Angiotensin II Type 1-Receptor Antagonist Candesartan Cilexetil Prevents Left Ventricular Dysfunction in Myocardial Infarcted Rats

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ABSTRACT—The purpose of this study was to analyze the effect of the angiotensin II type 1-receptor antagonist candesartan cilexetil on left ventricular systolic and diastolic function and mRNA expression of contractile proteins, collagen, and Ca^{2+} handling protein in myocardial-infarcted rats. After myocardial infarction, the animals were randomly assigned to candesartan cilexetil-treated or untreated groups (MI). We performed Doppler-echocardiographic examination and measured the hemodynamics at four and twelve weeks after myocardial infarction. Following these measurements, their cardiac mRNA was analyzed. At four weeks in MI, left ventricular end-diastolic dimension increased (Control, 6.2 ± 0.6 mm; MI, 8.7 ± 0.6 mm; $P < 0.01$), fractional shortening decreased (Control, $41 \pm 5\%$; MI, $16 \pm 3\%$; $P < 0.01$) and E wave deceleration rate increased (Control, 14.3 ± 2.0 m/sec²; MI, 23.3 ± 2.3 m/sec²; $P < 0.01$). Candesartan cilexetil significantly prevented these changes. The mRNA expressions of β -myosin heavy chain, α -skeletal actin, atrial natriuretic peptide, and collagens I and III in the non-infarcted left ventricle and right ventricle were increased at four weeks and were significantly suppressed by treatment with candesartan cilexetil. At four weeks, Na^+ - Ca^{2+} exchanger mRNA expression was increased, and candesartan cilexetil suppressed this increase. At twelve weeks, sarcoplasmic reticulum Ca^{2+} -ATPase mRNA expression in the infarcted region including the adjacent non-infarcted left ventricle and right ventricle were decreased and candesartan cilexetil restored it to the control level. Candesartan cilexetil prevented the systolic and diastolic dysfunction and abnormal cardiac mRNA expression in myocardial-infarcted rats.

Keywords: Ventricular remodeling, Angiotensin II type 1-receptor antagonist, Echocardiography, Gene expression, Diastolic function

Angiotensin-converting enzyme (ACE) inhibitor reduces morbidity and mortality in patients with chronic heart failure and systolic left-ventricular dysfunction as well as in patients who have had a myocardial infarction (1–4). The benefits of ACE inhibitors have been mostly attributed to blockade of angiotensin II production and/or inhibition of kinin destruction. Orally active, non-peptide angiotensin II type 1 (AT1)-receptor antagonists such as losartan or candesartan cilexetil can block this receptor specifically (5, 6). Since both ACE inhibitor and AT1-receptor antagonist block the renin-angiotensin system at different levels, both may have a cardioprotective effect. Recently, in the ELITE study, treatment with losartan was associated with unexpected lower mortality than that found with captopril in heart failure patients

(7).

Losartan improved hemodynamics (8) and also prevented left ventricular remodeling after myocardial infarction (9). Our previous study showed that candesartan cilexetil improved hemodynamics and prevented left ventricular dilation (10) and mRNA expressions of fetal contractile proteins and atrial natriuretic peptide (ANP) in the noninfarcted region after myocardial infarction (11). As many as one-third of patients presenting with heart failure are thought to have symptoms attributable to diastolic dysfunction (12). It has become apparent that abnormalities of diastolic filling of the heart play just as great a role, if not a greater role, than systolic function in producing the signs and symptoms of heart failure (13, 14). However, the effect of AT1-receptor antagonist on left ventricular diastolic function after myocardial infarction is not clear. The purpose of this study was to assess

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the effect of candesartan cilexetil on left ventricular systolic and diastolic function after myocardial infarction and cardiac gene expression of contractile protein, collagen and intracellular calcium handling proteins, such as sarcoplasmic reticulum (SR) Ca^{2+} -ATPase and Na^{+} - Ca^{2+} exchanger, by Northern blot analysis.

MATERIALS AND METHODS

Each animal received humane care in compliance with the "Principles of Laboratory Animal Care" of the National Society of Medical Research and the "Guide for the Care and Use of Laboratory Animals" of the National Academy of Sciences.

