Effects of Angiotensin-converting Enzyme Inhibition on Changes in Left Ventricular Myocardial Creatine Kinase System After Myocardial Infarction: Their Relation to Ventricular Remodeling and Function

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We assessed the effects of angiotensin-converting enzyme (ACE) inhibition on changes in the myocardial intracellular creatine kinase (CK) system in relation to left ventricular (LV) remodeling and function in heart failure after myocardial infarction (MI) in rats. We compared the findings at 4 weeks after MI to those at 12 weeks after MI. LV weight and chamber size were significantly increased and percent fractional shortening (%FS) was decreased in untreated MI rats compared with normal control animals both at 4 and 12 weeks after MI. Animals with MI and treated with the ACE inhibitor temocapril showed significantly reduced LV weight and chamber size and increased %FS compared with untreated MI rats at 12 weeks after MI, but not at 4 weeks after MI. At 4 weeks after MI, no significant changes were found in the total creatine and relative distribution of each CK isoenzyme in either the temocapril-treated or untreated animals with MI compared with the normal controls. In contrast, at 12 weeks after MI, untreated MI rats showed significant reductions in the total creatine and mitochondrial and MM-CK fractions and increases in the MB- and BB-CK fractions compared with the controls. The alterations in the mitochondrial and MB-CK fractions were significantly attenuated after 12 weeks of ACE inhibition. Thus, LV myocardial energy metabolism is progressively impaired and its alteration is not related to the magnitude of geometric changes and LV dysfunction after MI. Most of the beneficial effects of ACE inhibition were observed at 12 weeks after MI. Our results may provide an insight into the therapeutic strategy of ACE inhibition in chronic heart failure after MI. (Jpn Heart J 2003; 44: 537-546)

Key words: Total creatine, Total creatine kinase, Creatine kinase isoenzyme, Chronic heart failure

Recent studies suggest that long-term angiotensin-converting enzyme (ACE) inhibition exerts beneficial effects on functional and structural changes1-4 as well as impaired myocardial energy metabolism5 in the setting of chronic heart failure
after experimental myocardial infarction (MI). Treatment with \( \beta \)-adrenergic receptor blockade is also accompanied by energetically favorable effects in the same condition.\(^5,6\) In general, alterations of energy metabolism in the chronically failing heart are characterized by diminished energy reserve of the noninfarcted left ventricular (LV) myocardium in association with a marked decrease in creatine phosphate (CP), total creatine (CR), total creatine kinase (CK), and mitochondrial CK isoenzyme activity and shifts of CK isoenzymes and lactate dehydrogenase isoenzymes.\(^5-14\) In human heart failure, it has been reported that alterations of the myocardial CK system are associated with a depressed total enzyme activity and changes in isoenzyme distribution and contribute to the pathogenesis of heart failure.\(^15,16\) However, there have been no detailed reports on the changes in myocardial energy metabolism after MI and their relation to LV remodeling and function in heart failure.

The present study was designed to assess changes in the CK system and effects of ACE inhibition in relation to the progression of LV remodeling and dysfunction secondary to MI. We compared the findings at 4 weeks after MI to those at 12 weeks after MI in a rat model of MI. We also examined the relationship between the myocardial CK system and LV function.

**METHODS**

**Experimental protocol:** Female Sprague-Dawley rats weighing 210-260 g were anesthetized with 30 mg/kg sodium pentobarbital given intraperitoneally. Complete occlusion of the proximal left coronary artery was performed under ventilation with a rodent ventilator. One day after coronary occlusion, the rats were randomized to either an ACE inhibitor group, (temocapril, 80 mg/L in drinking water, \( n = 10 \)) or placebo group (\( n = 9 \)) for 4 weeks. Other rats subjected to coronary ligation were randomly assigned to either temocapril (80 mg/L in drinking water, \( n = 11 \)) or placebo (\( n = 13 \)) groups for 12 weeks. Sham-operated rats received control chow and served as controls. The rats underwent echocardiographic studies and then were killed. The wet weights of the individual cardiac chambers were measured. The noninfarcted LV myocardium was rapidly frozen in liquid nitrogen. The frozen myocardium was stored at \(-80^\circ\text{C}\) for biochemical analyses. Other parts of the LV myocardium were fixed by immersion in 10% formalin, sliced into 3 pieces in parallel to the atroventricular groove, and embedded in paraffin for morphological investigation.\(^17,18\) The experimental protocol was approved by the Animal Subjects Committee of Shinshu University.

