Circ J 2002; **66:** 959–964

Re-Expression of Fetal Troponin Isoforms in the Postinfarction Failing Heart of the Rat

Sang-Hyun Kim, MD; Hyo-Soo Kim, MD*†; Myoung-Mook Lee, MD*†

Molecular switches between the troponin T and I isoforms are known to occur in various conditions, but the results from studies of failing human hearts with various etiologies are contradictory and it is not certain whether troponin isoform changes occur. Therefore, the molecular switching of troponin isoforms during normal development and heart failure (HF) after myocardial infarction were investigated in Sprague-Dawley rats at the fetal, neonate, and normal adult stages, and in a postinfarction adult HF group. During normal development, switching from the fetal to the adult pattern of the troponin T and I isoforms was observed. Immunoblotting of postinfarction failing hearts revealed a marked increase in the fetal isoform of cardiac TnT (cTnT) (fetal/adult cTnT isoforms: normal adult = 0.61±0.09 vs postinfarction HF = 1.59±0.13, $p<0.001$). Also, the amount of the adult troponin I (TnI) isoform decreased significantly in the postinfarction failing heart. In the semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) with glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) as an internal standard, the mRNA of fetal cTnT increased in the postinfarction failing heart (fetal cTnT/GAPDH: control = 0.22 vs HF rat = 0.84, $p<0.05$). Therefore, molecular switching of the troponin T and I isoforms occurred during the normal development of the rat, and there was re-expression of the fetal pattern of the isoforms in the postinfarction failing heart of the adult rat. (*Circ J* 2002; **66:** 959–964)

Key Words: Dedifferentiation; Postinfarction heart failure; Rat; Troponin isoform

Troponin is a regulatory protein and constituent of the myofibrillar contractile complex. It consists of 3 subunits, troponin C, T, and I. The troponin complex controls muscular contraction through the regulation of myofibrillar responsiveness to calcium and adrenergic stimulation[1,2] Troponin C is the binding site for calcium, which is necessary for excitation–contraction coupling of the muscle. Troponin T and I regulate calcium binding to troponin C through 3-dimensional conformational change, and so confer calcium sensitivity to myofilaments[3–5]

Multiple isoforms of the troponin subunits have been identified in fast and slow skeletal muscles and in the myocardium of the rabbit[6,7] rat[8] and human[9] Moreover, the functional properties of the contractile apparatus may be determined by the composition of its structural and regulatory proteins, such as the troponin complex[1,5,9,10]

It is known that alternative RNA splicing of the troponin T gene produces multiple cardiac troponin T isoforms[1,11,12] and that the expressions of cardiac and slow skeletal troponin I isoforms from different genes are regulated by many transcription factors, such as GATA-4[13,14]

In the myocardium of the rat, there are 2 isoforms of cardiac troponin T, fetal and adult, and 2 isoforms of troponin I, slow skeletal, and cardiac. Moreover, the relative expression of the troponin T and I isoforms is known to change significantly during normal development[10,15,16]

In addition, changes of troponin isoform expression have been reported in various other conditions. In cases of hypothyroidism[17] cardiac hypertrophy of the left ventricle[18,19] and some cases of heart failure[9,20,21] reprogramming of cardiac gene expression produced shifts in the expressions of the troponin I or T isoforms. This expressional change is the re-expression of slow skeletal troponin I, which is not present in the myocardium of the normal adult rat. Myofibrils with fetal patterns of troponin isoforms (predominantly fetal cardiac troponin T and slow skeletal troponin I) had lower responsiveness to calcium and β-adrenergic stimulation, and a lower value of maximal ATPase activity than those with adult patterns of troponin isoforms (predominantly adult cardiac troponin T and cardiac troponin I). However, they showed less reduced responsiveness to calcium and maximal ATPase activity under stressful conditions such as acidosis, and hypoxia[1,22]

However, in studies of failing human hearts with diverse etiologies, it is not definite whether a change of the troponin isoforms develops because results from the studies have been contradictory. In addition, it is unclear whether changes in troponin isoform expression occur in the postinfarction failing heart. Therefore, we examined the molecular switches for troponin T and I isoforms in the postinfarction failing heart of the rat.

Methods

Sample Preparation and Hemodynamic Measurements

We chose female Sprague-Dawley rats (200–250 g body weight) as a model for normal adult heart (control, n=5) and non-infarcted myocardium of the postinfarction failing heart (postinfarction HF rat, n=5). In addition, we studied the fetal heart and the 1-day-old postnatal heart to detect the changes in troponin T and I expression during development.

