Jpn. J. Pharmacol. **86**, 203 – 214 (2001)

Possible Roles of Cardiac Chymase After Myocardial Infarction in Hamster Hearts

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Received July 5, 2000 Accepted March 13, 2001

ABSTRACT—The significance of cardiac chymase after myocardial infarction (MI) was evaluated using a hamster model of MI. At 1, 3, 7, 14, 28 and 56 days after MI, tissues were removed for measurements of angiotensin-converting enzyme (ACE) and chymase activities. The mean infarct size 3 days after left coronary artery ligation was 47.3 ± 5.9% of the left ventricle circumference. The ratio of left ventricle weight to body weight was significantly increased from 3 days after MI. The level of plasma renin activity in the MI hamsters was significantly increased at the early phase of MI (1 – 3 days), while no significant changes in plasma ACE activity were observed. The ACE activity in the infarcted left ventricle was significantly increased starting from 3 days after MI and this increase was sustained up to 28 days. The chymase activity in the infarcted left ventricle was significantly increased starting from 1 day after MI and this increase was sustained up to 56 days. The number of chymase-positive mast cells in the infarcted left ventricle was significantly higher than in the sham group 3 and 7 days after operation. Treatment with an angiotensin (Ang) II type 1 receptor antagonist (candesartan cilexetil, 10 mg/kg per day) starting 3 days before the induction of MI significantly reduced the mortality rate during 14 days of observation following MI, whereas treatment with an ACE inhibitor (lisinopril, 20 mg/kg per day) did not. A significant improvement in hemodynamics (maximal negative and positive rates of pressure development, left ventricular systolic pressure and end-diastolic pressure, mean arterial blood pressure) was observed by the treatment with candesartan cilexetil, but not with lisinopril, 3 and 14 days after MI. These results suggested that Ang II produced by chymase may participate in the pathophysiologic state after MI in hamsters.

Keywords: Chymase, Angiotensin-converting enzyme, Angiotensin, Myocardial infarction

After myocardial infarction (MI), two different pathophysiological states may occur in the infarcted and non-infarcted compartments of the heart. In the infarcted myocardium, tissue-repairing processes are initiated after cardiac myocyte necrosis in order to preserve structural integrity and these processes commonly are associated with the activation of the local angiotensin-converting enzyme (ACE)-dependent angiotensin (Ang) II-generating system (1 – 3), suggesting that Ang II participates in this healing process. In the non-infarcted myocardium, cardiac hypertrophy appears to be due to the compensatory mechanism that attempts to maintain systemic perfusion and is favorable for the prognosis of MI (4). However, cardiac hypertrophy is usually accompanied by a deposition of extracellular matrix protein, and this adverse remodeling may lead to an increase in cardiac stiffness and a decrease in contractile behavior, and ultimately compensatory heart failure will develop into decompensatory heart failure (5). Whether or not this structural remodeling is associated with the activation of the local ACE-dependent Ang II-generating system is still controversial (1 – 3, 6). However, increasing evidence has confirmed the fact that the intervention of this Ang II action with ACE inhibitors or Ang II type 1 receptor antagonists improves cardiac function, and this structural remodeling leads to reduced mortality rates of patients (7, 8) as well as in animals (9 – 11). These findings indicate that Ang II plays an important role in this pathophysiological stage.

Recently, a chymase-dependent Ang II-forming pathway was found in human cardiovascular tissues (12). In an extract of human heart tissues, it was reported that chymase accounts for >90% of Ang II formation. However, the biochemical and physiological roles of chymase are different

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in various animal species (13). Human, monkey, dog and hamster chymases cleave the Phe^{8}-His^{9} bond of Ang I and produce Ang II efficiently (14 – 17), whereas rat chymase hydrolyzes the Tyr^{4}-Ile^{5} bond of Ang I, yielding inactive fragments (18). Previously, we reported that chymase activity was significantly increased in dog hypertrophied vessels after balloon injury (19) and during a cardiac fibrosis-developing period caused by chronic pressure overload (20) as well as a cardiomyopathy-developing period in hamsters (21), suggesting that Ang II formation accelerated by the local activation of chymase may play an important role in tissue remodeling. In fact, we recently obtained additional information on the relationship between activated chymase and tissue remodeling. In a dog jugular vein grafted to the carotid artery, we found that progressive proliferation of the grafted vein is mainly associated with an increase of chymase activity and chymase inhibitor treatment alone significantly prevented this proliferation (22), implying that the activation of chymase plays a major role in this structural remodeling.

So far, although the significance of the local activation of the ACE-dependent Ang II-generating system in MI has been most intensively studied, there is little or no information on the relationship between the chymase-dependent Ang II-forming pathway and MI. In this study, we performed a comparative investigation to clarify the time-dependent changes of heart chymase, ACE and plasma renin in experimental heart failure induced by the ligation of the left coronary artery (LCA) in hamsters. On the other hand, if the chymase-dependent Ang II-forming pathway participates in the pathophysiology after MI, we proposed that the effects of Ang II receptor blockade or ACE inhibition alone on the mortality rate and cardiac function will be different in this model. Therefore, we compared the effects of the Ang II type 1 receptor antagonist candesartan cilexetil and the ACE inhibitor lisinopril on the mortality rate and cardiac function during the acute phase of MI in hamsters.

MATERIALS AND METHODS

Candesartan cilexetil was kindly donated by Takeda Chemical Industries, Ltd. (Osaka). Lisinopril was purchased from Sigma (St. Louis, MO, USA).

Male Syrian hamsters (SLC, Shizuoka) aged 6 weeks and weighing 90 – 110 g were used. The experimental procedures for animals were conducted in accordance with the guidelines of Osaka Medical College for medical experiments approved by the ethics committee, which included outside members. The hamsters were fed regular hamster chow, had free access to tap water, and were housed in a temperature-, humidity- and light-controlled room.

Myocardial infarction

The animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and ventilated by positive pressure through an endotracheal tube attached to a small animal respirator (NEMI Scientific Inc., Medway, MA, USA). A left-sided thoracotomy was performed via the fourth intercostal space and the lungs were retracted to expose the heart. After opening the pericardium, the LCA was ligated near its origin using a 7-0 silk suture. Coronary ligation was considered successful when the anterior wall of the left ventricle (LV) turned pale, and then the thoracotomy site was closed in layers. Another group of hamsters underwent a sham ligation; a similar surgical procedure was applied, except that the suture was not tightened around the coronary artery (sham). After surgery, the hamsters received food and water ad libitum. It was reported that the mortality rate in rats within the first 48 h after MI was about 40% (6, 10), but in hamsters, it was less than 5%.

