Changes in Hsp60 Level of the Failing Heart Following Acute Myocardial Infarction and the Effect of Long-Term Treatment with Trandolapril

Wakako TOGA,[a] Kouichi TANONAKA,[b] and Satoshi TAKEO*,[b]

[a] *Tsukuba Research Institute, Novartis Pharma K.K.; Ohkubo 8, Tsukuba, Ibaraki 300–2611, Japan: and* [b] *Department of Molecular and Cellular Pharmacology, Tokyo University of Pharmacy and Life Sciences; 1432–1 Horinouchi, Hachioji, Tokyo 192–0392, Japan.* Received August 19, 2006; accepted October 23, 2006; published online October 24, 2006

Changes in heat shock protein (Hsp) 60 of the viable left ventricular muscle (viable LV) after myocardial infarction in rats and the effect of the angiotensin I-converting enzyme inhibitor (ACEI) trandolapril were examined. Myocardial infarction was induced in rats by ligation of the left coronary artery. The coronary artery-ligated (CAL) and sham-operated (Sham) rats were orally treated with 3 mg/kg/d trandolapril from the 2nd to 8th week after surgery. Hemodynamic parameters and tissue weights of the left and right ventricles of the animals at the 8th week after CAL (8w-CAL rats) showed signs indicating chronic heart failure. An increase in Hsp60 content, a decrease in mitochondrial oxygen consumption rate (OCR), and an increase in the mitochondrial thiobarbiturate-reacting substance (TRS) of the viable LV were detected. Eight weeks after CAL. Long-term treatment of the CAL rats with trandolapril improved the hemodynamic parameters, attenuated the CAL-induced increase in Hsp60 content, the decrease in mitochondrial OCR, and the increase in the mitochondrial TRS content of the viable LV at the 8th week after myocardial infarction. The increase in Hsp60 content was closely related to the decrease in the mitochondrial OCR and to a rise in the LVEDP of the CAL animal at the 8th week after myocardial infarction. These results suggest that a series of pathophysiological alterations, including a reduction in mitochondrial function, appearance of reactive oxygen stress, and production of Hsp60 is involved in the development of cardiac failure and that trandolapril is beneficial for preventing these alterations.

Key words angiotensin I-converting enzyme inhibitor; heart failure; Hsp60; mitochondria; myocardial infarction; trandolapril

An exposure of cells, tissue, or whole body to various kinds of sublethal stress such as heat shock, toxins, heavy metal ions, and chemicals results in the induction of heat shock proteins (Hsps). Hsps are considered to be involved in the maturation of newly synthesized proteins and in the degradation of damaged proteins, leading to cell proliferation, differentiation, adaptation to various circumstances, and protection of cells against stress-induced damage. In a previous study, we examined the beneficial roles of Hsp70 in the viable left ventricle in the pathogenesis of chronic heart failure.[1] Hsp60 is a chaperon located in the mitochondrial matrix space under physiological conditions,[2,3] and it may be involved in protein transport from the cytosol into mitochondria across the mitochondrial inner membrane.[4] Recently, Knowlton *et al.* showed an increase in the Hsp60 content of the human heart at the end stage of both ischemia- and dilatation-elicited cardiomyopathies.[5] Several investigators have reported that antibodies against Hsp60 appeared in the serum of the patients with acute coronary syndrome or hypertension.[6,7] In a previous study, we showed a decrease in high-energy phosphates of the failing heart following acute myocardial infarction.[8] These findings suggest that an increased level of myocardial Hsp60 plays some roles in the development of heart failure. Thus, in the present study, we examined the relationship between the Hsp60 content and mitochondrial function in the viable cardiac muscle after myocardial infarction.

In earlier reports on our ongoing series of studies on myocardial infarction, we showed that when rats with myocardial infarction were treated for 6—10 weeks with angiotensin I-converting enzyme inhibitor (ACEI) trandolapril, there were improvements in their hemodynamics, energy metabolism, β-adrenoceptor signaling, and heat shock-induced Hsp72 production.[9—11] However, the effects of long-term treatment with trandolapril on the increase in myocardial Hsp60 after myocardial infarction remain unclear. Therefore, the second purpose of the present study was to examine the effect of trandolapril on the increase in Hsp60 during the development of heart failure after myocardial infarction.

MATERIALS AND METHODS

Animals Male Wistar rats (SLC, Hamamatsu, Japan), weighing 210—240 g, were used in the present study. The animals were conditioned according to 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). The protocol of this study was approved by the Committee of Animal Use and Welfare of Tokyo University of Pharmacy and Life Sciences.