(Received February 26, 2002; revised manuscript received July 9, 2002; accepted July 17, 2002)

*Department of Internal Medicine, Boramae Municipal Hospital and *Department of Internal Medicine, Seoul National University College of Medicine, †Cardiovascular Lab, Clinical Research Center, Seoul National University Hospital, Seoul, Korea*

Mailing address: Hyo-Soo Kim, MD, PhD, Director of Cardiovascular Laboratory, Clinical Research Center, Associate Professor, Division of Cardiology, Department of Internal Medicine, Seoul National University College of Medicine, #28 Yongon-dong, Chongno-gu, Seoul 110-744, Korea. E-mail: hyosoo@snu.ac.kr

Table 1 Primer Sequences Used in RT-PCR

Primer	*Position*	*Sequences*	*Product size*
fTnT1	*antisense*	*5'-AGACTGGAGCGAAGAAGGAAG-3'*	*320 bp*
fTnT2	*sense*	*5'-TGTTCTGCAAGTGAGCCTCGATC-3'*	
tTnT1	*antisense*	*5'-CGAGAGAAGGAAAFFCAFAACC-3'*	*250 bp*
tTnT2	*sense*	*5'-GGTCTTCATTCAGGTGGTCGATG-3'*	
ssTnI1	*antisense*	*5'-TGCCTCCACAACACGAGAGAGATC-5'*	*314 bp*
ssTnI2	*sense*	*5'-AAGCACCTCTACTGCAAGGTTGGG-3'*	
cTnI1	*antisense*	*5'-TCTCTACCTCTGGAGATCAGCATGG-3'*	*167 bp*
cTnI2	*sense*	*5'-TGAAGTTTTCTGGAGGCGGAG-3'*	
GAPD1	*antisense*	*5'-GCCAAGGATATCCATGACAACT-3'*	
GAPD2	*sense*	*5'-CAGGGTCGACCTTGCCCACAGCCTT-3'*	

Myocardial infarction was induced with left coronary artery ligation using Prolene 6-0 through a left thoracotomy under anesthesia. After an intramuscular injection of ketamine (7.52×10^{-2} mg/g of body weight) and xylazine (7.52×10^{-3} mg/g of body weight) to the right gluteal muscle, rats were intubated endotracheally and ventilated mechanically by a rodent ventilator (model 683, Harvard Apparatus). Left thoracotomy was performed at the fourth intercostal space, and left coronary artery ligation was carried out between the right ventricular outflow tract and the left atrial appendage. Rats were raised for 3 months post infarction under the same conditionss as the control group that underwent a sham operation.

After 3 months, hemodynamic parameters such as left ventricular end diastolic pressure (LVEDP), aortic pressure and heart rate were measured with a polygraph model 7 (Grass Instruments) through cannulation of the right carotid artery with a saline-filled polyethylene catheter (PE50). Hearts were arrested in diastole by the direct injection of 2–3 ml of 2 mol/l KCl into the left ventricle, and excised to examine the extent of the infarction and wall thinning with subsequent confirmation through histologic staining. The hearts were then stored at –70°C in a refrigerator or in formalin for immunoblotting, reverse transcriptase-polymerase chain reaction (RT-PCR) and histologic examination. We prepared rat soleus muscle for the experiments with slow skeletal muscle. All animal experiments and care were conducted according to the institutional guide for the care and use of laboratory animals.

Histologic Examination

The infarcted hearts was stained with hematoxylin–eosin (H&E) and Masson's trichrome to visualize the extent and location of the infarction, compensatory hypertrophy, and dilatation of the noninfarcted segment of the left ventricle after postinfarction remodeling.

Protein Preparation and Immunoblotting

Pieces of myocardium were cut from noninfarcted segments, and after homogenization, mixed with 4 ml of prechilled skinning solution (75 mmol/l potassium acetate [$KC_2H_3O_2$], 5 mmol/l potassium EGTA, 15 mM potassium phosphate, 5 mmol/l $MgCl_2$, 5 mmol/l potassium ATP, 1% Triton X-100, and 15 mmol/l MOPS, pH 7.0) and shaken on ice for 10 min. Protease inhibitors were added to all solutions (0.5 μg/ml antipain, 0.5 μg/ml chymostatin, 2 μg/ml pepstatin, 0.5 μmol/l phenylmethylsulfonyl fluoride, 2.0 μg/ml leupeptin). The sample was centrifuged, and the pellet was washed twice in 4 ml of Krebs-Henseleit solution (pH 7.4) with vortexing and placed in 400 μl of sample buffer (2.0% sodium dodecyl sulfate [SDS], 50 mmol/l Tris [pH 6.8], 20% glycerol, 0.0125% bromophenol blue and 100 mmol/l dithiothreitol). Samples was then heated at 100°C for 2 min and the supernatant was captured and stored at –70°C for immunoblotting.[6,20]

Next, 30 μg of extracted proteins from the myocardium of each group were loaded and separated by sodium dodesylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE), which consisted of 8% stacking gel and 12% separating gel, for 2 h at 40 V and for 90 min at 60 V (Miniprotean II electrophoresis kit, Bio-Rad–, Richmond, CA, USA). After electrophoresis, proteins were transferred onto a nitrocellulose membrane, and blocked overnight with 5% skim milk in 0.2% Tris buffered saline-Tween 20 (TBS-Tween 20). Immunoblottings were performed with previously characterized monoclonal antibodies to cardiac troponin T or I (Biopacific) diluted in 0.2% TBS-Tween 20 solution. Antibody binding was visualized using chemiluminescence with peroxidase-linked secondary antibody (Amersham).

