Should increasing the dose or adding an AT₁ receptor blocker follow a relatively low dose of ACE inhibitor initiated in acute myocardial infarction?

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Abstract

Objective: Based on currently available clinical evidence, we should use high-dose angiotensin converting enzyme inhibitor (ACE-I) for patients with acute myocardial infarction (MI), initiating it at incremental doses to avoid excessive hypotension. Recent animal studies with acute MI models failed to demonstrate the superiority of the combination therapy of ACE-I and angiotensin receptor blocker (ARB) to high-dose ACE-I treatment with comparable blood pressure reductions, which however might be attributed to the initiation of the targeted doses from the beginning. The aim of this study was to compare the effect of increasing the dose of ACE-I with that of adding ARB following a relatively low dose of ACE-I on the survival and left ventricular (LV) remodeling after MI.

Methods: Rats underwent left coronary artery ligation and were treated with either ACE-I temocapril (5 mg/kg/day) or vehicle for 2 weeks, which was initiated 3 days after the surgery. The rats treated with temocapril were further randomly assigned to receive either high-dose temocapril (10 mg/kg/day) or combination therapy (temocapril 5 mg/kg/day + olmesartan 2.5 mg/kg/day), which was continued for another 6 weeks.

Results: Both treatments similarly reduced the blood pressure, improved survival and ameliorated LV enlargement. In contrast, several parameters of LV function were significantly ameliorated only by the high-dose ACE-I but not by the combination therapy.

Conclusions: After the initiation of a relatively low dose of ACE-I in acute MI, increasing the dose of ACE-I or adding ARB may equally improve survival and LV remodeling in the setting of an equal hypotensive effect. Further study with a longer treatment protocol is required to determine whether the several favorable effects on LV function elicited only by the high-dose ACE-I treatment provide further beneficial effects on survival and LV remodeling compared with the combination therapy.

Keywords: ACE inhibitors; Infarction; Remodeling; Renin angiotensin system; Ventricular function

1. Introduction

Several earlier clinical trials demonstrated that initiating angiotensin converting enzyme inhibitors (ACE-I) early after acute myocardial infarction (MI) is beneficial in terms of survival and cardiovascular events [1,2]. Although monotherapy with angiotensin type 1 receptor blocker (ARB) has been suggested to elicit similar effects as ACE-I on mortality and morbidity in patients with chronic heart failure [3], in a more recent study regarding high-risk patients after acute MI [4], all-cause mortality tended to be higher in patients treated with losartan than in those treated with captopril. In the Val-HeFT study [5], the addition of valsartan to standard heart failure therapy including ACE-I...
significantly improved the combined end-point of mortality and morbidity in patients with chronic heart failure. The subgroup analysis, however, revealed that the addition of valsartan elicits beneficial effects predominantly in patients with nonischemic cardiomyopathy rather than in patients with coronary heart disease.

In an experimental animal study, Kim et al. demonstrated that combined administration of ACE-I and ARB improved survival and diastolic dysfunction in Dahl salt-sensitive hypertensive rats more effectively than either high-dose ACE-I or high-dose ARB therapy alone despite comparable hypotensive effects [6]. In contrast, Mankad et al. [7] and Cavasin et al. [8] have shown no additional benefit on survival and LV remodeling from the combination therapy compared with high dose ACE-I treatment, both of which were initiated 2–7 days after the onset of acute MI and elicited similar reductions in blood pressure. The difference in the animal models, chronic hypertensive and acute MI models, might explain at least partially the conflicting results.

As the superiority of ARB has not been proved in patients with acute MI, we should use high dose ACE-I [9] initiating it at incremental doses to avoid excessive hypotension in the acute phase of MI [10]. It is not known, however, whether adding ARB to ACE-I, which is initiated in the acute phase of MI, is more beneficial than increasing the dose of ACE-I because there is no currently available evidence. Furthermore, the failure to demonstrate the superiority of combination therapy to high-dose ACE-I treatment in acute MI models in the earlier experimental studies [7,8] might be attributed to the initiation of the targeted doses from the beginning. To address these questions, we compared the effect of increasing the dose of ACE-I with that of adding ARB on the survival and LV remodeling after MI following a relatively low dose of ACE-I in acute MI.