Operation Myocardial infarction of rats was produced by occlusion of the left ventricular coronary artery according to the method described previously.[8] The left coronary artery was ligated approximately 2 mm from its origin with a suture under artificial ventilation with air (CAL rats). We eliminated animals from the present study according to 2 criteria, *i.e.*, the absence of an abnormal Q wave (greater than 0.3 mV) in ECG (lead I) measured 2 d after myocardial infarction and a more than 10 g increase in body weight at 14 d after CAL.[12] By these criteria, approximately 65% of the CAL rats were consistently selected and these animals showed approximately 40% infarct area covering approximately 40% of their left ventricle. Sham-operated rats (Sham rats) were treated in a similar manner except that CAL was not performed.

Drug Treatment Oral treatment of the selected CAL rats with 3 mg/kg per day of trandolapril (Aventis Pharma Japan, Tokyo, Japan) was performed from the 2nd to the 8th week after the operation. Trandolapril was suspended in

* To whom correspondence should be addressed. e-mail: takeos@ps.toyku.ac.jp

0.25% carboxymethylcellulose sodium for the oral administration. In a preliminary study, the effect of various doses of trandolapril ranging from 0.3 to 10 mg/kg/d on the increase in myocardial Hsp60 in CAL rats was examined. We found that the dose of 3 mg/kg/d was the most effective one for preventing the increase in Hps60 content of the CAL rats at the 8th week. This dose of the agent employed were similar to that for studying the effects of trandolapril on hemodynamic parameters in previous studies.[9,13] Treatment with the drug starting at an earlier time after CAL increased the mortality of CAL animals.

Measurement of Hemodynamic Parameters Two and 8 weeks after the operation, CAL (2w- and 8w-CAL) and Sham (2w- and 8w-Sham) rats were anesthetized with a gas mixture of nitrous oxide–oxygen (3 : 1) and 0.5—2.5% enflurane at a flow rate of 600 ml/min through a mask loosely placed over the nose ($n=15$ each).[12] The pO_2, pCO_2, and pH of the blood were 95—110 mmHg, 35—41 mmHg, and 7.37—7.41, respectively. A microtip pressure transducer (SPC 320, Miller Instrument, Houston, TX, U.S.A.) was introduced into the left ventricle through the right carotid artery to measure left ventricular systolic and end-diastolic pressures (LVSP and LVEDP, respectively). The arterial blood pressure and heart rate (HR) were measured by means of a pressure transducer attached to a cannula placed into the right femoral artery.[9]

Western Immunoblot After determination of the hemodynamics, the heart was quickly excised. The isolated hearts were divided into the infarct area and the viable left ventricle including the intermediate septum (viable LV), and then their tissue weights were measured. The myocardium was used for Western blot analysis of Hsp60 and for determination of the mitochondrial activity. The tissue was homogenized in a suspension buffer (320 mM sucrose, 10 mM Tris–HCl, pH 7.4). The protein concentration of the fraction was determined by the method of Lowry *et al.*[14] and then the proteins were used for Western immunoblot analysis of Hsp60, performed with some modifications according to the method described earlier.[1]

For determination of myocardial Hsp60, the homogenate was electrophoresed through a 10% SDS-polyacrylamide gel and the protein was subsequently transferred to a polyvinylidene difluoride (PVDF) membrane (Immobilon, Millipore Co., Bedford, MA, U.S.A.). The membrane was then incubated with antibody against Hsp60 (MAB3104, Chemicon, Temecula, CA, U.S.A.) that had been diluted to 1 : 3000 in Tris-buffered saline containing 10% Block Ace^R (Dainippon Pharm, Osaka, Japan) and 0.1% Tween 20. After incubation with the antibody for 12 h at 4°C, the separated protein on the membrane was detected by using as secondary antibody and ECL^R reagent (Amersham Pharmacia Biotech, Buckinghamshire, U.K.). Quantification was performed by the method described previously.[1]