Preparation of tissue samples

Hamsters were sacrificed 1, 3, 7, 14, 28 and 56 days after MI. For determination of plasma renin activity (PRA) and plasma ACE activity, trunk blood was collected and the plasma was separated by centrifugation at 3000 rpm for 15 min at 4°C, and then 1-ml samples were stored at –80°C. For determination of heart chymase and ACE activities, the hearts were also harvested and divided into infarcted or non-infarcted (sham) LV, septum and right ventricle (RV), and then these tissues were frozen at –80°C until the biochemical assays. For the histopathological study, the hearts were harvested 3 and 7 days after MI, and four transverse slices, approximately 3-mm-thick, were cut from the apex to the base. These slices were fixed in methanol-Carnoy's fixative (60% methanol, 30% chloroform and 10% glacial acetic acid) and embedded in paraffin.

Histopathological examination and morphometric analysis

Four 5-μm serial sections were cut from each slice. To measure infarct size, every first section was stained with hematoxylin and eosin and every second section, with azan Mallory stain. To identify the distribution of chymase in the post-MI hearts, the third section was used for immunohistochemical staining. The sections were incubated with a 1:100 dilution of the anti-hamster chymase antibody (which was raised against rabbit by immunizing SPYVPWINIV IKASS, which is a C-terminal amino acid residue from position 212 to 226 of hamster chymase (21), kindly given to us by Otsuka Pharmaceutical Co., Ltd., Tokushima) for 1 h at room temperature, followed by reaction with a labelled streptavidin-biotin peroxidase kit (Dako LSAB kit; Dako Corporation, Carpinteria, CA, USA) with 3-amino-9-ethylcarbazole color development. The sections were faintly counterstained with hematoxylin. A positive control

study using hamster tongue tissue confirmed intense staining in chymase-positive mast cells. Additionally, the specificity and the results obtained with the anti-hamster chymase antibody were checked by omitting the primary antibody and using a non-immune rabbit serum (Dako Corporation) as the negative control. The neutralization of anti-hamster chymase antibody with the relevant peptide was performed on sections of hamster tongue tissue, and the positive cells disappeared after this neutralization (data not shown). The fourth section was stained with toluidine blue to identify mast cells.

The infarct size was determined in four slices from each heart on the azan Mallory-stained sections using a computerized morphometry system, MacSCOPE Ver 2.2 (Mitani Co., Fukui). Infarct size was expressed in percentage of the LV circumference. The numbers of mast cells and chymase-positive mast cells within the LV area of four slices obtained from each heart were quantified, using the computerized morphometry system, and expressed as the absolute number of stained mast cells per mm^2 of LV. The observer was blind to data regarding the treatment of the animal. Intraassay variance was determined on the basis of triplicate measurements. The coefficient of variation among measurements was 0.431.

Biochemical assays

The ACE and chymase activities were measured according to the methods described previously (22, 23). In brief, the tissues were minced and homogenized in 5 vol (w/v) of 20 mM Tris-HCl buffer, pH 8.3, containing 5 mM $Mg(CH_3COO)_2$, 30 mM KCl, 250 mM sucrose and 0.5% Nonidet P-40. The supernatant was used for the measurement of ACE and chymase activities.

ACE activity was measured by incubating the tissue extracts for 30 min at 37°C with 5 mM hippuril-His-Leu as a substrate in 100 mM phosphate buffer, pH 8.3, containing 600 mM NaCl (23). The enzyme reaction was terminated by addition of 3% metaphosphoric acid (w/v) and the reaction mixture was placed in ice water for 10 min. After centrifugation of the mixture at 15,000 rpm for 5 min, we applied 50 μl of the supernatant to an octadecyl silica reversed-phase column (4.6 mm × 25 cm; Tosoh, Tokyo), which had been equilibrated with 10 mM KH_2PO_4 and CH_3OH (1:1, pH 3.0), and eluted it with the same solution at a rate of 0.3 ml/min. Hippuric acid was detected by ultraviolet absorbance at 228 nm. One unit of ACE activity was defined as the amount of enzyme that cleaved 1 μM hippuric acid/min.

Chymase activity was measured by incubating the tissue extracts for 30 min at 37°C with 0.77 mM Ang I in 150 mM borax-borate buffer, pH 8.5, containing 5 mM ethylenediaminetetraacetic acid (EDTA), 8 mM bipyridine, and 0.77 mM diisopropylfluorophosphate (DFP), as described previously (22). A blank reaction was carried out with the addition of 0.5 mM chymostatin, a chymase inhibitor. The EDTA and bipyridine at these concentrations could sufficiently inhibit metalloproteases (such as ACE and carboxypeptidases) and aminopeptidases, respectively. The DFP at this concentration had no affect on chymase activity, but it could sufficiently inhibit other serine proteases (22). The enzyme reaction was terminated by addition of 15% trichloroacetic acid (w/v) and the reaction mixture was placed in ice water for 10 min. After centrifugation of the reaction mixture at 15,000 rpm for 5 min, we applied 50 μl of the supernatant to an octadecyl silica reversed-phase column (4.6 mm × 25 cm, Tosoh), which had been equilibrated with 30% methanol in 10 mM phosphoric acid, and eluted it with a linear gradient of 30 – 90% methanol in 10 mM phosphoric acid at a rate of 1 ml/min. Ang II was detected by ultraviolet absorbance at 226 nm. One unit of chymase activity was defined as the amount of enzyme that cleaved 1 μM Ang II/min.

The PRA was measured by radioimmunoassay of (^{125}I)-Ang I using an SRL kit (TFB, Tokyo). The protein concentration was measured by the bicinchoninic acid protein assay reagent (Pierce Chemical Co., Rockford, IL, USA) using bovine serum albumin as a standard.