2. Methods

2.1. Materials

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and was approved by the ethical committee on Animal Experiments of Tohoku University school of Medicine. The proximal portion of the left coronary artery was ligated in 8-week-old male Wistar rats as described earlier by Pfeffer et al. [11] and modified by us [12,13]. ACE-I temocapril hydrochloride (temocapril) and ARB olmesartan medoxomil (olmesartan) [14] were kind gifts from Sankyo (Tokyo, Japan).

2.2. Treatment protocols

The treatment protocol is shown in Fig. 1. Rats were treated with either ACE-I temocapril (5 mg/kg/day) or vehicle for 2 weeks, which was initiated 3 days after the coronary ligation. The rats treated with temocapril were then further randomly assigned to receive either high-dose temocapril (10 mg/kg/day) or combination therapy (temocapril 5 mg/kg/day + ARB olmesartan 2.5 mg/kg/day). The treatment with high-dose temocapril or the combination therapy was continued for another 6 weeks.

In our preliminary study, temocapril 5 mg/kg/day initiated 3 days after the coronary ligation and continued for 8 weeks significantly lowered the blood pressure, elicited a favorable but not significant trend with regard to survival, but did not ameliorate LV remodeling (LV weight and LV pressure–volume relationship) and LV dysfunction (echocardiography) compared with MI rats treated with vehicle (data not shown). We chose 2.5 mg/kg/day of ARB olmesartan to add to 5 mg/kg/day of temocapril for the combination therapy because 2.5 mg/kg/day of olmesartan+5 mg/kg/day of temocapril was equivalent to 10 mg/kg/day of temocapril in terms of lowering the blood pressure in MI rats.

2.3. LV pressure measurement

Invasive hemodynamic measurements were made at the end of the 8-week treatment protocol. Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and LV pressure was measured by a 2F micropip catheter transducer (SPC-320, Millar Instruments, Houston, TX, USA) inserted from the right carotid artery. The hemodynamic measurements were recorded and analyzed by a
data recording and analysis system (MacLab, ADInstruments Pty., Castle Hill, Australia).

2.4. Pressure–volume relationship

The LV pressure–volume relationship was obtained as reported earlier by Isoyama et al. [16] using isolated hearts at 4 °C with stepwise increments of 0.05 ml of cold saline at intervals of 15 s up to 50 mmHg of LV pressure. This procedure was repeated three times within 15 min of cardiac arrest and showed that the results were reproducible.

2.5. Histology

After weighing the heart, it was sectioned transversely into four 2-mm slices from the apex to base and fixed with neutral buffered 10% formalin and embedded in paraffin. A section (4 μm) was cut from each slice and stained with hematoxylin–eosin and Masson-trichrome. Details for infarct size measurement using computer-assisted planimetry are described elsewhere [17] by us as well as by Pfeffer et al. [11]. The myocyte width in noninfarcted myocardium (ten cells for each of four slices taken from a heart) was measured as described previously [17]. The myocardial collagen volume fraction (percent fibrosis) was determined by quantitative morphometry of the sirius-red stained sections as described previously [18]. The percent fibrosis determined by this morphometric approach has been shown to be correlated with the hydroxyproline concentration of the myocardium [19]. In brief, the areas of red-staining collagen fibers were digitized using a software system (Fuji, Tokyo, Japan) based on their color emission. The percent area of fibrosis was calculated as the sum of all connective tissue areas (excluding the infarcted and perivascular areas) divided by the total areas (excluding the infarcted and perivascular areas) in the visual field. Five fields were evaluated in each of four sections, and the values were averaged to obtain the collagen volume fraction of each heart.

2.6. Echocardiography

Transthoracic echocardiographic studies were performed in a separate group of rats at 8 weeks after the initiation of the treatment using an echocardiographic system equipped with a 10-MHz phased-array transducer (Toshiba, Tokyo, Japan) as described by Litwin et al. [20]. In brief, rats were lightly anesthetized with intraperitoneal injection of ketamine HCl (25–50 mg/kg) and xylazine (5–10 mg/kg). M-mode tracings were recorded to measure LV end-diastolic and end-systolic dimensions, and LV posterior wall thickness at end-diastole. Pulse-wave Doppler spectra (E and A waves) of mitral inflow were recorded using the apical 4-chamber view.

2.7. Neurohormonal profiles

Just before the hearts were isolated, the abdominal aorta was punctured and blood was withdrawn into precooled tubes, centrifuged at 4 °C, and the plasma was stored at −80 °C until analysis. The plasma levels of atrial natriuretic peptide (ANP) and norepinephrine were determined using immunoradiometric assay and high-performance liquid chromatography, respectively. The plasma level of bradykinin was determined by radioimmunoassay–polyethylene glycol method.