Measurement of Mitochondrial Oxygen Consumption in Skinned Bundles The mitochondrial oxygen consumption rate (OCR) of skinned bundles, an index for the functional activity of mitochondria, was determined by the method of Sanbe *et al.*[8] The myocardial bundles were prepared from the viable LV. The bundles were transferred into relaxing medium A of the following composition (mM): EGTA, 10; $MgSO_4$, 3; taurine, 20; dithiothreitol, 0.5; imidazole, 20; potassium 2-(*N*-morpholino)-ethanesulfonate, 160; ATP, 5; CP, 15 (pH 7.0). The bundles were incubated for 20 min in the medium A additionally containing 75 μg/ml saponin. After incubation, the myocardial bundles were washed with fresh medium B (medium A without ATP and CP but supplemented with 0.5% bovine serum albumin). The OCR of the skinned bundles was determined in medium B at 30°C by means of a Clark-type electrode (Central Kagaku, Tokyo). The basal OCR (V_0) was measured following the addition of 5 mM glutamate, 3 mM malate, and 3 mM KH_2PO_4. Total (maximal) OCR (V_{max}) was measured after the further addition of 1 mM ADP and 7.5 mM creatine. The ADP-stimulated OCR (V_{ADP}) of the skinned bundles was taken as the difference between V_{max} and V_0. After the determination of the OCR, the skinned bundles were solubilized with 1 N NaOH, and then the protein concentration was determined.

Measurement of Mitochondrial Thiobarbiturate-Reacting Substance (TRS) Content In another experiment, the level of TRS, a marker of free-radical formation, in the perfused heart was determined by the method of Ohkawa *et al.*[15] After anesthesia with 50 mg/kg pentobarbital, i.p., the heart was quickly removed and the mitochondrial fraction was prepared from the viable LV according to the method described previously.[16] Briefly, the viable LV was homogenized in ice-cold buffer containing 180 mM KCl, 10 mM EGTA (pH 7.4), and 0.5% fatty acid-free BSA. After centrifugation of the homogenate at 1000×g for 10 min, the resultant supernatant solution was centrifuged at 8000×g for 10 min. The crude mitochondria in the pellet were suspended in the buffer and again centrifuged at 8000×g for 10 min. Finally the mitochondria were resuspended in suspension buffer (320 mM sucrose, 20 mM Tris–HCl, pH 7.4) and used for the measurement of mitochondrial activity.

The prepared mitochondrial fraction (approximately 1 mg protein) was mixed with 0.5 ml of 1.15% KCl solution. A 200-μl aliquot of the homogenate was added to the reaction medium of the following composition: 40 μl of 8.1% SDS, 300 μl of 20% acetic acid, and 300 μl of 0.67% thiobarbituric acid. The reaction mixture was then incubated at 95°C for 60 min. The red pigment produced during the incubation was extracted with 1 ml of *n*-butanol, and its absorbance at 532 nm was measured by using a spectrophotometer (U-Best 30, JASCO, Hachioji, Japan). The myocardial content of TRS as malondialdehyde was calculated as described previously.[17]

Statistics The results were expressed as means±S.E.M. The statistical significance of differences in hemodynamic parameters, tissue weight, infarct size, myocardial Hsp60 content, and OCR of myocardial skinned bundles was estimated by using two-way analysis of variance (ANOVA) followed by Fisher's PLSD correction for multiple pairwise comparisons. The relationship between 2 parameters was calculated by the least squares method. Differences with a probability of 5% or less were considered to be significant ($p<0.05$).

RESULTS

Hemodynamics Table 1 shows changes in hemodynamic indices of the CAL and Sham rats at the 2nd and 8th weeks after the operation. The animals were treated with either trandolapril or vehicle. As compared with those of the

Table 1. Changes in Hemodynamic Parameters of Control, Sham-Operated (Sham), and Coronary Artery-Ligated (CAL) Rats Untreated (Un) or Treated with Trandolapril (Tra)

		MAP (mmHg)	HR (beats/min)	LVSP (mmHg)	LVEDP (mmHg)	+LV dP/dt (mmHg/min)	−LV dP/dt (mmHg/min)
Control		110±2	405±5	141±3	1.9±0.4	11002±314	10027±345
2nd week							
Sham		108±2	400±4	139±3	1.8±0.5	10934±312	9798±300
CAL		101±2#	397±4	134±4	20.7±0.9#	7902±251#	5141±255#
8th week							
Sham	Un	116±2	402±4	149±3	1.2±0.5	12490±345	10555±293
	Tra	95±2*	399±3	125±2*	1.2±0.4	11396±337	10454±365
CAL	Un	106±3#	396±3	135±2#	32.9±1.1#	8038±234#	5075±257#
	Tra	91±3#*	401±3	114±2#*	16.9±1.1#*	8384±197#	5869±195#

Each value represents the mean±S.E.M. of 6 experiments. # Significantly different from the corresponding sham-operated rats ($p<0.05$). * Significantly different from the corresponding drug-untreated rats ($p<0.05$). Abbreviations: MAP, mean arterial pressure; HR, heart rate; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure.