**Echocardiographic studies:** Transthoracic echocardiography was performed using a commercially available system (SSH-30A, Toshiba, Tokyo Japan), with a 9.0 MHz transducer or SONOS 5000, Hewlett-Packard Co., Andover, MA, with
a 12.0 MHz transducer). Animals were anesthetized with a mixture of ketamine hydrochloride (100 mg/kg) and xylazine (10 mg/kg) given intraperitoneally. From the M-mode imaging, the LV internal dimensions were measured and the percent fractional shortening (%FS) was calculated.\textsuperscript{19,20) MI size measurement:} Three-\(\mu\)m thick sections derived from 3 slices of the LV were cut and then mounted and stained with Azan-Mallory stain and analyzed blindly to assess MI size. The percentage of MI size was determined from the ratio of the sum of the scar lengths along the endocardial and epicardial surfaces to the sum of the total endocardial and epicardial circumferences.\textsuperscript{17,18,20) High performance liquid chromatographic (HPLC) analyses:} The noninfarcted LV myocardial tissue was homogenized in 0.42 N perchloric acid, keeping the sample temperature at 4°C, and an aliquot of the homogenate was removed for protein determination. The homogenate was neutralized and centrifuged. The supernatant was used for measuring total CR, including free CR and CP, by HPLC, as previously described.\textsuperscript{21,22) The HPLC system consisted of two pumps and a spectrophotometer with a recorder (LC-6A, Shimadzu, Kyoto, Japan) and a reversed-phase column (Licrosorb RP-18, 10 \(\mu\)m, Kanto Chemicals, Tokyo). Enzyme analyses:} Each sample with 20 to 25 mg of tissue was homogenized in 66 mMol phosphate buffer solution (pH 7.4) containing 1 mMol EGTA, 1 mMol beta-mercaptoethanol, and 0.1\% Triton X-100 at 4°C using an Ultra Turrax IKA CO & KG, Germany homogenizer. An aliquot for protein measurements was taken before the addition of Triton. All samples were kept on ice. Total CK activities and protein concentrations were measured spectrophotometrically (7170 auto-analyzer, Hitachi, Tochigi, Japan). The relative distribution of 4 CK isoenzymes, including the mitochondrial, MM-, MB-, and BB-CK fractions, was measured using a Rapid Electrophoresis System as a separation unit and an REP CK isoforms kit for agarose gel and incubation solution (Helena Diagnostica GmbH, Germany), as described elsewhere.\textsuperscript{6) Electrophotograms were analyzed by quantifying the separate isoenzyme bands using a densitometer. All of the biochemical values are expressed as per mg protein with the aid of the biuret method. Data analyses and statistics:} Data are presented as the mean \(\pm\) SD. One-way analysis of variance was used to analyze the differences in the data among the groups. A probability value of < 0.05 was accepted as statistically significant.

\textbf{RESULTS}

\textbf{Infarct size, cardiac weights, and echocardiographic data:} The average MI size, ranging from 35 to 50\%, was similar between animals both at 4 weeks and 12 weeks after MI. LV weight, absolute and normalized to body weight (BW), was significantly increased in untreated MI rats compared with normal control ani-
mals both at 4 and 12 weeks after MI. This parameter was significantly decreased at 12 weeks after MI, but not at 4 weeks after MI, in MI rats treated with the ACE inhibitor temocapril (Table).

LV end-diastolic dimension (EDD), absolute and normalized to BW, was significantly increased and LV %FS was decreased in the untreated MI rats compared with the normal control animals both at 4 weeks and 12 weeks after MI. Although there were no significant differences in these measurements between the MI rats treated with and without ACE inhibition at 4 weeks after MI, temocapril-treated animals with MI showed significantly reduced LVEDD and increased %FS compared with untreated MI rats at 12 weeks after MI (Table).

**Total CR levels and CK activities and isoenzyme distribution:** As shown in the Table and Figure 1, the total CR levels were decreased by 8.7% ($P = NS$) at 4 weeks and by 37.5% ($P < 0.01$) at 12 weeks after MI in the untreated MI rats compared with the normal control animals. There were significant reductions in total CK activity both at 4 weeks ($P < 0.05$) and 12 weeks ($P < 0.01$) after MI. These

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Control = normal control animals; Untreated MI = untreated rats with myocardial infarction (MI); Temocapril-treated MI = rats with MI treated with temocapril. Values are mean ± SD.

BW = body weight; EDD = end-diastolic dimension; FS = fractional shortening; LV = left ventricle.

*$P < 0.05$ and $**P < 0.01$ vs normal control animals. $+P < 0.05$ and $++P < 0.01$ vs untreated MI rats.
decreased levels of total CR and CK activity were suppressed by 12 weeks of treatment with ACE inhibition (Figure 1).