Experimental protocol

Male Wistar rats, weighing 290–310 g (Clea Japan, Inc., Osaka), were used in the present experiments. Myocardial infarction was produced in rats by the previously described method of coronary artery ligation (15, 16). The same surgical procedures were also performed (Control: four weeks, $n=8$; twelve weeks, $n=8$). Myocardial-infarcted rats, which survived 24 hr after surgery, were randomly separated into treated and untreated groups (MI: four weeks, $n=8$; twelve weeks, $n=9$). Treated groups of rats were orally administered the AT1-receptor antagonist candesartan cilexetil (1 mg/kg) in a volume of 2 ml/kg, by gastric gavage once per day for four and twelve weeks after myocardial infarction (candesartan cilexetil: four weeks, $n=9$; twelve weeks, $n=10$). The untreated groups were administered vehicle (5% gum arabic solution) in the same manner as candesartan cilexetil. There were no significant differences in body weight and blood pressure among the above groups prior to drug treatment.

Doppler-echocardiographic studies

In this study, we performed transthoracic echocardiography on each rat by modifying the procedure used in the previously described study (17). Rats were lightly anesthetized by an intraperitoneal injection of ketamine hydrochloride (25 to 50 mg/kg) and xylazine (5 to 10 mg/kg) and placed on a specially designed apparatus. Echocardiograms were performed with a commercially-available echocardiographic system equipped with a 7.5-MHz phased-array transducer (SONOS 2500; Hewlett Packard, Andover, MA, USA). M-mode tracings were recorded through the anterior and posterior LV walls at a paper speed of 100 mm/sec. Moving the transducer toward the cardiac apex and angling anteriorly enabled the acquisition of an apical two-chamber view. The high-frequency neonatal transducers used in this study allowed us to obtain good-quality transthoracic images of

the beating rat heart. All measurements were performed by two observers and analyzed by use of the analysis software present on the echocardiography machine.

Pulsed-wave Doppler spectra of mitral inflow were recorded from the apical four-chamber view, with the sample volume placed near the tips of the mitral leaflets and adjusted to the position at which the velocity was maximal and the flow pattern laminar. The sample volume was set at the smallest size available. All Doppler spectra were recorded on paper at 100 mm/sec and analyzed off-line as previously described. The numbers represent the mean of at least three consecutive cardiac cycles.

Hemodynamic studies

One day after the echocardiogram, the rats were anesthetized by intraperitoneal injection of pentobarbital sodium (35 mg/kg body weight). Aortic and left ventricular pressures were recorded by inserting a polyethylene tubing catheter into the right carotid artery and advanced into the aorta and then into the left ventricle. Central venous pressure (CVP) was measured by cannulating the right external jugular vein with a PE-50 tubing catheter, which was advanced to the region of the thoracic vena cava. Aortic and central venous pressures were recorded as the mean values determined by electronic averaging, and left ventricular end-diastolic pressure (LVEDP) was obtained by averaging the values for 10 beats. Heart rate was determined from the tracing of aortic pressure.

After these measurements, MI rats and sham-operated rats were decapitated, the chest and abdominal wall were incised, and the heart was quickly removed. The left ventricle, which was separated from the atria and the right ventricle, was opened with an incision along the septum from base to apex. Myocardial infarction size was measured as previously described (16). Rats with less than 20% of the infarction size were excluded from analysis. After determination of the infarction size, the left ventricle was divided into two regions, the myocardial-infarcted myocardium including two mm around the infarcted region (adjacent non-infarcted region) and the remote non-infarcted region. Because the infarcted myocardium contains less mRNA and no myocytes, the adjacent non-infarcted region was included in the determination of mRNA expression in infarcted myocardium and designated as "Adjacent". Contractile protein, ANP and calcium handling protein are located in the tissue surrounding the infarct region. However, mRNA expression of collagen in the designated "Adjacent" region represents both the infarct region and the tissue around it. After weighing, the tissues were rapidly frozen in liquid nitrogen and stored at -80°C until use.