Administration of lisinopril and candesartan cilexetil

Lisinopril (20 mg/kg) and candesartan cilexetil (10 mg /kg) were orally administered starting from 3 days before the induction of MI by gastric gavage once per day and continued for 3 or 14 days after MI. Three days after continuous treatment, lisinopril and candesartan cilexetil suppressed the pressor responses to Ang I (300 ng/kg, i.v.) and Ang II (100 ng/kg, i.v.) to 21.2 ± 11.2% and 24.4 ± 6.5%, respectively, as compared with the vehicle-treated group of anesthetized normotensive hamsters 24 h after final treatment. Both drugs also reduced blood pressure to a similar extent (lisinopril, 96 ± 9 mmHg, n = 3; candesartan cilexetil, 100 ± 6 mmHg, n = 3; vehicle, 110 ± 3 mmHg, n = 3). It has also been reported that both drugs at these dosages were high enough to improve cardiac function or survival rate in a rat MI model (10, 24 – 26).

Hemodynamic measurement

Hemodynamic measurements were performed in four groups (sham-operated hamsters, vehicle-, lisinopril- and candesartan cilexetil-treated MI hamsters) 3 and 14 days after operation. The hamsters were re-anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and the trachea was intubated. A polyethylene catheter that consisted of PE 10 tubing welded to PE 50 tubing (Clay Adams, Parsippany, NJ, USA) was introduced into the right carotid artery. Then, the catheter was connected to a pressure transducer (TP-200T; Nihon Kohden, Tokyo) and the mean arterial

blood pressure (MABP) was measured. After this procedure, the thorax was opened under positive-pressure respiration and a catheter was inserted into the LV chamber via its apex, where left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), as well as maximal positive and negative rates of pressure development (+dP/dt and −dP/dt) were measured. At the end of the experiment, blood and tissue samples were harvested for later enzymatic and morphometric analyses.

Survival studies

The effects of lisinopril and candesartan cilexetil on survival were assessed in three groups (vehicle-, lisinopril- and candesartan cilexetil-treated MI hamsters) and were followed for 14 days after MI. During the treatment period, the cages were inspected daily for animals that had died. Fourteen days after MI, the hemodynamic parameters were calculated in all surviving animals and the hearts were also harvested finally for later morphometric analysis.

Statistical analyses

All numerical data shown in the text are expressed as the mean ± S.E.M. Significant differences between the mean values of two groups were evaluated by Student's *t*-test for unpaired data. Significant differences among the mean values of multiple groups were evaluated by 1-way ANOVA followed by a post-hoc analysis (Fisher's test). Survival data are presented as Kaplan-Meier curves. The survival curves of individual groups were compared by the log-rank test. $P<0.05$ was used as the threshold for statistical significance.

RESULTS

In the present study, the location of the infarct and its size were determined with the use of azan staining. The mean infarct sizes at 3 and 7 days after LCA ligation were 47.3 ± 5.9% and 44.6 ± 5.1% of LV circumference, respectively, and all infarctions were transmural, involving only the free wall of the LV.

As shown in Table 1, there was no significant difference in body weight between the MI and sham groups until 2 months after the operation. However, a significant increase in the ratio of LV weight to body weight was observed starting from 3 days after LCA ligation, compared with that of the unligated hamsters.

The level of PRA in the MI hamsters was significantly increased 1 and 3 days after operation, compared with that in the unligated hamsters. However, from 7 days to 56 days after the operation, the PRA in MI hamsters did not differ from that of unligated hamsters (Table 1). There was no significant difference between the plasma ACE activities of LCA-ligated and unligated hamsters throughout the experimental period (Table 1).

Figure 1 shows the changes of cardiac ACE activities of LCA-ligated and unligated hamsters throughout the experimental period. The ACE activity was significantly increased in the infarcted LV at 3 days (27.3-fold), 7 days (7.7-fold), 14 days (3.2-fold) and 28 days (1.8-fold) after MI (Fig. 1B). On the other hand, the chymase activity was promptly increased in the infarcted LV at 1 day (2.9-fold, $P<0.05$) after LCA ligation and reached a peak 3 days (22.7-fold) after LCA ligation (Fig. 2B). In contrast to the ACE activity, the chymase activity in the infarcted LV was still higher 56 days (1.9-fold) after MI, compared with the sham-operated hamsters (Fig. 2B). Both ACE and chymase activities in the non-infarcted septum and RV in the ligated animals were unchanged at all experimental times, compared with the sham group (Figs. 1A, 1C, 2A and 2C).

Immunohistochemical studies using an epitope antibody to hamster chymase were performed to localize chymase protein in the sham-operated hearts. As shown in Fig. 3A, some chymase staining was found 7 days after the sham operation at subepicardial sites of the LV, primarily adjacent

Table 1. Body weight (BW), heart weight, plasma renin activity (PRA) and plasma angiotensin-converting enzyme (ACE) activities of the sham-operated (sham) and myocardial-infarcted (MI) hamsters

		Days					
	Group	1	3	7	14	28	56
BW (g)	Sham	118.3 ± 3.6	110.8 ± 4.4	117.5 ± 4.5	126.7 ± 6.0††	148.3 ± 4.8†††	166.2 ± 3.7†††
	MI	117.7 ± 2.5	112.5 ± 3.4	118.3 ± 4.2	130.8 ± 3.0	154.2 ± 2.0†††	162.7 ± 5.2†††
LV/BW (mg/g)	Sham	1.67 ± 0.05	1.69 ± 0.04	1.96 ± 0.05†††	2.05 ± 0.07†††	1.82 ± 0.025†	1.55 ± 0.03
	MI	1.71 ± 0.03	1.94 ± 0.09*	2.33 ± 0.07*,†††	2.37 ± 0.09*,†††	2.03 ± 0.05*,†	1.84 ± 0.04*
PRA (ng/ml per hour)	Sham	1.18 ± 0.12	1.01 ± 0.11	1.12 ± 0.13	1.03 ± 0.21	1.05 ± 0.03	1.06 ± 0.05†
	MI	2.88 ± 0.52*	2.09 ± 0.13*,†	1.26 ± 0.16†††	1.05 ± 0.07†††	1.22 ± 0.17†††	1.08 ± 0.03†††
Plasma ACE (mU/mg protein)	Sham	0.41 ± 0.03	0.42 ± 0.03	0.37 ± 0.01	0.51 ± 0.03††	0.37 ± 0.02	0.48 ± 0.02
	MI	0.40 ± 0.03	0.45 ± 0.03	0.36 ± 0.01	0.49 ± 0.03†	0.43 ± 0.02	0.47 ± 0.03

Results are the mean ± S.E.M. of six experiments. LV, left ventricle. *$P<0.05$, versus sham; $^{\dagger}P<0.05$, $^{\dagger\dagger}P<0.01$, $^{\dagger\dagger\dagger}P<0.001$, versus 1 day.