Table 2. Changes in Body and Tissue Weights and Myocardial Infarct Size of Control, Sham-Operated (Sham), and Coronary Artery-Ligated (CAL) Rats Untreated (Un) or Treated with Trandolapril (Tra)

		Body weight (g)	LV weight (mg)	LV/BW (mg/g)	Lung weight (g)	Lung/BW (mg/g)	Infarct size (% LV)
Control		229±3	431±9	1.88±0.07	0.83±0.03	3.62±0.13	0
2nd week							
Sham		257±6	511±11	1.78±0.06	0.92±0.02	3.57±0.14	0
CAL		229±5#	508±21	2.22±0.03#	2.28±0.11#	9.77±0.21#	41.4±1.7
8th week							
Sham	Un	323±7	619±9	1.92±0.06	1.04±0.03	3.21±0.11	0
	Tra	295±4*	494±11*	1.67±0.06*	0.98±0.05	3.32±0.08	0
CAL	Un	290±3#	604±16	2.08±0.07	2.88±0.11#	9.93±0.55#	42.3±0.99
	Tra	271±3#*	508±15*	1.87±0.05*	1.76±0.10#*	6.49±0.21#*	41.7±1.55

Each value represents the mean±S.E.M. of 6 experiments. # Significantly different from the corresponding sham-operated group ($p<0.05$). * Significantly different from the corresponding drug-untreated group ($p<0.05$). Abbreviations: BW, body weight; LV, left ventricular tissue including the intermediate septum.

Sham rats, the MAP and LVSP of the 2w- and 8w-CAL rats were decreased, whereas the HR did not change throughout the experiment. In contrast, the LVEDP of the 2w-CAL rats was increased to become 12-fold over the Sham value, and then further increased at the 8th week after CAL (to 20-fold over the Sham value). There were no significant changes in these hemodynamic parameters of the Sham rats throughout the experiment.

When CAL and Sham rats were treated with either trandolapril or vehicle, the MAP and LVSP of both groups were decreased as compared with those of the corresponding drug-untreated 8w-CAL or 8w-Sham rats. The value for the LVEDP of the 8w-CAL rats treated with trandolapril was similar to that of the 2w-CAL rats. Treatment of the Sham rats with trandolapril did not affect their LVEDP throughout the experiment.

Tissue Weight The left ventricular tissue and lung weights of rats at the 2nd and 8th week after sham and CAL operations are shown in Table 2. The values for the body weight of the 2w- and 8w-Sham rats were approximately 120 and 150%, respectively, of the control value, whereas the value of the 8w-Sham rats treated with trandolapril was decreased. There were no significant differences in the left ventricular weight between the 2w-CAL and 2w-Sham rat or between the 8w-CAL and 8w-Sham untreated rats. The body weight of the trandolapril-treated CAL rats was smaller than that of the untreated 8w-CAL rats. The left ventricular (LV) weight-to-body weight ratio (LV/BW) of the 2-w CAL rat did not significantly differ from that of the 8w-CAL rat. Treatment with trandolapril significantly attenuated the increase in the LV/BW of the 8w-CAL rat.

Both lung weight and the ratio of lung weight-to-body weight (Lung/BW) of the 2w- and 8w-CAL rats were significantly increased compared with those of the corresponding Sham group. Treatment with trandolapril significantly attenuated the increase in the Lung/BW of the 8w-CAL rat.

In another set of experiments, myocardial infarct areas were determined. The infarct areas of the 2w- and 8w-CAL rats covered approximately 40% of the left ventricle, whereas no infarction was detected in the myocardium of the control and Sham rats. Treatment with the drug did not affect the infarct size in the CAL rats.

Hsp60 Content in the Viable Left Ventricular Muscle Figure 1 shows changes in the Hsp60 content of the viable left ventricular muscle (viable LV) in Sham and CAL rats at the 2nd and 8th week after the operation. The myocardial Hsp60 content of the Sham rat was similar to that of the control rat throughout the experiment. The Hsp60 content of the 2w-CAL rat was approximately 100% of the value for the 2w-Sham rat. In contrast, the content of the 8w-CAL rat was increased to approximately 190% of that for the 8w-Sham rat.