Northern blot hybridization

The method of RNA extraction and Northern blot hybridization was previously described in detail (18).

The sequence of the oligonucleotide probes used were as follows:

α -MHC, 5'-TTGTGGGATAGCAACAGCGA-3'

β -MHC, 5'-GTCTCAGGGCTTCACAGG-3'

α -skeletal actin, 5'-GCAACCATAGCACGATGGTC-3'

α -cardiac actin, 5'-TGCACGTGTGTAAACAAACT-3'

18S, 5'-ACGGTATCTGATCGTCTTCAACC-3'

The cDNA probes used were rat $\alpha 1$ (I) collagen cDNA (1.3-kb PstI/BamHI fragment) (19), mouse $\alpha 1$ (III) collagen cDNA (1.8-kb EcoRI/EcoRI fragment) (20), rat ANP cDNA (0.825-kb fragment) (21), SR Ca^{2+} -ATPase cDNA (2.0-kb fragment) (22) and Na^{+} - Ca^{2+} exchanger cDNA (1.2-kb fragment) (23), rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1.3-kb PstI/PstI frag-

ment) (24) and rat 18S ribosomal RNA. To evaluate mRNA levels, an optical scanner (EPSON GT-8000; Seiko, Tokyo) was utilized for digitizing autoradiograms. The autoradiogram bands in the digitized image were measured for their density with the use of a public domain NIH image program and a computer (Macintosh LC-III; Apple Computer, Inc., USA). For all RNA samples, the density of an individual mRNA band was divided by that of a 18S ribosomal RNA band, to correct for differences in RNA loading and/or transfer.

Statistics

Results were each expressed as a mean $\pm$ S.E. Statistical significance was determined by ANOVA and Duncan's multiple range test. The differences were considered statistically significant at a value of $P < 0.05$.

Table 1. Ventricular weights, hemodynamics and myocardial infarction size in untreated (MI) and AII-receptor antagonist (AII ant)-treated rats after myocardial infarction

	4 weeks			12 weeks		
	Control	MI	AII ant	Control	MI	AII ant
n	8	8	9	8	9	10
Body weight (g)	374 $\pm$ 10	380 $\pm$ 9	370 $\pm$ 8	416 $\pm$ 8	408 $\pm$ 10	406 $\pm$ 8
LV/BW (g/kg)	1.98 $\pm$ 0.02	2.19 $\pm$ 0.03**	1.93 $\pm$ 0.03 ^{††}	2.04 $\pm$ 0.04	2.32 $\pm$ 0.06**	2.15 $\pm$ 0.05 ^{††}
RV/BW (g/kg)	0.52 $\pm$ 0.02	0.72 $\pm$ 0.02**	0.56 $\pm$ 0.01 ^{††}	0.54 $\pm$ 0.01	0.89 $\pm$ 0.07**	0.59 $\pm$ 0.02 ^{††}
MAP (mmHg)	111 $\pm$ 3	106 $\pm$ 2	98 $\pm$ 3*	114 $\pm$ 3	109 $\pm$ 2	96 $\pm$ 4*
HR (beats/min)	354 $\pm$ 5	350 $\pm$ 6	352 $\pm$ 8	354 $\pm$ 5	360 $\pm$ 6	366 $\pm$ 10
LVEDP (mmHg)	4 $\pm$ 1	21 $\pm$ 3**	6 $\pm$ 1 ^{††}	4 $\pm$ 1	25 $\pm$ 3**	6 $\pm$ 1 ^{††}
CVP (mmHg)	1 $\pm$ 1	5 $\pm$ 1**	2 $\pm$ 1 ^{††}	2 $\pm$ 0	6 $\pm$ 3**	2 $\pm$ 1 ^{††}
MI size (%)	—	38 $\pm$ 3	40 $\pm$ 4	—	40 $\pm$ 4	41 $\pm$ 3

* $P < 0.05$, ** $P < 0.01$ vs Control; ^{††} $P < 0.01$ vs MI. MAP: mean aortic pressure, HR: heart rate, MI size: myocardial infarction size.