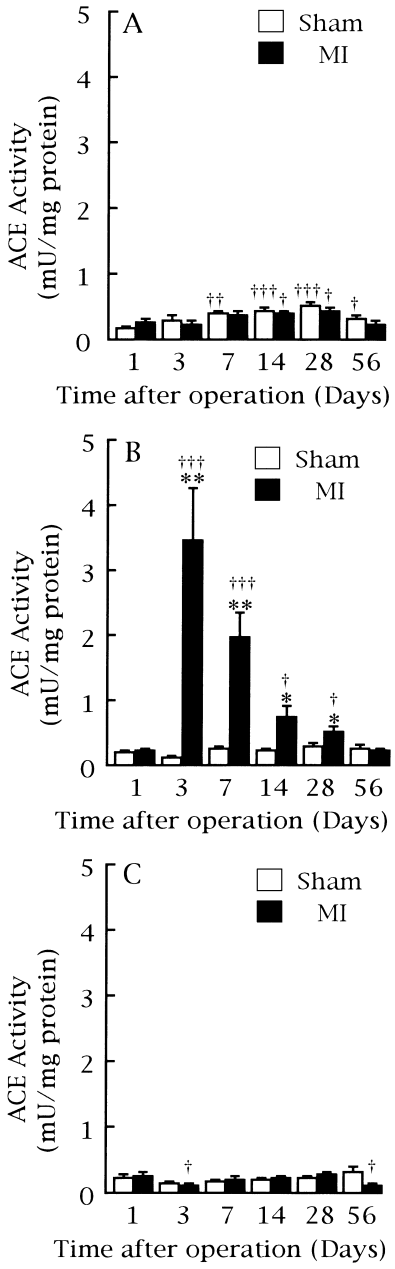

Fig. 1. Bar graphs show ACE activities in the right ventricle (A), left ventricle (B) and septum (C) of sham-operated (sham) and myocardial-infarcted (MI) hamsters 1, 3, 7, 14, 28 and 56 days after operation. Each bar represents the mean ± S.E.M. of data for six hamsters. *P<0.05, **P<0.01, versus sham; $^{\dagger}P$<0.05, $^{\dagger\dagger}P$<0.01, $^{\dagger\dagger\dagger}P$<0.001, versus 1 day.

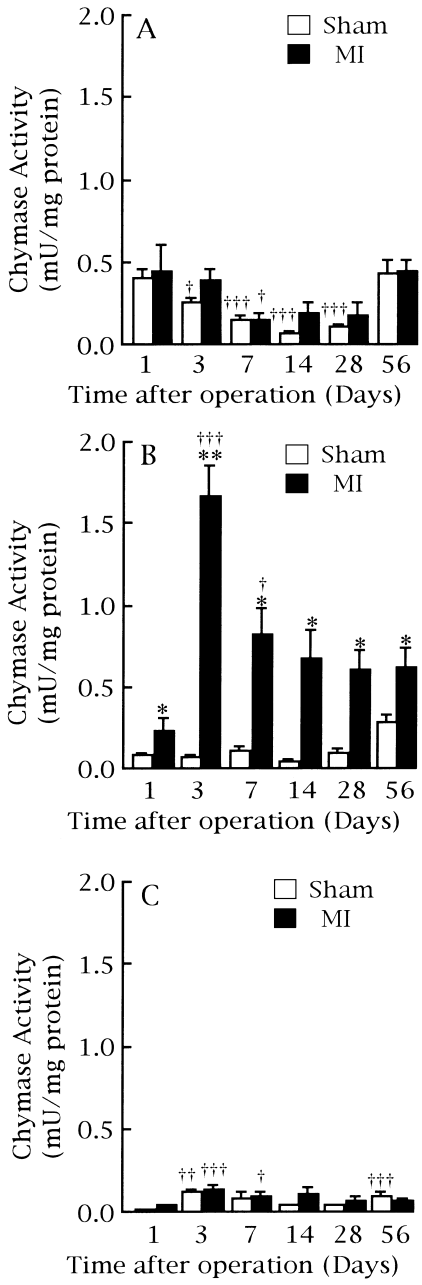

Fig. 2. Bar graphs show chymase activities in the right ventricle (A), left ventricle (B) and septum (C) of sham-operated (sham) and myocardial-infarcted (MI) hamsters 1, 3, 7, 14, 28 and 56 days after operation. Each bar represents the mean ± S.E.M. of data for six hamsters. *P<0.05, **P<0.01, versus sham; $^{\dagger}P$<0.05, $^{\dagger\dagger}P$<0.01, $^{\dagger\dagger\dagger}P$<0.001, versus 1 day.

to vessels. Consecutive sections processed with toluidine blue staining revealed that mast cells were the source of chymase (Fig. 3B). Ventricular sections from MI hamster hearts stained with hematoxylin-eosin revealed that cardiac necrosis was present 7 days after operation at the LV free wall and that it was transmural (Fig. 3C). As indicated with azan staining, fibrillar collagen had accumulated at this site, replacing the lost myocytes (Fig. 3D). Consecutive sections stained with anti-hamster chymase antibody revealed intense chymase staining at subepicardial sites of the infarcted LV (Fig. 3E), but not at these sites in the non-infarcted LV. Consecutive sections also stained with toluidine blue indicated that chymase was located mainly in mast cells (Fig. 3F). The numbers of both chymase-positive mast cells