2.8. RNA dot blot analysis

Total RNA was extracted from noninfarcted LV of three MI rat groups as well as from LV of sham-operated rats. The total RNA was applied (3 μg/dot) to nylon membranes. The membranes were hybridized with 32P-labeled antisense oligonucleotide (β-myosin heavy chain) and cDNA (ANP, transforming growth factor-β, prepro-endothelin, collagen type I and III, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH)) probes and quantified using a phosphoimager. GAPDH was used as an internal standard.

2.9. Statistical analysis

We used the software for statistics (Statview version 4.5, SAS Institute San Francisco, CA, USA) for the statistical analysis. The data from the time course of the tail-cuff blood pressure and pressure–volume relationship were analyzed by two-way ANOVA with repeated measures. For other data, statistical significance was determined by one-way ANOVA followed by Fisher’s exact test. Survival were analyzed by the standard Kaplan–Meier analysis with log-rank test. Data are expressed as mean±S.E.M. A value of P<0.05 was considered significant.

3. Results

3.1. Survival rate

For the protocols to investigate the survival rate, tail-cuff blood pressure, LV pressure measurement, LV pressure–volume relationship, and gene expression, 94 rats underwent coronary artery ligation. Before they were randomized to the three treatment groups, ten died within 3 days of the surgery. The early mortality rate of 10.6% was almost identical to that in our previous study [17]. The number of rats that survived until the end of the 8-week treatment protocol was 18 in the vehicle group (69.2%), 26 in the high-dose ACE-I group (92.9%), and 27 in the combination therapy group (90.0%). The Kaplan–Meier survival curve during the 8-week treatment protocol showed that both the high-dose ACE-I and combination treatments in MI rats significantly and similarly improved
3.2. Blood pressure

The tail-cuff systolic blood pressure measured once a week during the 8-week treatment protocol was significantly and equally decreased in both treatment groups compared with the vehicle group (Fig. 3). The heart rate did not differ among the three groups during the treatment (data not shown).

3.3. LV pressure measurement

The LV pressure measurement was performed just before the isolation of the hearts in the rats examined for the LV pressure–volume relationship. Both the high-dose ACE-I and combination treatments significantly lowered the LV peak-systolic pressure compared with the vehicle group (Table 1). The LV end-diastolic pressure (LVEDP) was significantly lower in the high-dose ACE-I group, but not in the combination therapy group, than in the vehicle group. Although LV +dP/dt\text{max} was similar among the three groups, that corrected for LV pressure [21–23] was significantly increased in the high-dose ACE-I group, but not in the combination therapy group, compared with the vehicle group. Compared with the vehicle group, LV –dP/dt\text{min} corrected for LV pressure [21,24] was significantly decreased in both treated groups, and \( \tau \), a time constant of the deceleration of LV pressure was significantly improved in the high-dose ACE-I group but not in the combination therapy group.

3.4. LV pressure–volume relationship

LV pressure–volume curves obtained using isolated hearts showed a significant and similar leftward shift in both treatment groups compared with the vehicle group, indicating that both treatments suppressed LV enlargement significantly and equally (Fig. 4).

<table>
<thead>
<tr>
<th>Hemodynamic data</th>
<th>Vehicle</th>
<th>ACE-I</th>
<th>Combination</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>12</td>
<td>16</td>
<td>17</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>418±13</td>
<td>426±12</td>
<td>433±9</td>
</tr>
<tr>
<td>Mean aortic pressure (mmHg)</td>
<td>100.8±3.6</td>
<td>86.6±3.8*</td>
<td>85.2±3.2*</td>
</tr>
<tr>
<td>LV peak systolic pressure (mmHg)</td>
<td>110.5±4.1</td>
<td>97.8±3.8*</td>
<td>94.1±3.1*</td>
</tr>
<tr>
<td>LV end-diastolic pressure (mmHg)</td>
<td>13.4±2.8</td>
<td>4.5±1.8</td>
<td>7.1±2.2</td>
</tr>
<tr>
<td>LV +dP/dt\text{max} (mmHg/s)</td>
<td>5267±255</td>
<td>5146±277</td>
<td>4683±259</td>
</tr>
<tr>
<td>LV –dP/dt\text{min} (mmHg/s)</td>
<td>143.9±22.5</td>
<td>212.1±22.0†</td>
<td>159.5±14.4</td>
</tr>
<tr>
<td>LV (+dP/dt/P)\text{max} (1/s)</td>
<td>3363±151</td>
<td>3503±164</td>
<td>3233±154</td>
</tr>
<tr>
<td>LV (–dP/dt/P)\text{min} (1/s)</td>
<td>70.1±11.6</td>
<td>144.2±11.0*</td>
<td>118.5±13.9†</td>
</tr>
<tr>
<td>( \tau ) (ms)</td>
<td>16.6±1.1</td>
<td>13.3±0.6*</td>
<td>14.9±0.9</td>
</tr>
</tbody>
</table>