Treatment of the Sham rat with trandolapril from the 2nd to 8th week after the operation did not alter the myocardial Hsp60 content. Treatment of the CAL rat with trandolapril attenuated the increase in the Hsp60 content of the viable LV

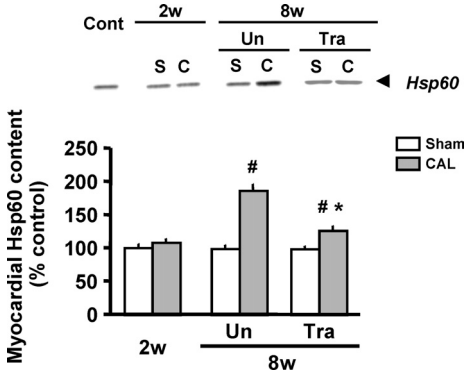

Fig. 1. Hsp60 Content in the Viable LV of the Sham and CAL Rats 2 Weeks (2w) and 8 Weeks (8w) after the Operation and the Effects of Trandolapril (Tra) on the Protein Content in the Viable LV of Sham and CAL Rats 8 Weeks after the Operation

Western blots in the upper panel indicate 60-kDa bands representing Hsp60 in the viable LV. "Un" indicates animals without drug treatment. Each value represents the mean±S.E.M. of 6 experiments. #$p<0.05$ *vs.* the corresponding sham-operated group. *$p<0.05$ *vs.* the corresponding drug-untreated group at the 8th week.

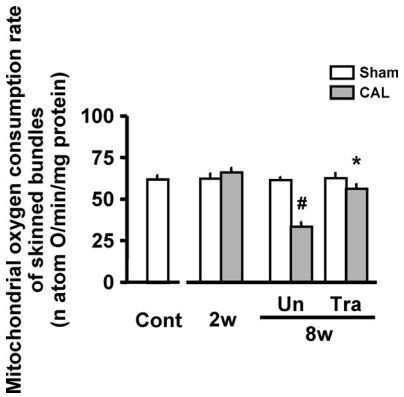

Fig. 2. Mitochondrial Oxygen Consumption Rate (OCR) of Skinned Bundles Prepared from the Viable LV of the Control, Sham, and CAL Rats 2 Weeks (2w) and 8 Weeks (8w) after the Operation and the Effects of Trandolapril (Tra) on the OCR in the Viable LV of Sham and CAL Rats 8 Weeks after the Operation

"Un" indicates animals without drug treatment. Each value represents the mean± S.E.M. of 6 experiments. #$p<0.05$ *vs.* the corresponding sham-operated group. *$p<0.05$ *vs.* the corresponding drug-untreated group at the 8th week.

(approximately 130% of that for the corresponding 8w-Sham rat).

Mitochondrial Oxygen Consumption The ADP-stimulated mitochondrial OCR of the left ventricular skinned bundles prepared from CAL and Sham rats is shown in Fig. 2. The OCR for the control animals was 62.2±2.5 nano-atom O/min/mg protein ($n=6$). There were no significant differences in the OCR of skinned bundles among control, 2w-, and 8w-Sham rats. The OCR of the 8w-CAL rat was decreased to approximately 55% of the value for the 8w-Sham rat, whereas the OCR value for the 2w-CAL rat tended to be greater than that of the 2w-Sham rat ($p=0.071$, $n=6$).

The mitochondrial OCR for the skinned bundles of the 8w-Sham rat treated with trandolapril was similar to that of the control animal. The OCR value for the 8w-CAL rat treated with the drug was approximately 90% of that for the corresponding Sham animal.

Relationship between Hsp60 Content and LVEDP or Mitochondrial OCR The Hsp60 content in the viable LV

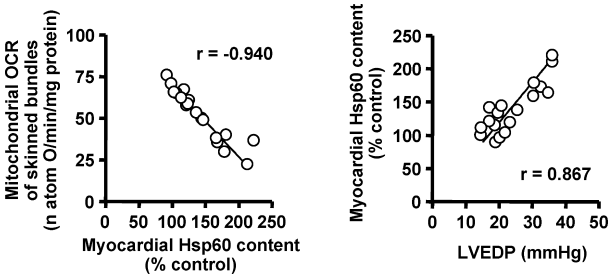

Fig. 3. Relationships between Either Mitochondrial Oxygen Consumption Rate (OCR; Left Panel) or Left Ventricular End-Diastolic Pressure (LVEDP; Right Panel) and Hsp60 Content in the Viable LV of the 2w-CAL Rat and 8w-CAL Rats Treated or Not with Trandolapril

There was a significant relationship between LVEDP or mitochondrial OCR and Hsp60 content in the viable LV ($n=18$; $p<0.05$).