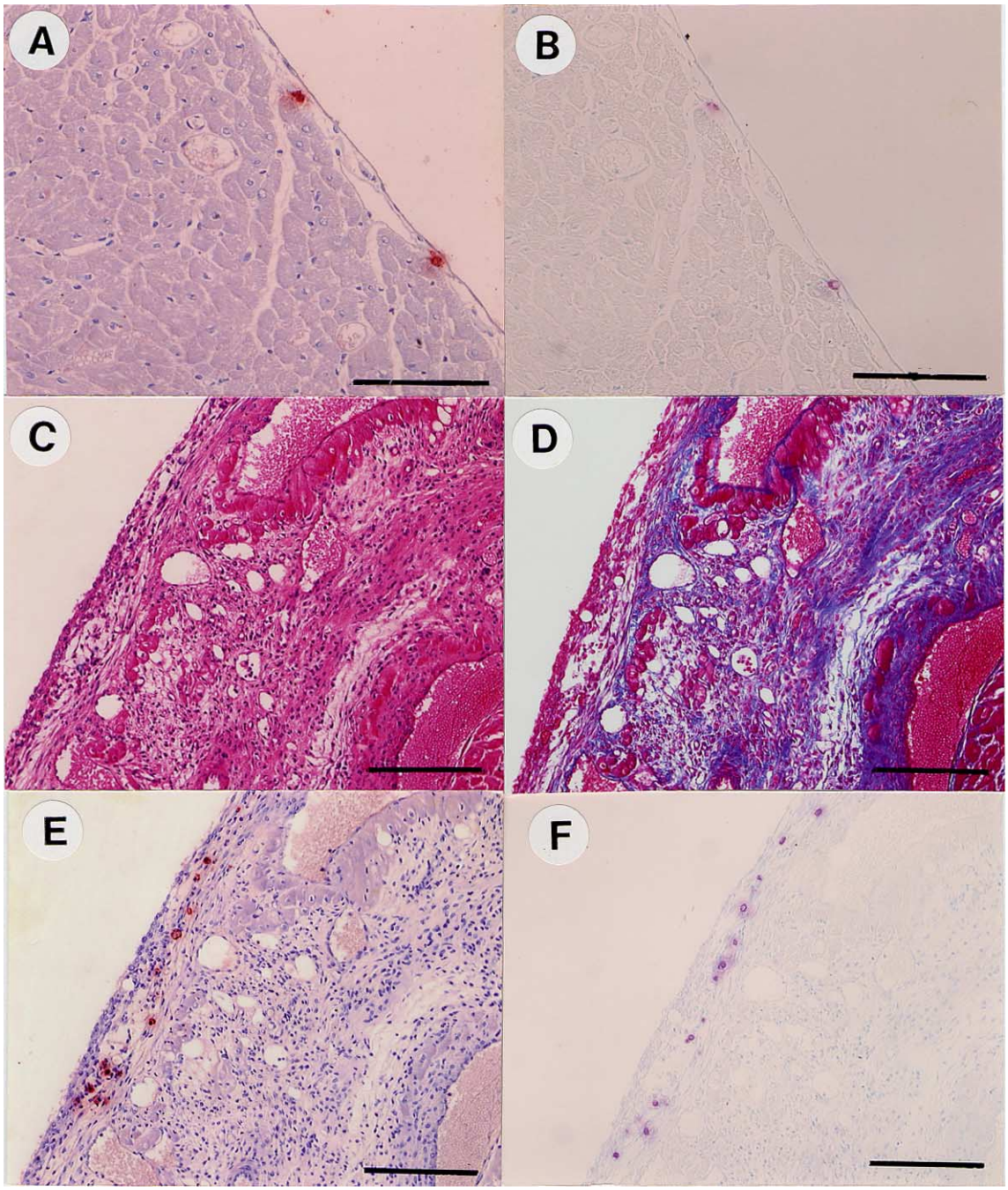

Fig. 3. Representative histological (B, C, D and F) and immunohistochemical (A and E) stainings of serial sections of the left ventricle (LV) obtained 7 days after operation in hamsters. A few chymase-positive cells were found at subepicardial sites of the LV, and these cells were localized adjacent to venules in the sham group (A). Consecutive sections processed with toluidine-blue staining revealed that chymase expression was consistent with the distribution of mast cells (B). MI hamster hearts stained with hematoxylin-eosin revealed that cardiac necrosis was present at the LV free wall and that it was transmural (C). Consecutive sections stained with azan Mallory showed fibrillar collagen had accumulated in these areas (D). Chymase-positive cells at the subepicardial site of infarction were significantly increased after MI (E), and toluidine-blue staining revealed that mast cells were the main source of chymase (F). Bar = 200 μm.

and mast cells in the infarcted LV were significantly higher than in the sham-operated control group 3 and 7 days after the operation (Fig. 4).

Figure 5 illustrates the survival curves in the vehicle-, lisinopril- and candesartan cilexetil-treated groups during the 14-day observation period after MI. During the 14-day

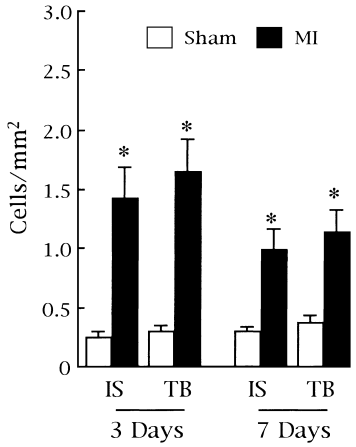

Fig. 4. Bar graphs show the numbers of chymase-positive cells and mast cells per mm^2 of the left ventricle (LV) at 3 and 7 days after operation. Each bar represents the mean ± S.E.M. of data for six hamsters. IS, immunohistochemical staining; TB, toluidine-blue staining; sham, sham-operated hamsters; MI, myocardial-infarcted hamsters. *P<0.05, versus sham.

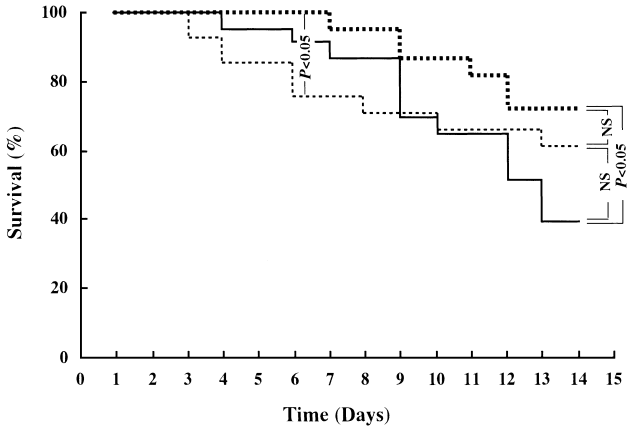

Fig. 5. Cumulative survival rate of infarcted hamsters treated with vehicle (solid line, n = 23) or lisinopril (20 mg/kg per day, thin broken line, n = 21) or candesartan cilexetil (10 mg/kg per day, thick broken line, n = 22). Treatment was initiated 3 days before operation.