RNA Preparation and Semi-Quantitative RT-PCR

A sample of rat myocardium or soleus muscle (100 mg) was homogenized with 1 ml of Ultraspec solution (Biotecx, Huston, TX, USA) containing guanidine salt and urea, and shaken frequently for 15 min after adding 0.2 ml of chloro-

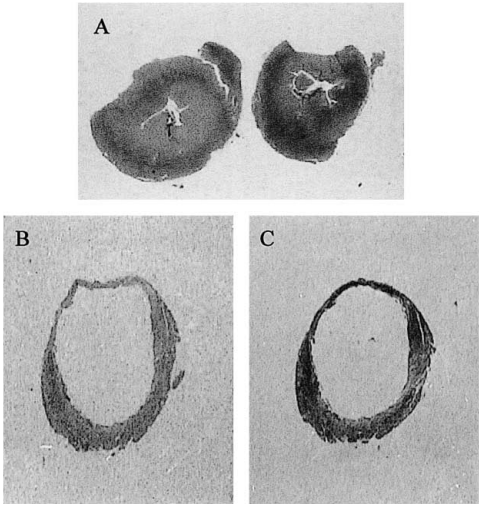

Fig 1. Hematoxylin–eosin staining of the normal adult heart (A), postinfarction failing heart (B) and Masson's trichrome staining of a postinfarction failing heart (C). Dilatation of the left ventricle and thinning of the anterior wall of the infarcted segment are shown. The fibrosis after myocardial infarction is shown in blue by Masson's trichrome staining.

form. The sample was then centrifuged at 12,000 G, for 4 min at 4°C, and the supernatant was removed, mixed with an isovolume of isopropanol, and then incubated in ice for 10 min. The sample was then centrifuged at 12,000 G at 4°C for 30 min and the resulting RNA pellet was washed twice with 75% ethanol and dried for 15 min. Finally, RNA was suspended in 0.5% SDS, and stored at –70°C after being quantified by spectrophotometry. Reverse transcription was performed with oligodeoxynucleotide, 10 mmol/l dNTP, and Moloney murine leukemia virus (MoMLV) reverse transcriptase. PCR was performed on 2 μl of the newly synthesized cDNAs with Taq DNA polymerase and oligonucleotide primers semi-quantitatively using glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) as an internal standard. RT-PCR primers were designed with acomputer program using a previously reported cDNA sequence and included the unique sequence of each troponin isoform. PCR conditions were altered to determine optimal conditions from 21 to 33 cycles: denaturation at 94°C for 30 s, annealing at 58°C for 1 min, and extension at 72°C for 1 min. PCR products were separated by electrophoresis in 3% agarose gel with ethidium bromide staining. The primers used in this study are shown in Table 1. Primers for fetal troponin T (fTnT1, fTnT2) yielded PCR products of 320 base pairs (bp), and primers for both fetal and adult troponin T (tTnT1, tTnT2) yielded PCR products of 250 bp. The primers of cardiac troponin I (cTnI1, cTnI2) produced a cDNA of 167 bp, and the primers of slow skeletal troponin I (ssTnI1, ssTnI2) produced a cDNA of 314 bp.

Statistical Analysis

Results are expressed as means ± SD or as the ratio of densitometry results from the densitometric scanning (Helena Lab, Beumont, TX, USA) of bands in western blotting and RT-PCR. Statistical significance was determined using Student's t-test and the Wilcoxon rank sum test. Differences were considered statistically significant when $p<0.05$.

Results

Group Characteristics and Hemodynamic Parameters

Three months after myocardial infarction, a significant difference was noticed in the body weights of the control group (n=5) and the postinfarction heart failure (HF) group (n=5); 270±10 g vs 245±25 g, respectively (p=0.02). The systolic pressure of the HF group was also lower than that of controls (90±10 mmHg vs 130±15 mmHg, p=0.01), and the heart rate of the HF group was higher (126±21 beats/min vs 110±20 beats/min). Mean LVEDP in the HF group increased to 21.4 mmHg, which was compatible with left ventricular failure after myocardial infarction (7.4 mmHg in the control group).