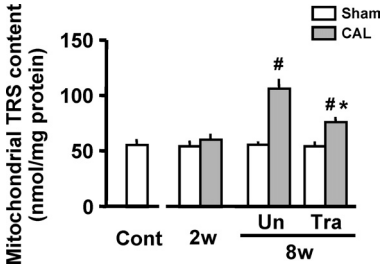

Fig. 4. Thiobarbiturate-Reacting Substance (TRS) Content of Mitochondrial Fraction Prepared from the Viable LV of the Control, Sham, and CAL Rats 2 Weeks (2w) and 8 Weeks (8w) after the Operation and the Effects of Trandolapril (Tra) on the Mitochondrial TRS Content in the Viable LV of Sham and CAL Rats 8 Weeks after the Operation

"Un" indicates animals without drug treatment. Each value represents the mean± S.E.M. of 6 experiments. #$p<0.05$ *vs.* the corresponding sham-operated group. *$p<0.05$ *vs.* the corresponding drug-untreated group at the 8th week.

of the 2w-CAL rat and the 8w-CAL rat treated without and with trandolapril was plotted against either the mitochondrial OCR or LVEDP ($n=18$, Fig. 3). The mitochondrial OCR of the viable LV after myocardial infarction was inversely related to the Hsp60 content ($r=-0.940$; left panel in Fig. 3), whereas the latter was positively correlated with the LVEDP ($r=0.867$; right panel in Fig. 3).

Mitochondrial TRS Content Figure 4 shows the mitochondrial TRS content of the viable LV of the Sham and CAL rats after the operation. The TRS content of the control rat was approximately 55 nmol/mg protein ($n=5$). The values for the mitochondrial TRS content of the 2w- and 8w-Sham rats were similar to the value of the control regardless of treatment or not with trandolapril. Mitochondrial TRS content of the 8w-CAL rat increased to approximately 2-fold the control value ($n=5$). In contrast, the mitochondrial TRS content of the viable LV in the CAL rat treated with trandolapril was approximately 135% of the control value.

DISCUSSION

In the present study, we observed decreases in the LVSP, positive dP/dt (+LV dP/dt), and negative LV dP/dt (−LV dP/dt) and an increase in the LVEDP of the 8w-CAL rat. These findings indicate possible signs of chronic heart failure in this model, and are consistent with those in our previous studies.[9—11,13,18] In this model, we also observed left ventricular dysfunction with a decrease in cardiac output

index at the 8th week after CAL, whereas the cardiac function of the 2w-CAL rat was compensated in previous studies.[8,12] Several clinical trials showed that ACEIs favorably affected the hemodynamics, improved the clinical symptoms,[19,20] reduced the overall mortality, and ameliorated the left ventricular dysfunction in patients with congestive heart failure.[20—22] Trandolapril attenuated the rise in LVEDP of the 8w-CAL rat, reduced the hypertrophy of the viable LV, and decreased the preload and afterload in the present as well as in previous studies.[9,23]

We observed a decrease in LV/BW ratio in trandolapril-treated Sham and CAL animals at the 8th week after the operation, which was associated with concomitant decreases in the body weight and left ventricular weight as compared with those of the corresponding untreated animal. It is generally admitted that ACEIs reduce a release of aldosterone from the adrenal cortex and exert the diuretic effect,[24] and thus elicit a decrease in the body weight of animals. Furthermore, ACEIs decrease blood angiotensin II levels and thus decline MAP of animals, which may lead to reduction in pressure overload *in vivo* animals. Such reduction is capable of decreasing the heart weight of ACEI-treated Sham and CAL animals. This may account for the decrease in the LV/BW ratio of the trandolapril-treated Sham and CAL animals. The structural and hemodynamic alterations as above suggest that long-term treatment with trandolapril is beneficial for the improvement of cardiac function and remodeling after myocardial infarction in chronic heart failure.