observation period, none of the sham-operated hamsters died (data not shown). None of the candesartan cilexetil-treated MI hamsters died during 6-day observation period. However, some of the lisinopril- and vehicle-treated MI hamsters were dead before 6 days after MI. Although the 6-day mortality rate of the vehicle-treated MI hamsters did not differ from the candesartan cilexetil-treated MI hamsters, the 6-day mortality rate in the lisinopril-treated MI hamsters was significantly higher than in the candesartan cilexetil-treated MI hamsters (P<0.05). The vehicle-treated MI hamsters exhibited a 14-day mortality rate of 61%. The 14-day mortality rate was somewhat reduced by the treatment with lisinopril in MI hamsters, but it did not reach statistical significance (38%; P = 0.298, vehicle versus lisinopril). On the other hand, candesartan cilexetil treatment reduced the 14-day mortality rate significantly compared with the vehicle-treated MI hamsters (27%; P<0.05, vehicle versus candesartan cilexetil). No significant differences were observed for the 14-day mortality rates between the lisinopril- and candesartan cilexetil-treated MI groups (P = 0.342).

The plasma ACE activities of the vehicle- and candesartan cilexetil-treated MI hamsters were similar to that of the sham-operated hamsters 3 days after operation (Fig. 6A). In contrast, lisinopril treatment inhibited the plasma ACE activity significantly, as compared with vehicle-treated MI hamsters (P<0.001). As shown in Fig. 6B, the cardiac ACE activity in the infarcted LV was increased significantly 3 days after MI (P<0.001, sham versus vehicle) and this increase was significantly inhibited by the lisinopril treat-

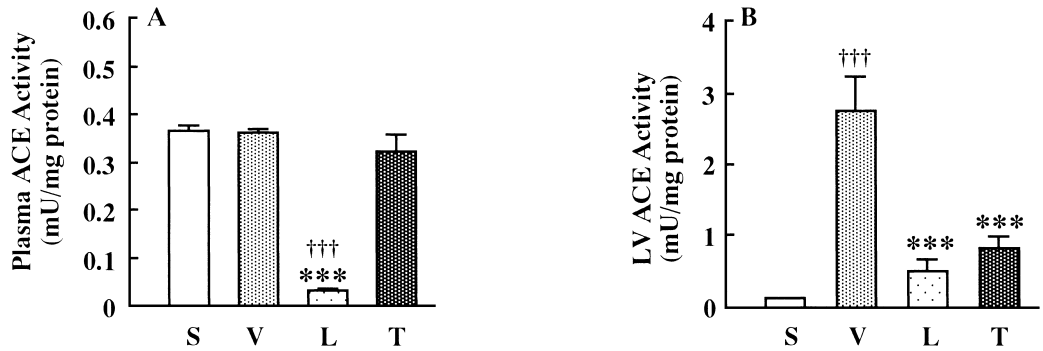

Fig. 6. Bar graphs show ACE activities in the plasma (A) and left ventricle (LV, B) of sham-operated hamsters (S) and myocardial-infarcted hamsters treated with vehicle (V) or lisinopril (20 mg/kg per day, L) or candesartan cilexetil (10 mg/kg per day, T) 3 days after operation. Each bar represents the mean ± S.E.M. of data for five to six hamsters. Treatment was initiated 3 days before operation. ***P<0.001, versus vehicle; †††P<0.001, versus sham.

ment (P<0.001, vehicle versus lisinopril). The cardiac ACE activity in the candesartan cilexetil-treated MI hamsters was significantly lower than in the vehicle-treated MI hamsters. There were no significant differences in ACE activity of the infarcted LV among the sham-operated hamsters, MI hamsters treated with lisinopril and candesartan cilexetil 3 days after operation.

Figure 7 shows changes of the hemodynamic parameters in the four groups of hamsters 3 and 14 days after operation. As can be seen, MI resulted in a significant decreases in heart rate, MABP, LVSP, +dP/dt and –dP/dt and in a slight increase in LVEDP at 3 and 14 days after MI. There were no significant differences in the heart rate among the vehicle-, lisinopril- and candesartan cilexetil-treated MI hamsters 3 and 14 days after operation. The MABP of the candesartan cilexetil-treated hamsters was still lower than that of the sham-operated animals (P<0.01, sham versus candesartan cilexetil), but the candesartan cilexetil treat-