Histology

Heart histology in the control group was examined with H&E staining (Fig 1A). Dilatation of the left ventricle and wall thinning of the left ventricular anterior wall in the postinfarction failing heart were observed (H&E; Fig 1B). Fibrosis after myocardial infarction was shown in the left ventricle by Masson's trichrome staining (Fig 1C). Postinfarction remodeling was therefore confirmed to have occurred after successful induction of myocardial infarction through ligation of the left coronary artery.

Western Blotting

Immunoblotting for cardiac troponin T showed 2 isoforms, the fetal troponin T isoform (45 kDa) and the adult troponin T isoform (42 kDa). The intensity of each band was measured by densitometry and it was found that the fetal isoform of cardiac troponin T predominated in fetal and neonatal hearts, but that the adult isoform predominated in the normal adult heart. In the postinfarction failing heart, the fetal isoform of cardiac troponin T

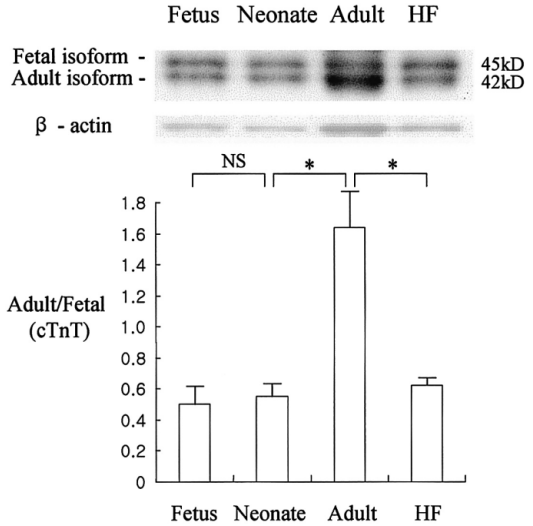

Fig 2. Immunoblots of protein extract of different rat myocardium with monoclonal antibody to cardiac troponin T (cTnT). Changes in the expression patterns of the fetal and adult troponin T isoforms were observed during development and in postinfarction heart failure. Compared with the normal adult heart, marked decreases in the adult isoform of TnT and marked increases in the fetal isoform of TnT were observed in the postinfarction failing heart of the rat (NS, not significant, *p<0.001).

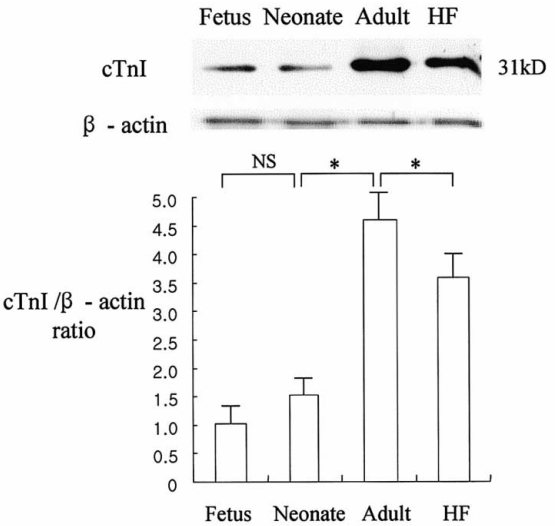

Fig 3. Immunoblots of protein extracts of different rat myocardium with monoclonal antibody to cardiac troponin I (cTnI). Changes in the expression pattern of cardiac troponin I isoform were observed during development and in postinfarction heart failure. Compared with the normal adult rat heart, a moderate decrease in the ratio of cTnI/β-actin was observed in the postinfarction failing heart of the rat (NS, not significant, *p<0.05).

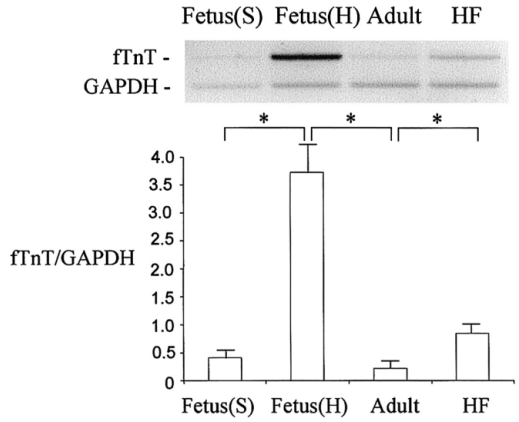

Fig 4. RT-PCR product of fetal cardiac troponin T mRNA. Fetal cardiac troponin T (fTnT) cDNA and GAPDH bands are shown. fTnT cDNA was amplified significantly in the fetal heart (Fetus (H)) and amplified only slightly in fetal slow skeletal muscle (Fetus (S)) and the normal adult heart (Adult). However, fTnT cDNA amplification was increased in the postinfarction rat heart (HF), which was similar to that observed in the fetal heart, compared with the normal adult heart (*p<0.05).