The lower level of Hsp60 was related to increased mitochondrial energy-producing ability and reduced LVEDP of the 8w-CAL rat heart. What are the roles of Hsp60 in the pathophysiological alterations seen in chronic heart failure? Since cardiac contraction requires energy that is mainly produced by mitochondria, the ability of the mitochondria to produce energy plays a critical role in the maintenance of cardiac contractile function. We showed that the mitochondrial OCR in the viable LV was lowered at the 8th week after CAL, whereas the mitochondrial activity of the 2w-CAL rat was not decreased. On the other hand, mitochondria are capable of producing reactive oxygen species such as superoxide and hydrogen peroxide. Free radicals generated from mitochondria may attack various intracellular organelles including the mitochondria themselves.[25—27] Recently, several investigators suggested that a decrease in the activity of mitochondrial complex I, a participant in oxidative phosphorylation, results in an enhanced production of reactive oxygen species in the ischemic border area of the ventricle during the development of heart failure following myocardial infarction.[28,29] Thus, we examined changes in production of TRS in the failing heart. We found that the TRS content of the 8w-CAL animal was increased, suggesting a putative relationship between the impairment of mitochondrial function and the production of reactive oxygen species in this model. Since Hsp60 is known to be located in mitochondria,[3] it is possible that the increase in Hsp60 content after myocardial infarction plays a significant role in the impairment of the mitochondrial function in the failing heart. Hsp60 may not only be involved in the transport of proteins from the cytosol to the mitochondrial matrix across the mitochondrial inner membrane but also prevent denaturation of intramitochondrial proteins and support the repair of damaged proteins. Therefore, it is possible that production of Hsp60 in the viable LV may be enhanced after myocardial infarction. In fact, the myocardial Hsp60 content was inversely related to the mitochondrial OCR at the 8th week after myocardial infarction, suggesting that an increase in Hsp60 in the viable LV leads to an augmentation of the oxidative stress to mitochondria. An increase in Hsp60 content in the viable LV was related to a rise in the LVEDP, a typical marker for the symptoms of chronic heart failure. Taken together, our data lead to the conclusion that the reduction in the mitochondrial OCR of the viable LV induces production of ROS, and then an increase in Hsp60 production. This sequence of events may lead to impairment of cardiac function and hemodynamics.

Xanthoudakis *et al.* reported that Hsp60 accelerated maturation of pro-caspase 3, one of the key enzymes for apoptosis induction, which may enhance apoptotic cell death.[30] In the present study, there was a significant relationship between an increase in Hsp60 content and a rise in LVEDP after myocardial infarction. Therefore, it is possible that production of Hsp60 in the viable LV leads to contractile failure after myocardial infarction.

In contrast, several investigators reported that Hsp60 prevented apoptotic cell death of cardiomyocytes.[31] The increase in Hsp60 in the failing heart can be a cellular defence mechanism against apoptotic cell death. If this is the case, an increase in Hsp60 in the failing heart in the present study is considered to be a marker for cellular defense against apoptotic cell death. Trandolapril may namely attenuate an increase in Hsp60 content in failing hearts, indirectly by reducing myocardial oxidative stress and improving mitochondrial function. Unfortunately, due to lack of evidence, we cannot determine at present whether an increase in Hsp60 is beneficial for cardioprotection in the development of chronic heart failure or not. A further study is required to elucidate the exact role of Hsp60 in the development of chronic heart failure.

In conclusion, long-term treatment with trandolapril reduced the increased production of Hsp60 in the failing heart, associated with improvement of hemodynamics, suppression of cardiac remodeling, preservation of mitochondrial energy-producing ability, and prevention of oxygen stress. Therefore, although the exact function of Hsp60 in failing hearts is unclear, the expression level of Hsp60 may be a useful marker for their mitochondrial function and oxygen radical formation.

Acknowledgements This work is in part supported by the Promotion and Mutual Aid Corporation for Private Schools of Japan.

REFERENCES

1) Tanonaka K., Furuhama K., Yoshida H., Kakuta K., Miyamoto Y., Takeo S., *Am. J. Physiol. Heart Circ. Physiol.*, **281**, H215—H222 (2001).
2) Cheng M. Y., Hartl F. U., Horwich A. L., *Nature* (London), **348**, 455—458 (1990).
3) Kreisel W., Hildebrandt H., Schiltz E., Kohler G., Spamer C., Dietz C., Mossner W., Heilmann C., *Acta Histochem.*, **96**, 51—62 (1994).
4) Koll H., Guiard B., Rassow J., Ostermann J., Horwich A. L., Neupert W., Hartl F. U., *Cell*, **68**, 1163—1175 (1992).
5) Knowlton A. A., Kapadia S., Torre-Amione G., Durand J. B., Bies R., Young J., Mann D. L., *J. Mol. Cell. Cardiol.*, **30**, 811—818 (1998).