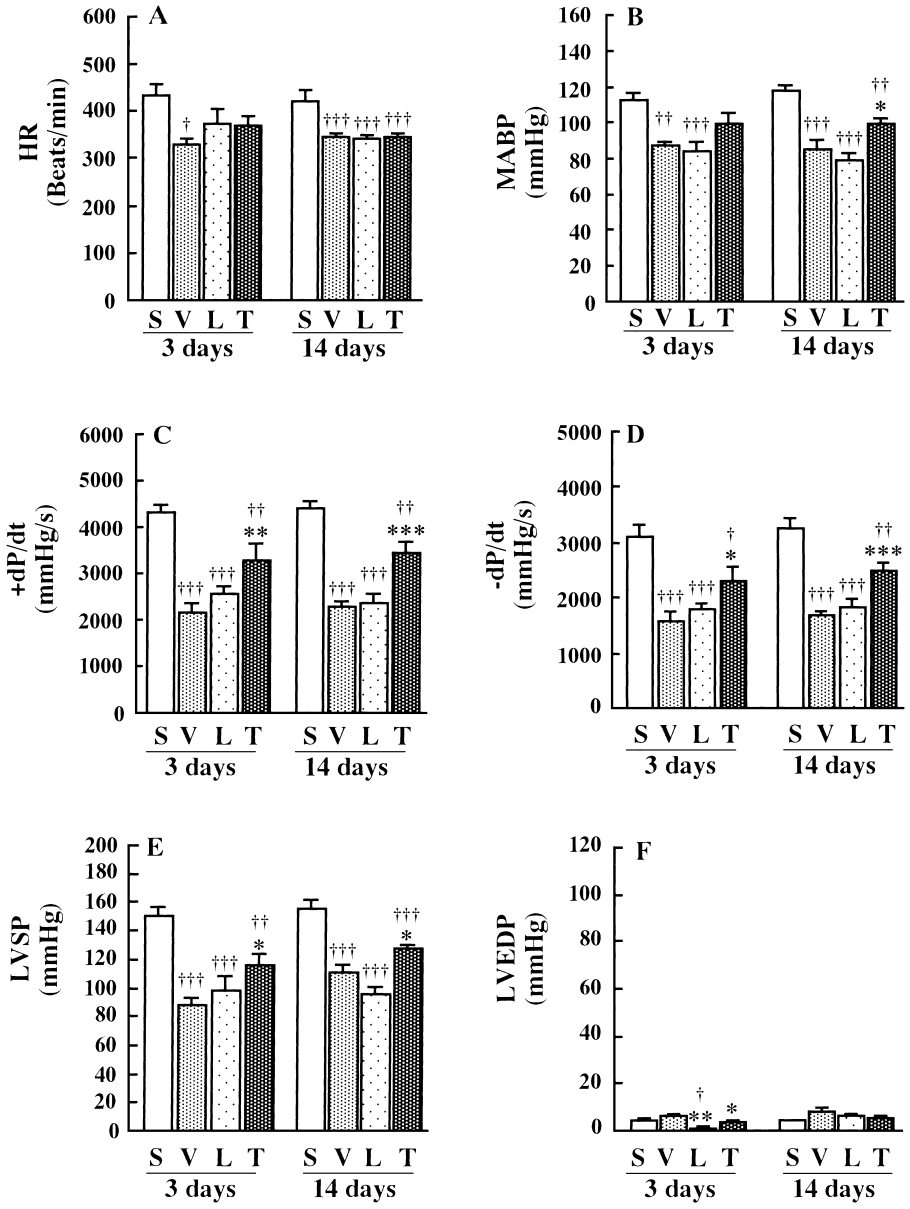

Fig. 7. Bar graphs show the hemodynamic parameters of sham-operated hamsters (S) and myocardial-infarcted hamsters treated with vehicle (V) or lisinopril (20 mg/kg per day, L) or candesartan cilexetil (10 mg/kg per day, T) 3 and 14 days after operation. Each bar represents the mean ± S.E.M. of data for six to sixteen hamsters. Treatment was initiated 3 days before operation. HR, heart rate (A); MABP, mean arterial blood pressure (B); +dP/dt and –dP/dt, maximal positive (C) and negative (D) rates of pressure development, respectively; LVSP, left ventricular systolic pressure (E); LVEDP, left ventricular end-diastolic pressure (F). *P<0.05, **P<0.01, ***P<0.001, versus vehicle; $^{\dagger}P$<0.05, $^{\dagger\dagger}P$<0.01, $^{\dagger\dagger\dagger}P$<0.001, versus sham.

ment attenuated the post-MI MABP lowering effect 14 days after MI (Fig. 7B; $P<0.05$, vehicle versus candesartan cilexetil). In contrast, the MABP of the lisinopril-treated hamsters did not differ from that of the vehicle-treated animals. When compared to the vehicle only treatment, candesartan cilexetil treatment, but not lisinopril treatment, significantly increased LVSP, as well as +dP/dt and –dP/dt 3 and 14 days after MI (C, D and E of Fig. 7). Both lisinopril and candesartan cilexetil treatment reduced LVEDP significantly 3 days after MI; however, the effects of the two drugs did not persist up to 14 days after MI (Fig. 7F).

The mean infarct sizes of the vehicle-treated MI hamsters 3 and 14 days after LCA ligation were $41.9 \pm 4.9\%$ and $39.8 \pm 3.7\%$ of the LV circumference, respectively. Lisinopril and candesartan cilexetil treatments did not affect the mean infarct size at 3 (lisinopril, $42.5 \pm 5.0\%$; candesartan cilexetil, $47.6 \pm 4.7\%$) and 14 (lisinopril, $41.2 \pm 3.9\%$; candesartan cilexetil, $35.3 \pm 2.6\%$) days after MI. The ratio of whole heart weight to body weight was reduced by the treatments with both lisinopril (lisinopril, 2.58 ± 0.05 mg/g; vehicle, 3.22 ± 0.15 mg/g; $P<0.001$, versus vehicle) and candesartan cilexetil (candesartan cilexetil, 2.83 ± 0.08 mg/g; $P<0.01$, versus vehicle) 14 days after MI.

DISCUSSION

In the present study, we demonstrated for the first time that cardiac chymase was activated in myocardial-infarcted regions in hamster during the early and later phases of MI, and this activation occurred earlier and lasted longer than ACE activation. Moreover, our present study also showed that if candesartan cilexetil treatment, but not lisinopril treatment, was initiated 3 days before MI, the cardiac function and survival were significantly improved during the 14-day observation period after MI in hamsters. These findings suggest that an increase of Ang II production via activated cardiac chymase may play an important role in the pathophysiological state after MI.

Many studies have demonstrated that the renin-angiotensin system of circulating and cardiac tissue was upregulated following MI (1 – 3, 25, 27). In agreement with the previous findings, our result also showed that ACE activity at the site of infarction was increased significantly, starting from 3 days after MI and lasting until 4 weeks after operation in hamsters. A significant increase of PRA was also observed during 3 days after the LCA ligation. On the other hand, cardiac chymase, which was recently found to play an important role in the formation of Ang II from Ang I in cardiovascular tissues (12 – 17), was also increased promptly in the infarcted myocardium starting from 1 day after LCA ligation and this activation lasted throughout the experimental period (56 days). Chymase is stored in the secretory granules of mast cells and can only exert its effects after degranulation from mast cells (28 – 30). Therefore, not only the number of mast cells, but also the degranulation of mast cells may affect the production rate of Ang II after MI. Previous reports have confirmed that cardiac ischemia and reperfusion or chronic ischemia can induce a degranulation of mast cells and an increase in the number of mast cells in infarcted hearts (31, 32). Our immunohistochemical and toluidine-blue staining also indicated that the increase of chymase activity mainly depended on the accumulation of chymase-positive mast cells at the site of infarction, although the increase in chymase activity was not in parallel with the increase of mast cells 3 days after MI. Recently, we found that tissue inhibitors of chymase, such as secretory leukocyte protease inhibitor-like protein, could affect tissue chymase activity in vivo (33). Moreover, the contents of chymase in human mast cells may be regulated by cytokines (34). Thus, changes in tissue chymase inhibitors or cytokines may affect chymase activity without a change in the number of mast cells, although this is currently not known in the present study.