ACE-I, angiotensin converting enzyme inhibitor; LV, left ventricular; LV + or –dP/dt\text{max or min}, the peak positive or negative first derivative of LV pressure; LV (+ or –dP/dt/P)\text{max or min}, the peak positive or negative first derivative of LV pressure corrected for LV pressure; \( \tau \), time constant of the deceleration of LV pressure. Values are mean±S.E.M.; *, \( P<0.01; \) †, \( P<0.05 \) vs. Vehicle.
3.5. MI size, heart weight, collagen volume fraction, and myocyte width

MI size determined at the end of the 8-week treatment protocol was similar among the 3 treatment groups (Table 2). Heart weight:body weight and heart weight:tibial length were significantly and equally reduced by 14% in both treatment groups compared with the vehicle group. Both the high-dose temocapril and the combination therapies significantly and similarly reduced the collagen volume fraction and the myocyte width in noninfarcted myocardium compared with the vehicle group.

3.6. Echocardiography

Table 3 shows the echocardiographic data obtained at the end of the 8-week treatment protocol. There was a trend favoring the high-dose ACE-I therapy over the combination therapy with respect to decreasing the LV end-diastolic dimension (LVIDd), but it was not statistically significant. Only increasing the dose of ACE-I, but not adding ARB, significantly improved the LV fractional shortening (LVFS) compared with the vehicle group. Both treatments significantly improved the E/A ratio compared with the vehicle group. The deceleration of E wave was significantly slower in the high-dose ACE-I group and tended to be slower in the combination therapy group ($P=0.07$) compared with the vehicle group.

3.7. Plasma concentrations of ANP, norepinephrine, and bradykinin

The measurement of the plasma concentrations of ANP and norepinephrine were performed in rats examined for the LV pressure–volume relationship. There were no differences in the plasma concentrations of ANP and norepinephrine among the three groups of MI rats (Table 4). The plasma concentration of bradykinin was determined in the rats subject to echocardiography. This was because we could not obtain a sufficient volume of blood from one rat to analyze these three parameters. Both the high-dose temocapril and combination therapies significantly and equally increased the plasma level of bradykinin compared with the vehicle treatment.

3.8. RNA dot blot analysis

RNA dot blot analysis was performed in the rats of the survival study that were not assigned to the LV pressure–volume study. Fig. 5 and Table 5 show the results of the RNA dot blot analysis in noninfarcted viable LV myocardium.
Table 4
Neurohormonal profile

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>ACE-I</th>
<th>Combination</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>4–9</td>
<td>6–13</td>
<td>6–10</td>
</tr>
<tr>
<td>Atrial natriuretic peptide (pg/ml)</td>
<td>2.47±0.58</td>
<td>2.52±0.47</td>
<td>1.90±0.60</td>
</tr>
<tr>
<td>Norepinephrine (ng/ml)</td>
<td>460±65</td>
<td>406±32</td>
<td>422±91</td>
</tr>
<tr>
<td>Bradykinin (pg/ml)</td>
<td>20.1±0.8</td>
<td>26.8±1.2*</td>
<td>30.5±2.0†</td>
</tr>
</tbody>
</table>

ACE-I, angiotensin converting enzyme inhibitor; values are mean±S.E.M.
*, P<0.05, †, P<0.01 vs. Vehicle.

Fig. 5. Representative mRNA dot blot analysis of the noninfarcted viable LV myocardium taken at the end of 8-week treatment protocol, as well as of the LV myocardium from sham-operated rats. Results of quantitative analysis are shown in Table 5.