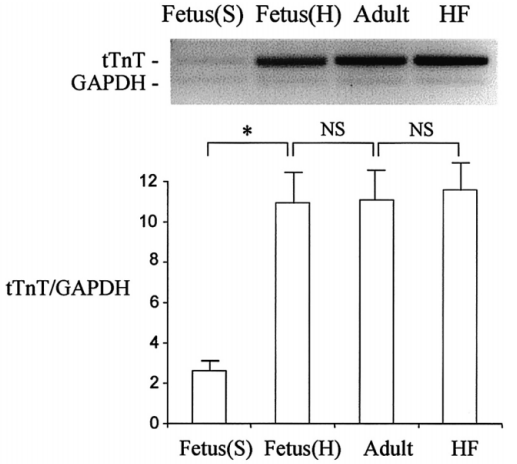

Fig 5. RT-PCR product of fetal and adult cardiac troponin T mRNA. Total (fetal and adult) cardiac troponin T (tTnT) cDNA and GAPDH bands are shown. With the exception of fetal slow skeletal muscle, adult cardiac troponin T cDNA was amplified to a similar degree in the fetal heart, normal adult heart and postinfarction failing heart (NS, not significant, *p<0.05).

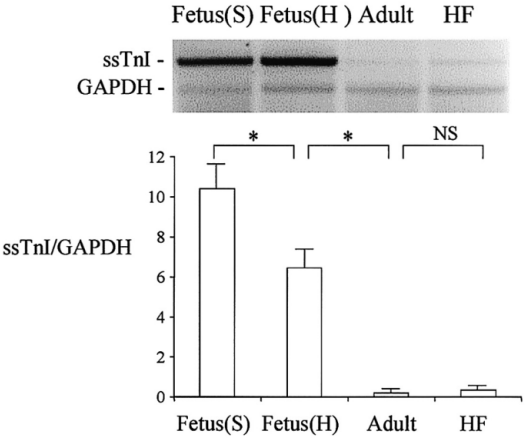

Fig 6. RT-PCR product of slow skeletal troponin I mRNA. Slow skeletal troponin I (ssTnI) cDNA and GAPDH bands are shown. Slow skeletal troponin I was amplified markedly both in fetal slow skeletal muscle and the fetal heart, but only slightly in the normal adult heart and postinfarction failing heart (NS, not significant, *p<0.05).

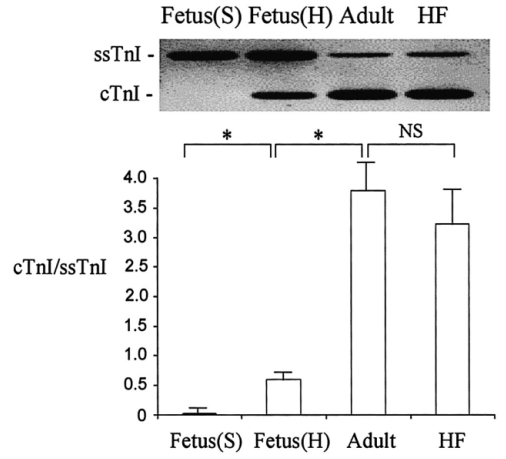

Fig 7. RT-PCR product of cardiac troponin I and slow skeletal troponin I mRNA. Cardiac troponin I (cTnI) and slow skeletal troponin I (ssTnI) cDNA bands are shown. The amplification of Cardiac troponin I cDNA, compared with slow skeletal troponin I occurred negligibly in fetal slow skeletal muscle, moderately in the fetal heart, and markedly in the normal adult heart, but was slightly decreased in the postinfarction failing heart (NS, not significant, *p<0.05).

increased markedly and reversed the ratio of fetal/adult cardiac troponin T isoforms, as shown in Fig 2 (control group = 0.61±0.09 vs the postinfarction HF group = 1.59± 0.13, p<0.001).

Immunoblotting for cardiac troponin I showed the isoform (31.5 kDa) of each group (Fig 3). The amount of cardiac troponin I isoform increased more so in normal adult hearts than in fetal or neonatal hearts, but decreased significantly in the postinfarction HF group (p<0.05). During normal development, switching from a fetal to an adult pattern of troponin T and I isoforms was observed by western blotting, and the isoform pattern specific for postinfarction HF was similar to that of the fetal heart rather than that of the normal adult heart.