Table 2. Doppler echocardiographic measurements in untreated (MI) and AII-receptor antagonist (AII ant)-treated rats after myocardial infarction

	4 weeks			12 weeks		
	Control	MI	AII ant	Control	MI	AII ant
n	8	8	9	8	9	10
LVDd (mm)	6.2 $\pm$ 0.6	8.7 $\pm$ 0.6**	7.1 $\pm$ 0.3 ^{††}	6.6 $\pm$ 0.3	10.5 $\pm$ 0.6**	8.0 $\pm$ 0.4 ^{††}
FS (%)	41 $\pm$ 5	16 $\pm$ 3**	31 $\pm$ 4 [†]	38 $\pm$ 6	13 $\pm$ 1**	24 $\pm$ 2** [†]
PW thickening (%)	59 $\pm$ 4	29 $\pm$ 7*	41 $\pm$ 10	57 $\pm$ 6	19 $\pm$ 6**	46 $\pm$ 8 [†]
E Velocity (cm/sec)	66 $\pm$ 2	98 $\pm$ 8**	81 $\pm$ 5 [†]	66 $\pm$ 8	109 $\pm$ 8**	87 $\pm$ 4 ^{††}
A Velocity (cm/sec)	36 $\pm$ 4	16 $\pm$ 2**	24 $\pm$ 2** [†]	38 $\pm$ 4	13 $\pm$ 2**	25 $\pm$ 4 ^{††}
E/A	2.3 $\pm$ 0.5	6.7 $\pm$ 0.9**	3.5 $\pm$ 0.4 ^{††}	2.2 $\pm$ 0.4	8.5 $\pm$ 1.2**	3.8 $\pm$ 0.6 ^{††}
E Deceleration (m/sec ²)	14.3 $\pm$ 2.0	23.3 $\pm$ 2.3**	15.3 $\pm$ 1.8 [†]	16.7 $\pm$ 2.5	28.7 $\pm$ 2.9**	19.5 $\pm$ 1.8 [†]

* $P < 0.05$, ** $P < 0.01$ vs Control; [†] $P < 0.05$, ^{††} $P < 0.01$ vs MI. FS: fractional shortening, PW: posterior wall.

Fig. 1. Examples of M-mode echocardiograms from sham-operated (Control), myocardial-infarcted rats (MI) and myocardial-infarcted rats treated with candesartan cilexetil (All ant) at twelve weeks. AW indicates the anterior wall and PW indicates the posterior wall. Note the left ventricular cavity dilatation, thinning, and akinesis of the anterior wall and thickening of the posterior wall in MI. Candesartan cilexetil prevented LV dilatation and the decrease of left ventricular PW thickness.

Fig. 2. Examples of the pulsed wave Doppler spectra of the mitral inflow pattern from sham-operated (Control), myocardial-infarcted rats (MI) and myocardial-infarcted rats treated with candesartan cilexetil (All ant) at four weeks. Compared with the Control, the mitral inflow pattern from the infarcted rat shows increased peak E wave velocity, rapid deceleration of the E wave and decreased peak A wave. The myocardial infarcted rat treated with candesartan cilexetil visually shows a relatively normal transmitral flow pattern.

RESULTS

Effect of candesartan cilexetil on ventricular weights and hemodynamics (Table 1)

In myocardial infarcted rats, left and right ventricular weight/body weight ratio increased at four and twelve weeks, and candesartan cilexetil significantly prevented these increases. Mean aortic pressure did not change in myocardial infarcted rats, while candesartan cilexetil significantly decreased it at four and twelve weeks after myocardial infarction. LVEDP and CVP increased in myocardial infarcted rats, and candesartan cilexetil significantly prevented these increases at four and twelve weeks after myocardial infarction.

Echocardiographic assessments of left ventricular geometry and function (Table 2)

Left ventricular cavity size significantly increased in myocardial infarcted rats at four and twelve weeks (LVDd: Control, 6.2 ± 0.6 mm versus MI, 8.7 ± 0.6 mm,

$P < 0.01$, at four weeks; Control, 6.6 ± 0.3 mm versus MI, 10.5 ± 0.6 mm, $P < 0.01$, at twelve weeks). Candesartan cilexetil significantly prevented the left ventricular cavity dilatation. Myocardial infarcted rats had significant systolic dysfunction, as evidenced by decreased fractional shortening, and candesartan cilexetil improved it at four and twelve weeks. Left ventricular posterior wall thickness decreased at four and twelve weeks. Candesartan cilexetil significantly prevented this decrease at twelve weeks (Fig. 1).