The β-myosin heavy chain and ANP mRNA levels in the vehicle group were significantly higher than those in the sham-operated rats. Both the high-dose temocapril and combination therapies significantly reduced the β-myosin heavy chain mRNA level, and tended to decrease the ANP mRNA level (P=0.06) compared with the vehicle. Transforming growth factor-β and prepro-endothelin mRNA levels in the vehicle group were significantly upregulated compared with the sham-operated rats, and were completely normalized by either treatment. Both collagen types I and III mRNA levels were significantly higher in the vehicle group than in the sham-operated rats. Increasing

Table 5
mRNA dot blot analysis in noninfarcted LV myocardium

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Vehicle</th>
<th>ACE-I</th>
<th>Combination</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>6</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>β-Myosin heavy chain</td>
<td>1.00±0.05</td>
<td>1.99±0.03*</td>
<td>1.13±0.04†</td>
<td>1.02±0.04†</td>
</tr>
<tr>
<td>Atrial natriuretic peptide</td>
<td>1.00±0.12</td>
<td>4.77±0.81*</td>
<td>3.04±0.82†</td>
<td>2.99±0.40†</td>
</tr>
<tr>
<td>Transforming growth factor-β</td>
<td>1.00±0.04</td>
<td>1.35±0.06*</td>
<td>0.92±0.04†</td>
<td>0.92±0.04†</td>
</tr>
<tr>
<td>Prepro-endothelin</td>
<td>1.00±0.08</td>
<td>1.33±0.07*</td>
<td>0.94±0.07†</td>
<td>0.88±0.06†</td>
</tr>
<tr>
<td>Collagen type I</td>
<td>1.00±0.06</td>
<td>1.40±0.08*</td>
<td>1.21±0.06†</td>
<td>1.15±0.04†</td>
</tr>
<tr>
<td>Collagen type III</td>
<td>1.00±0.11</td>
<td>1.50±0.17*</td>
<td>1.02±0.05†</td>
<td>1.20±0.12</td>
</tr>
</tbody>
</table>

ACE-I indicates angiotensin converting enzyme inhibitor. The mRNA levels were corrected for GAPDH mRNA levels in individual rats. Values are mean±S.E.M.
*, P<0.01; †, P<0.05 vs. Sham; ‡, P<0.01, §, P<0.05 vs. Vehicle.
the dose of ACE-I significantly decreased both the collagen types I and III mRNA levels compared with the vehicle group, while adding ARB significantly reduced the collagen type I mRNA level but not the collagen type III mRNA level.

4. Discussion

The present experimental study demonstrated that increasing the dose of ACE-I or adding ARB similarly improved the survival and ameliorated LV remodeling in rats first treated with a relatively low dose of ACE-I that was initiated 3 days after the onset of MI.

Mankad et al. [7] and Cavasin et al. [8] have shown that there was no additional benefit in terms of survival and LV remodeling from the combination therapy with ACE-I and ARB compared with high-dose ACE-I treatment, both of which were initiated 2–7 days after the onset of acute MI and elicited similar reductions in blood pressure. We hypothesized that this failure to demonstrate the superiority of the combination therapy to the high-dose ACE-I treatment in these experimental studies might be attributed to the initiation of the targeted doses from the beginning of the combination therapy in the relatively early phase of acute MI. In the present study, therefore, the treatment was initiated with a relatively low dose of ACE-I which had been confirmed in our preliminary experiment to provide a favorable but not significant trend with regard to survival but no beneficial effects in terms of LV remodeling and LV function despite a significant reduction in blood pressure. We increased the dose of ACE-I or added ARB after the 2-week initial treatment with a relatively low dose of ACE-I. The results, however, indicated that there was no additional benefit in terms of survival and LV remodeling from adding ARB compared with increasing the dose of ACE-I. The results in the present study may not be in agreement with the report by Kim et al. [6] which demonstrated that combined administration of ACE-I and ARB improved LV diastolic dysfunction and survival in Dahl salt-sensitive hypertensive rats more effectively than either treatment alone did despite comparable hypotensive effects. The difference in the models, chronic hypertensive and acute MI models, might explain partially at least the different results. Several investigators reported favorable effects of the combination therapy with ACE-I and ARB compared with ACE-I treatment alone in rat models of MI [25,26]. The dose of ACE-I employed in the combination therapy group, however, was the same as that in the ACE-I group, which might elicit different levels of inhibition of the renin–angiotensin system as well as those of the afterload reduction.