RT-PCR

RT-PCR using primer for the fetal isoform of cardiac troponin T (fTnT1, fTnT2) proved effective in 21 cycles. The cDNA of fetal cardiac troponin T was found to be amplified more so in the fetal heart than in fetal slow skeletal muscle or the normal adult heart. However, in the postinfarction failing heart, the gene expression of fetal cardiac troponin T increased significantly compared with the normal adult heart. In the densitometry results, the ratio of fetal cardiac troponin T cDNA/GAPDH was 0.41 in fetal slow skeletal muscle, 3.73 in the fetal rat heart, 0.22 in the normal adult heart, and 0.84 in the postinfarction HF rat, as shown in Fig 4 (p<0.05).

The primer for all cardiac troponin T isoforms (tTnT1, tTnT2) amplified both fetal and adult cardiac troponin T isoforms. The cDNA of the cardiac troponin T isoforms were amplified to a lesser extent in the fetal slow skeletal muscle than in the fetal heart, the normal adult heart or the

postinfarction failing heart. Therefore, considering the prior RT-PCR results for fetal cardiac troponin T, the gene expression of the adult cardiac troponin T isoform was found to be increased in the normal adult heart, and to be significantly decreased in the fetal and postinfarction failing heart (Fig 5). Therefore, during normal development from the fetus to the adult rat, the gene expression of fetal cardiac troponin T decreased, but the gene expression of adult cardiac troponin T increased significantly. In the postinfarction failing heart, the cDNA of adult cardiac troponin T decreased, and the gene expression of fetal cardiac troponin T increased, in the same manner as shown by western blotting.

The primer for slow skeletal troponin I (ssTnI1, ssTnI2) amplified the cDNA effectively within 25 cycles. Gene expression of slow skeletal troponin I increased in the fetal heart and slow skeletal muscle, but decreased in the normal adult heart, and was not significantly increased in postinfarction HF. The densitometry result was 10.4 in fetal slow skeletal muscle, 6.4 in the fetal rat heart, 0.20 in the normal adult rat heart, and 0.32 in the postinfarction failing heart (Fig 6).

RT-PCR with the primer for both cardiac troponin I and slow skeletal troponin I (cTnI1, cTnI2) was effective within 25 cycles. Cardiac troponin I cDNA was found to be amplified slightly in the fetal heart and fetal slow skeletal muscle. Both the normal adult heart and the postinfarction failing heart group showed a similar large amplification of cardiac troponin I cDNA (Fig 7).

Discussion

The troponin complex (ie, troponin T, I, and C) is the major regulator of muscular contractile function. Several isoforms of troponin T and I have been reported in the myocardium of various animals, including humans. Five isoforms of cardiac troponin T have been found in the rabbit;[6,7] and 4 isoforms of cardiac troponin T and 2 isoforms of troponin I have been found in the human heart.[9,20,21,23] In the rat, 2 cardiac troponin T isoforms (fetal, and adult) and 2 troponin I isoforms (slow skeletal, and cardiac) of the myocardium have been reported.[15,16,24] Moreover, the isoforms of troponin T and I are known to be one of the determinants of changed contractile characteristics during various conditions such as normal development, and cardiac hypertrophy.[1,3,5,10,22]

Changes in the troponin isoform expression during development and postinfarction heart failure were studied in the present experiment. The fetal isoform of cardiac troponin T (fTnT) and the slow skeletal isoform of troponin I (ssTnI) predominated in fetal and neonatal rat hearts, but the adult isoform of cardiac troponin T (aTnT) and the cardiac isoform of troponin I (cTnI) predominated in the normal adult heart. Switching from the fetal to the adult pattern of troponin T and I isoforms was observed during development, and re-switching to the fetal pattern occurred in postinfarction heart failure. This study showed, for the first time, that the molecular switching from the fetal to the adult pattern of troponin T and I isoforms during normal rat development was reversed during postinfarction heart failure.

The switching from the fetal to the adult pattern of troponin T and I isoforms that occurs during normal development was observed by western blotting. Moreover, it was found that the troponin isoform pattern specific for postinfarction heart failure was similar to that of fetal heart rather than that of normal adult heart. In RT-PCR, the isoform switching of cardiac troponin T during normal development and postinfarction heart failure followed the same pattern as shown by western blotting. However, in the case of the RT-PCR of troponin I isoforms, the amplification of isoform mRNA in postinfarction heart failure did not have the same pattern as that shown in the fetal rat heart. Therefore, it is proposed that the regulatory mechanism of cardiac troponin I gene expression of rat may involve a post-transcriptional modification of mRNA. More studies are required to clarify this issue.