Examples of pulsed-wave Doppler recordings of mitral inflow from three groups at four weeks are shown in Fig. 2. In myocardial infarcted rats, peak early diastolic filling wave (E wave) velocity increased and atrial filling wave (A wave) velocity decreased at four weeks, resulting in a marked increase in the ratio of E wave to A wave velocity (Control; 2.3 ± 0.5 versus MI, 6.7 ± 0.9 , $P < 0.01$). Deceleration rate of the E wave became rapid (Control, 14.3 ± 2.0 m/sec² versus MI, 23.3 ± 2.3 m/sec², $P < 0.01$). Candesartan cilexetil significantly prevented the worsen-

Fig. 3. Typical autoradiograms of Northern blot analysis of the noninfarcted adjacent and remote LV mRNAs for β -myosin heavy chain (β -MHC), α -skeletal actin, atrial natriuretic polypeptide (ANP), collagen types I and III, GAPDH and 18S ribosomal RNA at four weeks after myocardial infarction. Adjacent: the infarcted region including the adjacent noninfarcted LV, Remote: the remote noninfarcted LV, RV: right ventricle, C: sham-operated rats, MI: myocardial-infarcted rats treated with vehicle, All ant: myocardial-infarcted rats treated with candesartan cilexetil.

ing of diastolic function at four and twelve weeks after myocardial infarction.

mRNA expressions of contractile proteins, ANP, collagens and Ca^{2+} handling proteins

The results of cardiac gene expressions at four and twelve weeks are shown in Figs. 3 and 4 and Tables 3 and 4. At four weeks, β -MHC, α -skeletal actin and ANP mRNA expressions were increased by 2.6-, 2.4- and 4.7-fold ($P < 0.01$), respectively, in the adjacent region; by 1.4-, 2.8-, 3.9-fold ($P < 0.01$), respectively, in the remote non-infarcted region; and by 2.2-, 3.4- and 6.7-fold ($P < 0.01$), respectively, in the RV. Candesartan cilexetil prevented increases of β -MHC, α -skeletal actin and ANP mRNA expression in the three regions. α -MHC mRNA expression was decreased by 0.4- and 0.5-fold ($P < 0.01$), respectively, in the adjacent region and RV. Candesartan cilexetil did not improve these changes. Collagen type I and III mRNA expressions significantly increased, and candesartan cilexetil prevented these increases significantly in all regions. $\text{Na}^+ - \text{Ca}^{2+}$ exchanger mRNA increased

significantly in all regions, while SR Ca^{2+} -ATPase mRNA did not change. Candesartan cilexetil inhibited the increase of $\text{Na}^+ - \text{Ca}^{2+}$ exchanger mRNA. GAPDH in the adjacent region decreased significantly (0.8-fold, $P < 0.05$).

At twelve weeks, α -skeletal actin and ANP mRNAs remained at increased levels, and candesartan cilexetil significantly prevented these increases in all regions. On the other hand, β -MHC mRNA returned to the control level in all regions. Collagen type I and III mRNAs also increased and candesartan cilexetil significantly prevented these increases in all regions. SR Ca^{2+} -ATPase mRNA decreased to 0.6- ($P < 0.01$) and 0.6- ($P < 0.05$) fold, respectively, in the adjacent region and RV, while there was no change in the remote non-infarcted region. Candesartan cilexetil significantly improved these decreases. $\text{Na}^+ - \text{Ca}^{2+}$ exchanger mRNA returned to the control level in the adjacent region and RV, while it remained increased by 1.8-fold in the remote non-infarcted region, and candesartan cilexetil prevented this increase.