The Val-HeFT study was a randomized clinical trial that evaluated the long-term effects of the addition of the ARB valsartan to standard therapy including ACE-I in patients with chronic heart failure [5]. The incidence of the combined end point of mortality and morbidity was significantly lower with valsartan than with the placebo. In this clinical trial, however, the dose of ACE-I was not increased in the placebo group although valsartan was added to the other group. The result might have been different if the dose of ACE-I had been further increased in the patients not assigned to the valsartan group. Furthermore, the subgroup analysis revealed that the addition of valsartan elicits beneficial effects predominantly in patients with nonischemic cardiomyopathy rather than in patients with coronary artery disease. It is possible, therefore, that the combination therapy with ACE-I and ARB may be effective predominantly in nonischemic LV dysfunction.

The previous meta-analysis [27] demonstrated that treatment with ACE-I should be initiated as early as within 24–36 h of the onset of MI to obtain maximal benefits if the patients are not suffering from hypotension. We, therefore, might have saved more rats with MI by initiating the treatment within 24–36 h of the surgery, and the results might have been different. In contrast, however, we might have lost more rats with severe hypotension by the treatment with ACE-I within 24–36 h of the surgery. This was the reason that we initiated the treatment 3 days after the coronary ligation.

The Kaplan–Meyer curves (Fig. 2) suggest that the initial treatment with 5 mg temocapril contributed more to the survival during the 8-week protocol than 5 mg of temocapril or 2.5 mg of olmesartan added to the initial treatment did, although our preliminary study failed to show a significant survival benefit with 5 mg of temocapril for 8 weeks. We believe, however, that the addition of 5 mg of temocapril or 2.5 mg of olmesartan greatly contributed to the amelioration of LV remodeling, because, in our preliminary study, 5 mg of temocapril administered for 8 weeks decreased LV weight/body weight by only 4%, which was not statistically significant.

The echocardiographic study demonstrated that only increasing the dose of ACE-I, but not adding ARB, improved the depressed LVFS in rats with MI compared with the vehicle group. The mechanism for this favorable effect of the high-dose ACE-I treatment on LVFS is not clear. As the systolic blood pressure was significantly but equally reduced in both treatment groups compared with the vehicle group, the result cannot be explained by a difference in the degree of the afterload reduction. ACE-I increases the level of bradykinin [28] which has been shown to elicit protective effects through an NO-dependent mechanism in myocardium subject to ischemia [29]. In the present study, the plasma bradykinin level was significantly but equally increased in both treatment groups compared with the vehicle group. Therefore, the improvement of the LVFS elicited only by the high-dose ACE-I therapy cannot be explained by differences in the plasma bradykinin levels. However, we cannot exclude the possibility that the high-dose ACE-I might elicit a higher tissue bradykinin level in myocardium than the combination
therapy did. In contrast to the result from echocardiography, the LV pressure measurement demonstrated similar $\text{LV} + \frac{dP}{dt_{\max}}$ among the three study groups. Similarly conflicting results in terms of $\text{LV} + \frac{dP}{dt_{\max}}$ and LVFS were reported by Litwin et al. [21]. As $\text{LV} + \frac{dP}{dt_{\max}}$ is highly sensitive to LV preload [22,23], we corrected LV $+ \frac{dP}{dt_{\max}}$ for LV pressure [21–23] and found that it was very consistent with the LVFS obtained by echocardiography (Table 3). These data suggest that LV systolic function was significantly ameliorated by the high-dose ACE-I treatment but not by the combination therapy.

Negative $\frac{dP}{dt_{\min}}$ corrected for LV pressure [21,24] was significantly decreased in the two treatment groups compared with the vehicle group. The $\tau$ value was significantly decreased in the high-dose ACE-I group, but not in the combination therapy group, compared with the vehicle group. The E/A ratio was significantly decreased in both treated groups, and the deceleration of E wave was significantly slower in the high-dose temocapril group and tended to be slower in the combination therapy group than in the vehicle group. These observations strongly suggest that LV filling was impaired in the vehicle group and that it was ameliorated in the both treatment groups, especially in the high-dose ACE-I group.