The switching of troponin I isoforms has also been reported in other pathologic conditions, such as hypothyroidism or pressure-overload hypertrophy of the left ventricle. Moreover, the content of cardiac troponin I has been reported to decrease in the rat myocardium with hypothyroidism, induced by the administration of propylthiouracil, as compared with the normal adult rat myocardium.[17] In addition, mutations of the cardiac troponin T gene have been reported in cases of familial hypertrophic cardiomyopathy.[25] Furthermore, it has been reported that decreased adult isoform, and increased fetal isoform of cardiac troponin T, as well as decreased cardiac troponin I isoform, occurred in a guinea pig model of heart failure, which was induced by pressure-overload using the aorta banding method.[10] It has also been shown that the expression of slow skeletal troponin I in the heart of adult transgenic mice reduced the force decline observed under acidic conditions.[26] Another study showed that depressed troponin I protein phosphorylation was associated with the functional characteristics of a failing heart.[27] Those studies suggest that the sequential activation and expression of different isoforms of a contractile gene family might be the one of mechanisms of regulating contractile properties in the heart under various pathologic conditions.

The functional significance of troponin isoform switching has been studied in several experiments. For example, it is known that troponin T and I isoforms are related to calcium sensitivity, maximal ATPase activity, and myocardial relaxation rate.[1,5,22] Therefore, the re-switching of troponin isoforms to fetal patterns during postinfarction HF, the so called 'dedifferentiation' process, may be either one of the basic mechanisms of pathologic change or an adaptive process induced by the pathologic changes of HF.

It is known that the DNA of troponin T of rat consists of 16 exons and 19,186 nucleotides, and that the expression of the troponin T isoform is regulated by the alternative RNA splicing of exon 4 and exon 12 nucleotides.[28] The switching of troponin I isoforms may be the result of post-transcriptional alteration of the troponin I gene.[10,16] Although the timing of isoform switching is different in the atrium, ventricle and conduction system, it is known that the switching of troponin isoforms in the rat occurs during the first 3 weeks of birth.[8,10,16]

The switching of troponin isoforms in the human heart has been studied on several occasions, but the results have produced different conclusions. In the failing human heart, it is not definite whether troponin isoform changes develop because results have been contradictory. Some studies[9,29] have shown a re-switching to the fetal pattern of troponin isoforms in the failing human heart with various etiologies, but others[21,23,30] found that re-switching occurred only in some circumstances.

We observed the changes in the troponin T and I

isoforms during normal rat development, and re-switching of the troponin isoforms to the fetal pattern during postinfarction heart failure in the rat. We report here for the first time upon changes of both the troponin T and I isoforms in postinfarction HF.

In the future, more comprehensive and extensive studies of the mechanisms of gene regulation of the troponin isoforms, changes in the troponin isoforms in human failing and postinfarction hearts, and the functional implications of troponin isoform switching should be undertaken to give a better understanding of the molecular mechanisms and pathogenesis of postinfarction HF.

Acknowledgments

This study was supported by the Korea Science and Engineering Foundation (KOSEF) through the Aging and Apoptosis Research Center at Seoul National University.

