI patients, it is important to note that LV remodeling may occur despite the persistence of patency of the infarct-related artery. Therefore pharmacotherapy for suppression of neurohumoral activation is essential for preventing LV remodeling after MI, and several large-scale clinical trials revealed significance of angiotensin receptor antagonists, ARBs. The Val-HeFT (Valsartan Heart Failure Trial) showed that valsartan significantly reduced the mortality and morbidity, and improved clinical signs and symptoms in heart failure patients, especially those who had low cardiac systolic function. Other trials reported that ARBs were as beneficial as ACE inhibitors in decreasing the rate of mortality, morbidity, and recurrent infarction in patients with MI complicated by LV systolic dysfunction.

Azilsartan medoxomil is a prodrug that is quickly hydrolyzed to the active moiety azilsartan. Azilsartan has a potent and highly selective AT1 receptor blocking effect with estimated bioavailability of 60% and elimination half-life of 12h, resulting that has long-lasting antihypertensive effect. A maximal dose of azilsartan medoxomil showed superior efficacy to both olmesartan and valsartan at their maximal, approved doses without increasing adverse events, and a dose of 40mg or 80mg of azilsartan had greater efficacy of blood pressure lowering than a 320mg dose of valsartan, the maximum approved dose for this drug. These findings demonstrated that the new ARB azilsartan might be more effective for decreasing blood pressure and safer compared to other ARBs. The long-lasting antihypertensive effect of azilsartan has been proven by the tight binding to and the slow dissociating from AT1 receptor compared with other ARBs by in vitro study. However, there was no report on the azilsartan’s effects to prevent cardiac remodeling after MI.

There were several reports showing that following experimental MI in rats, renin, ACE, and AT1 receptor expression is significantly increased at the infarcted myocardium, coincident with inflammatory response and the initial accumulation of fibrillar collagen. Moreover, elevated angiotensin II concentration is found at the infarct site, suggesting that the cardiac RAAS rather than the circulating system may play an important role in myocardial remodeling after MI. The first component to local angiotensin II generation is provided by activated macrophages, and in an autocrine manner, macrophage-derived angiotensin II stimulates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase expression and reactive oxygen species (ROS) production. Excessive ROS production in the infarcted heart provides mitochondrial DNA damage and defects in the electron transport function, which leads to progression of LV remodeling and heart failure. Moreover, cardiac angiotensin II also influences the fibrous tissue formation by autocrine and paracrine manner at the site remote to the myocardial infarcted area. A classical ARB drug, losartan, significantly reduced cardiac fibrotic change at the site remote to the myocardial infarcted area compared to non-treated MI mice. These results suggest that not only circulating angiotensin II but also local cardiac RAAS is an important therapeutic target for preventing cardiac remodeling after MI. Moreover, excessive circulating angiotensin increases vascular tone, and hypertension accelerates cardiac remodeling after MI. In this study, a low dose of azilsartan attenuated cardiac remodeling after MI independently from blood pressure lowering. The results in Group H (high dose of azilsartan) might have been influenced by the blood pressure lowering effect. On the other hand, the same effect in Group L (low dose of azilsartan) might demonstrate directly the suppressive effect of cardiac RAAS. Low dose azilsartan without blood pressure lowering might have a sufficient effect on the suppression of cardiac remodeling after MI.

CONCLUSION

In the present study, cardiac remodeling evaluated by histological examination and echocardiography after MI was attenuated by administration of the new ARB, azilsartan. This effect was observed in low dose administration of azilsartan without blood pressure lowering.

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