The pressure–volume curves in the present study indicate that both treatments equally attenuated LV enlargement compared with no treatment. In contrast, LVEDP was significantly lower only in the high-dose ACE-I group, but not in the combination therapy group, than in the vehicle group. These data suggest that LV in the high-dose ACE-I group was more unloaded in terms of preload than that in the combination therapy group. One possible explanation might be that different degrees of dilation of the venules between the two treatment groups elicited different LV preload levels. These data from the LV pressure–volume relationship combined with those of LVEDP, however, may conflict with the data from the echocardiography, which demonstrated no significant difference in LVIDd among the three groups. Mulder et al. [30] reported that bosentan significantly ameliorated LV dilatation in rats with MI as evidenced by a significant shift of the LV pressure–volume curve, significantly lowered LVEDP, but did not decrease LVIDd as measured by echocardiography. In our experiment as well as in that by Mulder et al. the pressure–volume curves of the treated group(s) were closer to those of the vehicle group around the pressure of LVEDP than at the higher LV pressure. This might explain why echocardiography failed to demonstrate a difference in LVIDd between the treated and untreated groups. Another possible explanation might be the relatively low number of rats subject to echocardiography compared with those subject to catheterization and the pressure–volume relationship. The results might be different with more echocardiographic data with regard to LVIDd. LV ventricles in the treated groups seemed to be stiffer than in the untreated group, especially at the higher LV pressure (Fig. 4). Pfeffer et al. [31] reported that chronic captopril treatment not only attenuated the LV dilation but also increased the LV chamber stiffness toward the values in noninfarcted rat ventricles. Ventricular chamber stiffness is related directly to myocardial stiffness and inversely to ventricular volume [31]. Inhibition of the renin–angiotensin system in rats with MI may prevent ventricular dilatation enough to prevent the resultant reduction in chamber stiffness.

It is well known that fetal genes are predominantly expressed in noninfarcted viable myocardium in infarcted hearts, and that both ACE-I and ARB treatments suppress the expression of these genes [32]. The present study confirmed the findings reported earlier. In our present study, both the high-dose ACE-I and combination therapies similarly decreased the collagen deposition and collagen type I mRNA level in the noninfarcted myocardium. In contrast, the high-dose ACE-I treatment, but not the combination therapy, significantly decreased the collagen type III mRNA level in the noninfarcted myocardium compared with the vehicle group. Collagen type I fibers have substantial tensile strength, whereas type III collagen fibers possess a resilience that is ideal for maintaining structural integrity and distensibility of the network [33]. Because of their different physical properties, alterations of the amounts may have a major impact on the diastolic and systolic function of the heart. As the deposition of each type of collagen depends on both the synthesis and degradation rates, further study is needed to determine the implication of the effect of the high-dose ACE-I treatment on the LV collagen type III mRNA level.

There are some limitations in the present study. First, we investigated only one protocol in terms of the doses of ACE-I and the combination as well as the duration of the treatment. We cannot exclude the possibility, therefore, that the results would have been different if we had employed different doses of ACE-I and ARB or a different treatment schedule. We chose 5 mg/kg/day temocapril to initiate the inhibition of ACE in the acute phase of MI because, in our preliminary experiment, this dose of temocapril elicited a significant but not a profound decline in arterial blood pressure in rats with acute MI. Furthermore, as described before, the treatment with 5 mg/kg/day temocapril for 8 weeks elicited only a favorable but not a significant trend with regard to survival, and did not ameliorate the LV remodeling and LV dysfunction. We chose 2.5 mg/kg/day olmesartan, which was added to 5 mg/kg/day temocapril, for the combination therapy group. Earlier studies by others [34,35] demonstrated that 1 mg/kg olmesartan is almost equivalent to 5 mg/kg temocapril in terms of the hypotensive effect in spontaneous hypertensive rats. We believe, therefore, that the 2.5 mg/kg/day olmesartan was sufficient to add to the combination group instead of 5 mg/kg/day temocapril which was added in the high-dose ACE-I group. Second, human hearts contain a dual pathway of angiotensin II formation in which the major angiotensin II-forming enzymes are ACE and chymase.
[36]. The results of the present study, therefore, cannot be simply extrapolated to the clinical settings.

In conclusion, following the initiation of a relatively low dose of ACE-I after MI, increasing the dose of ACE-I and adding ARB may equally improve survival and ameliorate LV enlargement in the setting of an equal reduction in arterial blood pressure. Several parameters of LV systolic and diastolic function, however, were ameliorated only by the high-dose ACE-I treatment but not by the combination therapy. Further study with a longer treatment protocol is required to determine whether the favorable effects on LV function elicited only by the high-dose ACE-I treatment provide further beneficial effects in terms of survival and LV remodeling compared with the combination therapy.

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