The activation of the RAS after MI appears to primarily maintain systemic homeostasis, but this compensatory activated RAS may also introduce other harmful actions. Ang II accelerates cardiac fibrosis in the non-infarcted myocardium, which is considered to be a major reason for the development of chronic heart failure after MI (5). On the other hand, Ang II also facilitates the release of norepinephrine from cardiac sympathetic nerve endings (35), which may be closely related with sudden cardiac death during the post-MI acute phase (36). In the present study, the death of vehicle-treated MI hamsters was seen as early as 3 days after MI and during this time, the cardiac ACE and chymase activities reached their peak levels. The PRA was also significantly higher than that in the sham-operated hamsters. Candesartan cilexetil treatment initiated 3 days before the LCA ligation significantly reduced the mortality during the 14-day observation period. Our results indicated that the elevation of Ang II concentration in the plasma and local cardiac tissue may result in a lethal effect after a permanent LCA ligation in the hamsters. Because death occurred in the early phase after MI in the hamsters treated with vehicle, these deaths may result from acute heart failure due to certain lethal arrhythmias. Indeed, it has been demonstrated that the Ang II type 1 receptor is involved in the occurrence of arrhythmias in an ischemia-reperfusion model of the Ang II type 1 receptor knockout mice (37). It was also reported that losartan treatment in elderly patients with chronic heart failure reduced mortality significantly and this reduction was primarily due to a decrease in the number of sudden deaths (8).

In the present study, compared with the MI hamsters

treated with vehicle, cardiac function was improved significantly in the MI hamsters treated with candesartan cilexetil, but not with lisinopril, as indicated by the significant increase in the LVSP, as well as the +dP/dt and −dP/dt at 3 and 14 days after MI. Thus, the improvement of cardiac function in MI hamsters treated with candesartan cilexetil may also lead to an increase in the survival rate in the present study.

Compared with the vehicle-treated MI hamsters, the cardiac ACE activity in the candesartan cilexetil-treated MI hamsters was significantly low in the present study. Although the reason was unclear, it seemed not to result from a direct inhibitory effect of candesartan cilexetil, because plasma ACE activity was not inhibited by this treatment. Recently, it was reported that the differentiation from monocytes to macropharges was mediated by Ang II (38). The strong expression of ACE in macropharges and the increase of macropharges in the infarcted-myocardium during the acute phase of the post-MI were also reported (39, 40). Therefore, the blockade of Ang II actions may affect the expression of ACE under a certain condition.

Numerous studies have demonstrated that the beneficial effects of ACE inhibitors on cardiac function and survival were similar to those of Ang II type 1 antagonists in the rat MI model (26, 41). Lisinopril treatment at a dose of 20 mg/kg per day or less has also been reported to improve cardiac function and survival in MI rats (10, 24). However, in the present study, lisinopril treatment failed to show significant benefits on cardiac function or survival compared to candesartan cilexetil treatment in the MI hamsters. The differences between lisinopril and candesartan cilexetil treatment may result from insufficient inhibition of ACE. However, our results showed that lisinopril inhibited the ACE activities of plasma and infarcted LV to an extent similar to that in the sham-operated hamsters 3 days after MI. In the lisinopril-treated normotensive hamsters, the pressor response to Ang I was suppressed about 79% at 24 h after the final treatment. A similar suppression of the pressor response to Ang II was also observed in the candesartan cilexetil-treated group. The MABP and ratio of whole heart weight to body weight in these hamsters also did not differ for the groups treated with the two drugs. Thus, the different effects of lisinopril and candesartan cilexetil were independent of the insufficient inhibition of ACE by lisinopril.

In hamsters, cardiovascular tissues possess both the ACE- and chymase-dependent Ang II-forming pathways like humans, whereas rats possess only the ACE-dependent Ang II-forming pathway. In the present study, although lisinopril treatment significantly inhibited both plasma and cardiac ACE, it did not affect cardiac chymase 3 days after MI (data not shown), suggesting that Ang II generation that bypasses ACE may still be present in the lisinopril-treated MI hamsters. This assumption is supported by the finding that the plasma Ang II concentration under long-term ACE treatment returns to normal levels after a significant drop during the initial phase of treatment (42). Furthermore, a major role of the chymase-generated Ang II on carrier-mediated norepinephrine release in a human model of myocardial ischemia has been reported recently (35). These findings, taken together with our results, suggest that the smaller effect on cardiac function and survival after MI in hamsters treated with lisinopril may be due primarily to the presence of continuous Ang II generation by the activated cardiac chymase.

Negative results of ACE therapy on cardiac function and survival in hamsters with acute MI were observed in this study. However, ACE inhibitor treatment has been reported to improve cardiac function and decrease mortality in patients with MI (43, 44). Unlike the present lisinopril therapy, these therapies are usually started several days after MI and last over a long period. Therefore, whether a deleterious effect of early intervention is masked by the long period of treatment is unknown. In the present study, the 6-day mortality rate of the lisinopril-treated MI hamsters was higher than the candesartan cilexetil-treated MI hamsters, although no significant differences were observed for the 6-day mortality rates between the vehicle- and candesartan cilexetil-treated MI groups, suggesting that early treatment of ACE inhibitor may have a disadvantageous effect after MI. In fact, improvement of abnormal remodeling in the viable myocardium has been reported to be responsible for the main beneficial effects of ACE inhibitors on cardiac function and survival after MI (5), indicating that appropriate timing may be very important to obtain the best therapeutic effect of ACE inhibitors in MI.

We conclude that the cardiac chymase activity is increased significantly at the site of infarction, and this activation takes place earlier and lasts longer than the increased ACE activity in hamsters with MI. Cardiac function and survival were improved significantly after blockade of Ang II in the receptors by candesartan cilexetil, whereas they were not improved after ACE inhibition with lisinopril. These results suggest that the increased Ang II production via activated cardiac chymase may play an important role in the pathophysiologic state after MI in hamsters, although final confirmation is needed by using chymase specific-inhibitors in this model.

Acknowledgments

We thank Takeda Chemical Industries Ltd. for kindly supplying Candesartan cilexetil. We also thank Otsuka Pharmaceutical Co., Ltd. for kindly providing anti-hamster chymase antibody.

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