Fig. 4. Typical autoradiograms of Northern blot analysis of the noninfarcted adjacent and remote LV mRNAs for sarcoplasmic reticulum (SR) Ca^{2+} -ATPase, $\text{Na}^+ - \text{Ca}^{2+}$ exchanger and 18S ribosomal RNA at four and twelve weeks after myocardial infarction. Adjacent: the infarcted region including the adjacent noninfarcted LV, Remote: the remote noninfarcted LV, RV: right ventricle, C: sham-operated rats, MI: myocardial-infarcted rats treated with vehicle, All ant: myocardial-infarcted rats treated with candesartan cilexetil.

Table 3. mRNA expression in untreated (MI) and AII-receptor antagonist (AII ant)-treated rats at 4 weeks after myocardial infarction (compared with the control)

	Adjacent		Remote		RV	
	MI	AII ant	MI	AII ant	MI	AII ant
n	8	9	8	9	8	9
α -MHC	0.4 $\pm$ 0.1**	0.4 $\pm$ 0.3**	0.9 $\pm$ 0.1	0.9 $\pm$ 0.1	0.5 $\pm$ 0.1**	0.6 $\pm$ 0.2**
β -MHC	2.6 $\pm$ 0.2**	2.0 $\pm$ 0.3*†	1.4 $\pm$ 0.1**	1.0 $\pm$ 0.1††	2.2 $\pm$ 0.3**	1.2 $\pm$ 0.1††
α -Cardiac actin	1.0 $\pm$ 0.1	1.0 $\pm$ 0.1	1.1 $\pm$ 0.1	1.0 $\pm$ 0.1	1.1 $\pm$ 0.1	1.0 $\pm$ 0.1
α -Skeletal actin	2.4 $\pm$ 0.1**	1.6 $\pm$ 0.1**††	2.8 $\pm$ 0.1**	1.1 $\pm$ 0.1††	3.4 $\pm$ 0.2**	1.3 $\pm$ 0.2††
ANP	4.7 $\pm$ 0.3**	3.3 $\pm$ 0.3**††	3.9 $\pm$ 0.5**	2.8 $\pm$ 0.2**†	6.7 $\pm$ 0.6**	4.0 $\pm$ 1.0**†
Collagen I	12.1 $\pm$ 0.1**	7.2 $\pm$ 0.5**†	3.6 $\pm$ 0.3**	2.4 $\pm$ 0.4**††	3.4 $\pm$ 0.3**	1.4 $\pm$ 0.4††
Collagen III	8.4 $\pm$ 0.3**	5.3 $\pm$ 0.4**††	2.2 $\pm$ 0.1**	1.4 $\pm$ 0.2**†	3.0 $\pm$ 0.2**	2.4 $\pm$ 0.2**†
SR Ca ²⁺ -ATPase	0.9 $\pm$ 0.1	1.0 $\pm$ 0.1	1.0 $\pm$ 0.1	1.0 $\pm$ 0.1	1.1 $\pm$ 0.1	1.1 $\pm$ 0.2
Na ⁺ -Ca ²⁺ exchanger	1.5 $\pm$ 0.1**	1.1 $\pm$ 0.2†	1.6 $\pm$ 0.1**	1.2 $\pm$ 0.1†	1.5 $\pm$ 0.1**	1.1 $\pm$ 0.1†

Control=1.0 $\pm$ 0.0–0.1. *P<0.05, **P<0.01 vs Control; †P<0.05, ††P<0.01 vs MI.

Table 4. mRNA expression in untreated (MI) and AII-receptor antagonist (AII ant)-treated rats at 12 weeks after myocardial infarction (compared with the control)