6) Wysocki J., Karawajczyk B., Gorski J., Korzeniowski A., Mackiewicz Z., Kupryszewski G., Glosnicka R., *Cardiovasc. Pathol.*, **11**, 238—243 (2002).
7) Kervinen H., Huittinen T., Vaarala O., Leinonen M., Saikku P., Manninen V., Manttari M., *Atherosclerosis*, **169**, 339—344 (2003).
8) Sanbe A., Tanonaka K., Hanaoka Y., Katoh T., Takeo S., *J. Mol. Cell. Cardiol.*, **25**, 995—1013 (1993).
9) Sanbe A., Tanonaka K., Kobayashi R., Takeo S., *J. Mol. Cell. Cardiol.*, **27**, 2209—2222 (1995).
10) Tanonaka K., Toga W., Yoshida H., Furuhama K., Takeo S., *Br. J. Pharmacol.*, **134**, 969—976 (2001).
11) Yoshida H., Takahashi M., Tanonaka K., Maki T., Nasa Y., Takeo S., *Br. J. Pharmacol.*, **134**, 150—160 (2001).
12) Yoshida H., Tanonaka K., Miyamoto Y., Abe T., Takahashi M., Anand-Srivastava M. B., Takeo S., *Cardiovasc. Res.*, **50**, 34—45 (2001).
13) Sanbe A., Takeo S., *Circulation*, **92**, 2666—2675 (1995).
14) Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. J., *J. Biol. Chem.*, **261**, 6300—6306 (1951).
15) Ohkawa H., Ohnishi N., Yagi K., *Analyt. Biochem.*, **95**, 351—358 (1979).
16) Iwai T., Tanonaka K., Inoue R., Kasahara S., Kamo N., Takeo S., *J. Mol. Cell. Cardiol.*, **34**, 725—738 (2002).
17) Tanonaka K., Iwai T., Motegi K., Takeo S., *Cardiovasc. Res.*, **57**, 416—425 (2003).
18) Yoshida H., Takahashi M., Koshimizu M., Tanonaka K., Oikawa R., Toyo-oka T., Takeo S., *Cardiovasc. Res.*, **59**, 419—427 (2003).
19) Pfeffer M. A., Braunwald E., Moyé L. A., Basta L., Brown E. J., Jr., Cuddy T. E., Davis B. R., Geltman E. M., Goldman S., Flaker G. C., Klein M., Lamas G. A., Packer M., Rouleau J., Rouleau J. L., Rutherford J., Wertheimer J. H., Hawkins M., *N. Engl. J. Med.*, **327**, 669—677 (1992).
20) McKelvie R. S., Yusuf S., Pericak D., Avezum A., Burns R. J., Probstfield J., Tsuyuki R. T., White M., Rouleau J., Latini R., Maggioni A., Young J., Pogue J., *Circulation*, **100**, 1056—1064 (1999).
21) Køber L., Torp-Pedersen C., Carlsen J. E., Bagger H., Eliasen, P., Lyngborg K., Videbak J., Cole D. S., Auclert L., Pauly N. C., Aliot E., Persson S., Camm A. J., *N. Eng. J. Med.*, **333**, 1670—1676 (1995).
22) Pitt B., Segal R., Martinez F. A., Meurers G., Cowley A. J., Thomas I., Deedwania P. C., Neyg D. E., Snavelyg D. B., Paul I., Chang P. I., *Lancet*, **349**, 747—752 (1997).
23) Takahashi M., Tanonaka K., Yoshida H., Oikawa R., Koshimizu M., Daicho T., Toyo-Oka T., Takeo S., *Cardiovasc. Res.*, **65**, 356—365 (2005).
24) Moore T. J., Williams G. H., Dluhy R. G., Bavli S. Z., Himathongkan T., Greefield M., *Circ. Res.*, **41**, 167—171 (1977).
25) Ide T., Tsutsui H., Kinugawa S., Suematsu N., Hayashidani S., Ichikawa K., Utsumi H., Machida Y., Egashira K., Takeshita A., *Circ. Res.*, **86**, 152—157 (2000).
26) Tsutsui H., Ide T., Shiomi T., Kang D., Hayashidani S., Suematsu N., Wen J., Utsumi H., Hamasaki N., Takeshita A., *Circulation*, **104**, 2883—2885 (2001).
27) Suematsu N., Tsutsui H., Wen J., Kang D., Ikeuchi M., Ide T., Hayashidani S., Shiomi T., Kubota T., Hamasaki N., Takeshita A., *Circulation*, **107**, 1418—1423 (2003).
28) Ide T., Tsutsui H., Kinugawa S., Utsumi H., Kang D., Hattori N., Uchida K., Arimura K., Egashira K., Takeshita A., *Circ. Res.*, **85**, 357—363 (1999).
29) Scheubel R. J., Tostlebe M., Simm A., Rohrbach S., Prondzinsky R., Gellerich F. N., Silber R. E., Holtz J., *J. Am. Coll. Cardiol.*, **40**, 2174—2181 (2002).
30) Xanthoudakis S., Roy S., Rasper D., Hennessey T., Aubin Y., Cassady R., Tawa P., Ruel R., Rosen A., Nicholson D. W., *EMBO J.*, **18**, 2049—2056 (1999).
31) Kirchhoff S. R,, Gupta S., Knowlton A. A., *Circulation*, **105**, 2899—2904 (2002).