At 4 weeks after MI, no significant changes were observed in the relative distribution of each CK isoenzyme in either the temocapril-treated or untreated animals with MI compared with the controls (Figure 2). On the other hand, at 12 weeks after MI, untreated MI rats showed significant reductions in the mitochondrial and MM-CK fractions and increases in the MB- and BB-CK fractions compared with the control animals. These alterations in the mitochondrial and MB-fractions reverted to the control levels after 12 weeks of ACE inhibition (Figure 2).

In MI rats treated with and without ACE inhibition for 12 weeks, the LV %FS was positively correlated with total CR, CK, and mitochondrial CK fraction and inversely correlated with the MB-CK fraction (Figure 3).

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**Figure 1.** Total creatine (CR) levels and creatine kinase (CK) activities in the residual myocardium among the 3 groups. Values are mean ± SD. *P < 0.05 and **P < 0.01 vs normal control rats and +++P < 0.01 vs untreated MI rats (U). MI = myocardial infarction; T = temocapril-treated MI rats.
Figure 2. Relative distribution of each CK isoenzyme among the 3 groups. Values are expressed as the percentage of total CK activity. Data represent the mean ± SD. *p < 0.05 and **p < 0.01 vs normal control rats and +p < 0.05 and ++p < 0.01 vs untreated MI rats.
In this study, we found progressive alterations of the myocardial intracellular CK system in a rat model of heart failure after MI. These changes were in agreement with previous findings in experimental heart failure and in end-stage human heart failure, and were characterized by a significant shift of cardiac CK isoenzymes towards the fetal BB- and MB-CK isoenzymes. This suggests a compensatory mechanism towards a more efficient cardiac CK system to improve cardiac energy metabolism in the LV dysfunctional condition. The CK
system was partially impaired at 4 weeks after MI in rats and the changes in the system were further prominent at 12 weeks after MI. In contrast, LV remodeling was accomplished and LV myocardial hypertrophy and systolic dysfunction were apparent at 4 weeks after MI, as demonstrated by histological and echocardiographic analyses. These observations suggest that LV myocardial energy metabolism is progressively impaired and its alteration is not related to the magnitude of geometric changes and LV dysfunction after MI. In addition, 12 weeks, not 4 weeks, of ACE inhibition was associated with a marked improvement of the impaired myocardial CK system. Thus, our results may provide insight into the therapeutic strategy of ACE inhibition in relation to myocardial energy metabolism in chronic heart failure after MI.

Relation of LV myocardial CK system to LV dysfunction and remodeling: It is generally recognized that there are increases in total CK activity and the mitochondrial CK fraction and decreases in fetal isoenzyme MB- and BB-CK fractions during the development of fetal to adult normal hearts. A number of investigators reported changes in the cardiac CK system in chronic heart failure after experimental MI in rats, usually at 8 weeks after coronary ligation. These included reductions in total CK and mitochondrial CK activities and decreased total CR levels, as shown at 12 weeks after MI in the present study. In addition, a decreased mitochondrial CK fraction and increased MB- and BB-CK fractions were also characterized. However, these data were limited to the relatively chronic stage of heart failure when most postinfarction adjustments of the cardiovascular system had been completed, and no reports have examined in detail the relation between the changes in the myocardial CK system to LV remodeling and dysfunction in vivo after MI in rats.

In the present study, from 4 to 12 weeks after MI, progressive abnormalities in the CK system developed despite no significant changes in the magnitude of LV myocardial hypertrophy, chamber size, or systolic dysfunction over the period. Our results clearly demonstrate that the CK system was slightly deranged at the early stage and marked impairment occurred at the late stage of heart failure secondary to MI. LV myocardial energy metabolism was progressively impaired and its alteration was not related to the magnitude of geometric changes and LV dysfunction.

Effects of ACE inhibition on myocardial CK system: It has been reported that 8 weeks of bisoprolol treatment early after MI is effective at preventing changes in the myocardial CK and lactate dehydrogenase systems. In the present study, after treatment with the ACE inhibitor temocapril, LV hypertrophy, chamber dilatation, and decreased systolic function were suppressed at 12 weeks after MI alone. This treatment was associated with improvement of the impaired myocardial CK system, including decreased total CR, CK, and mitochondrial CK activi-
ties and increased MB-CK fractions. These findings are in accordance with those of previous studies in which the CK system was examined at 8 weeks after MI. In our study, however, the favorable effects on the CK system were not apparent at 4 weeks after MI. Thus, our results may provide insight into the therapeutic strategy of ACE inhibition in relation to myocardial energy metabolism in heart failure after MI.

Another point of interest in the present study was the finding that significant correlations were found between the LV %FS and several variables of the myocardial CK system. This implies that LV systolic function is closely related to the myocardial CK system in this rat model of heart failure following MI.

**REFERENCES**