	Adjacent		Remote		RV	
	MI	AII ant	MI	AII ant	MI	AII ant
n	8	9	8	9	8	9
α -MHC	0.7 $\pm$ 0.2	0.6 $\pm$ 0.2	0.9 $\pm$ 0.1	1.0 $\pm$ 0.1	0.5 $\pm$ 0.2*	0.7 $\pm$ 0.1
β -MHC	1.2 $\pm$ 0.2	1.0 $\pm$ 0.2	1.0 $\pm$ 0.1	0.9 $\pm$ 0.1	1.3 $\pm$ 0.1	1.1 $\pm$ 0.2
α -Cardiac actin	1.1 $\pm$ 0.1	1.0 $\pm$ 0.1	1.1 $\pm$ 0.1	1.1 $\pm$ 0.1	1.1 $\pm$ 0.1	1.0 $\pm$ 0.1
α -Skeletal actin	1.6 $\pm$ 0.2**	1.2 $\pm$ 0.1†	1.3 $\pm$ 0.1*	0.9 $\pm$ 0.1†	1.4 $\pm$ 0.2*	1.1 $\pm$ 0.1††
ANP	2.7 $\pm$ 0.5**	1.5 $\pm$ 0.2†	2.2 $\pm$ 0.2**	1.4 $\pm$ 0.3†	3.0 $\pm$ 0.7**	1.4 $\pm$ 0.4†
Collagen I	2.2 $\pm$ 0.1**	1.2 $\pm$ 0.1††	1.4 $\pm$ 0.1*	1.0 $\pm$ 0.1†	1.5 $\pm$ 0.2*	0.8 $\pm$ 0.1††
Collagen III	2.5 $\pm$ 0.3**	1.2 $\pm$ 0.1††	1.5 $\pm$ 0.2*	1.2 $\pm$ 0.1†	1.9 $\pm$ 0.2**	1.0 $\pm$ 0.2††
SR Ca ²⁺ -ATPase	0.6 $\pm$ 0.1**	0.9 $\pm$ 0.1††	1.0 $\pm$ 0.1	1.1 $\pm$ 0.1	0.6 $\pm$ 0.2*	0.9 $\pm$ 0.1†
Na ⁺ -Ca ²⁺ exchanger	0.9 $\pm$ 0.1	1.0 $\pm$ 0.1	1.8 $\pm$ 0.1**	1.2 $\pm$ 0.2†	1.3 $\pm$ 0.3	1.1 $\pm$ 0.2

Control=1.0 $\pm$ 0.0–0.1. *P<0.05, **P<0.01 vs Control; †P<0.05, ††P<0.01 vs MI.

DISCUSSION

Doppler-echocardiography is currently the primary technique used for evaluating left ventricular diastolic function (25). Increased peak E wave velocity, decreased peak A velocity (or absent A wave), and rapid E-wave deceleration were observed in our rats, and these changes are in accordance with alterations of transmitral flow profiles observed in patients with heart failure with restrictive filling pattern. In experimental and clinical studies, ACE inhibitor treatment is reported to prevent diastolic dysfunction by using Doppler-echocardiography (26, 27). Candesartan cilexetil almost completely normalized the restrictive LV diastolic filling pattern, indicating that candesartan cilexetil might prevent diastolic dysfunction

after myocardial infarction.

There are several potential mechanisms whereby candesartan cilexetil might significantly improve diastolic dysfunction. First, we considered the potential effects of alterations in preload. Simply reducing LV filling pressure could explain a large portion of the improvement in the LV filling pattern. Central blood volume and venous tone are important components of preload. Both of these abnormalities may be corrected by candesartan cilexetil. Second, changes in the passive elastic properties of the myocardium could alter LV filling characteristics in diastole (28). The interstitial fibrosis is believed to increase cardiac stiffness and impair diastolic dysfunction (29, 30). In this study, the increased mRNAs of collagen types I and III were inhibited by candesartan cilexetil. The inhi-

bition of the interstitial collagen accumulation by candesartan cilexetil may prevent diastolic dysfunction after myocardial infarction. Third, a reduction of myocyte hypertrophy during candesartan cilexetil treatment may decrease LV chamber stiffness. In hypertrophic hearts, abnormalities of diastole are common. The prevention of cardiac hypertrophy in non-infarcted myocardium may contribute to improved LV filling characteristics. Fourth, we considered that the prevention of SR dysfunction contributed the prevention of diastolic dysfunction. Myocardial relaxation depends on the function of SR Ca^{2+} -ATPase, which mediates the reuptake of Ca^{2+} into the SR following each systole (31). Decreased expression of the SR Ca^{2+} -ATPase gene has been observed experimentally (32) and clinically in failing hearts (33). In this study, we demonstrated that candesartan cilexetil prevented the decrease of SR Ca^{2+} -ATPase mRNA expression in adjacent myocardium at twelve weeks, thereby indicating that the decrease of SR Ca^{2+} -ATPase activity may be prevented by candesartan cilexetil.