References

1. Nassar R, Malouf NN, Kelly MB, Oakeley AE, Anderson PAW. Force-pCa relation and troponin I isoforms of rabbit myocardium. *Circ Res* 1991; **69:** 1470–1475.
2. Solaro RJ, Van Eyk J. Altered interactions among thin filament proteins modulate cardiac function. *J Mol Cell Cardiol* 1996; **28:** 217–230.
3. McConnell BK, Moravee CS, Bond M. Troponin I phosphorylation and myofilament calcium sensitivity during decompensated cardiac hypertrophy. *Am J Physiol* 1998; **274:** H385–H396.
4. Wang J, Jin JP. Conformational modulation of troponin T by configuration of the NH2-terminal variable region and functional effects. *Biochemistry* 1998; **37:** 14519–14528.
5. Zhang R, Zhao J, Mandveno A, Potter JD. Cardiac troponin I phosphorylation increases the rate of cardiac muscle relaxation. *Circ Res* 1995; **76:** 1028–1035.
6. Anderson PAW, Moore GE, Nassar RE. Developmental changes in the expression of rabbit left ventricular troponin T. *Circ Res* 1988; **63:** 742–747.
7. Anderson PAW, Oakeley AE. Immunological identification of five troponin T isoforms reveals an elaborate maturational troponin T profile in rabbit myocardium. *Circ Res* 1989; **65:** 1087–1093.
8. Saggin L, Gorza L, Ausoni S, Schiaffino S. Troponin I switching in the developing heart. *J Biol Chem* 1989; **264:** 16299–16302.
9. Anderson PAW, Malouf NN, Oakeley AE, Pagani ED, Allen PD. Troponin isoform expression in human: A comparison among normal and failing heart, fetal heart, and adult and fetal skeletal muscle. *Circ Res* 1991; **69:** 1226–1233.
10. Schiaffino S, Gorza L, Ausoni S. Troponin isoform switching in the developing heart and its functional consequences. *Trends Cardiovasc Med* 1993; **3:** 12–17.
11. Forza H, Townsend PJ, Carrier L, Barton PJ, Mesnard L, Bahrend E, et al. Genomic organization, alternative splicing and polymorphisms of the human cardiac troponin T gene. *J Mol Cell Cardiol* 1998; **30:** 1247–1253.
12. Jin JP. Alternative RNA splicing-generated cardiac troponin T isoform switching: A non-heart-restricted genetic programming synchronized in developing cardiac and skeletal muscles. *Biochem Biophys Res Commun* 1996; **225:** 883–889.
13. Bhavsar PK, Brand NJ, Yacoub MH, Barton PJR. Isolation and characterization of the human cardiac troponin I gene (TNNI3). *Genomics* 1996; **35:** 11–23.
14. Murphy AM, Tompson WR, Peng LF, Jones L 2nd. Regulation of the rat cardiac troponin I gene by the transcription factor GATA-4. *Biochemistry* 1997; **322:** 393–401.
15. Ausoni S, de Nardi C, Moretti P, Gorza L, Schiaffino S. Developmental expression of rat cardiac troponin I mRNA. *Development* 1991; **112:** 1041–1051.
16. L'Ecuyer TJ, Schulze D, Lin JJC. Thin filament changes during in vivo rat heart development. *Pediatr Res* 1991; **30:** 228–232.
17. Dieckman LJ, Solaro RJ. Effect of thyroid status on thin filament Ca^{2+} regulation and expression of troponin I in perinatal and adult rat hearts. *Circ Res* 1990; **67:** 344–351.
18. Izumo S, Nada-Ginard B, Mahdavi V. Protooncogene induction and reprogramming of cardiac gene expression produced by pressure overload. *Proc Natl Acad Sci USA* 1988; **85:** 339–343.
19. Gulati J, Akella AB, Nicole SD, Stare V, Siri F. Shifts in contractile regulatory protein subunits troponin T and troponin I in cardiac hypertrophy. *Biochem Biophys Res Commun* 1994; **202:** 384–390.
20. Anderson PAW, Greig A, Mark TM, Malouf NN, Oakeley AE, Ungerleider RM, et al. Molecular basis of human cardiac troponin T isoforms expressed in the developing, adult, and failing hearts. *Circ Res* 1995; **76:** 681–686.
21. Mesnard L, Logeart D, Tarianx S, Diriong S, Mercardier JJ, Samson F. Human cardiac troponin T: Cloning and expression of new isoforms in the normal and failing heart. *Circ Res* 1995; **76:** 687–692.
22. Blanchard EM, Solaro RJ. Inhibition of the activation and troponin I calcium binding of dog cardiac myofibils by acidic pH. *Circ Res* 1984; **55:** 382–391.
23. Mesnard-Rouiller L, Mercadier JJ, Butler-Browne G, Heimburger M, Logeart D, Allen PD, et al. Troponin T mRNA and protein isoforms in the human left ventricle: Pattern of expression in failing and control hearts. *J Mol Cell Cardiol* 1997; **29:** 3043–3055.
24. Martin AF, Ball K, Gao L, Kumar P, Solaro RJ. Identification and functional significance of troponin I isoforms in neonatal rat heart myofibrils. *Circ Res* 1991; **69:** 1244–1252.
25. Thierfelder L, Watkins H, MacRae C, Lamas R, McKenna W, Vosberg HP, et al. Alpha-tropomyosin and cardiac troponin T mutations cause familial hypertrophic cardiomyopathy: A disease of sarcomere. *Cell* 1994; **77:** 701–712.
26. Wolska BM, Vijayan K, Arteaga GM, Konhilas JP, Phillips RM, Solaro RJ, et al. Expression of slow skeletal troponin I in adult transgenic mouse heart muscle reduces the force decline observed during acidic conditions. *J Physiol* 2001; **536:** 863–870.
27. Bodor GS, Oakeley AE, Allen PD, Crimmins DL, Ladenson JH, Anderson PAW. Troponin I phosphorylation in the normal and failing adult human heart. *Circulation* 1997; **96:** 1495–1500.
28. Jin JP, Huang QQ, Yeh HI, Lin JJC. Complete nucleotide sequence and structural organization of rat cardiac troponin T gene: A single gene generates embryonic and adult isoforms via developmentally regulated alternative splicing. *J Mol Biol* 1992; **227:** 1269–1276.
29. Wolff MR, Buck SH, Stoker SW, Greaser ML, Mentzer RM. Myofibillar calcium sensitivity of isometric tension is increased in human dilated cardiomyopathies. *J Clin Invest* 1996; **98:** 167–176.
30. Townsend PJ, Barton PJR, Yacoub MH, Farza H. Molecular cloning of human cardiac troponin T isoforms: Expression in developing and failing heart. *J Mol Cell Cardiol* 1995; **27:** 2223–2236.