The Na^{+} - Ca^{2+} exchanger is a membrane component that mediates the facilitated bidirectional exchange of Na^{+} for Ca^{2+} across the sarcolemmal membrane (34, 35). Of the Ca^{2+} flux involved in excitation-contraction coupling, about 80% occurs across the SR membrane, and 20% of the Ca^{2+} flux is moved across the sarcolemma by two transport systems, the Na^{+} - Ca^{2+} exchanger and the plasma membrane Ca^{2+} pumps (34, 35). Studer et al. reported that Na^{+} - Ca^{2+} exchanger gene expression is enhanced in failing human hearts (36). They speculated that this increase in expression may compensate for depressed SR function via diastolic Ca^{2+} removal. In our study, Na^{+} - Ca^{2+} exchanger gene expression was enhanced in the myocardium without any change in the gene expression of SR Ca^{2+} ATPase at four weeks. The present work demonstrates that Studer's opinion is not the general rule in heart failure.

Another interesting aspect of the present work was the finding of a trend toward a decrease in relative wall thickness of the non-infarcted myocardium, and candesartan cilexetil improved global and regional systolic function after myocardial infarction. The decrease in LVPW % thickness in myocardial infarcted rats implies that the limits of compensation have been reached in the non-ischemic region. This in turn contributes to continuous afterload mismatch and provides an extrinsic mechanism for impairment of systolic function in the non-infarcted region. The decrease in afterload by candesartan cilexetil is reflected in our study by an increased fractional shortening and a decreased mean arterial pressure. Treatment also had an effect on preload, as evidenced in our study by a decrease in left ventricular diastolic diameter and a decreased LVEDP. This indicates a

shift of the pressure volume loop down and to the left, back to a more efficient and economic work range. This return to a more economic work will enable the heart to eject more forcefully and decrease the end-systolic volume.

Continued hypertrophy that is inadequate for normalizing increased wall tension activates an initially fetal program of gene expression, exemplified by an increase in β -MHC and α -skeletal actin and accompanied by an increase in the expression of ANP (11, 37, 38). Myosin and actin isoform shifts improve cardiac muscle efficiency and better correspond to the new velocity of contraction. In addition, ANP is upregulated in the ventricle with increased work load (39), which probably contributes to the increased level of circulating peptide that in turn tends to decrease preload and afterload. Both phenotypic conversions and ventricular production of ANP would normalize the working conditions of the cardiac pump. However, these changes become ineffective during the transition from hypertrophy to failure.

We have previously reported that fetal genes (β -MHC, α -skeletal actin, ANP) are predominantly expressed in adjacent non-infarcted myocardium (40). Left ventricular remodeling after myocardial infarction is accompanied by severe hypertrophy and dysfunction of the myocardium adjacent to the infarct (41). A specific and complex response to the mechanical and/or neurohumoral stimuli triggered by the left ventricular remodeling process may exist between the adjacent and remote myocardium. Candesartan cilexetil treatment not only prevented cardiac hypertrophy but also inhibited the gene expression of β -MHC, α -skeletal actin and ANP in both regions. These results support the theory that the AT1 receptor may be involved in the shift to fetal phenotype of myocytes after myocardial infarction as well as after ventricular hypertrophy.

In addition, candesartan cilexetil inhibited the increase of right ventricular weight and fetal and collagen gene expressions. These parameters of the right ventricle appear to be a useful marker of right ventricular remodeling in this model (42), and we showed that candesartan cilexetil prevents the right ventricular remodeling.

In summary, our study demonstrated that candesartan cilexetil prevents systolic and diastolic dysfunction and abnormal mRNA expression of the myocardium in infarcted rats. It follows that an intriguing possibility exists that suitable candesartan cilexetil administration after myocardial infarction may overcome systolic and diastolic dysfunction, thereby altering the natural course towards heart failure.

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