J Vet Clin 25(3) : 152-158 (2008)

Journal of Veterinary Clinics
www.ksvc.or.kr

Alterations of Proteins in Artificially Induced Chronic Myocardial Infarction in Rats

Mi-Jin Lee, Hyun-Jin Tae, Do-Hyeon Yu, Ying-Hua Li, Jong-Hyun Lee, Ji-Seon Yoon, Seok-Won Lee, In-Shik Kim and Jin-Ho Park

College of Veterinary Medicine and Bio-Safety Research Institute, Chonbuk National University, Jeonju 561-756, Korea

(Accepted: June 5, 2008)

Abstract : We investigated the changes of protein in chronic MI which was occurred with long-term ischemia, without reperfusion. Sprague Dawley (SD) rats were divided into the sham group and the experimental groups (MI groups). The sham group was treated only thoracotomy without ligation for left main descending artery (LMDA) of left coronary artery (LCA), and the experimental groups (MI7d, ligation of LMDA for 7 days and MI30d, ligation of LMDA for 30 days) were conducted an artificial chronic MI. The change of proteins according to passage of times was compared and analyzed on first and second dimension (1 and 2D) sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis. Among total 46 spots expressed differentially in the sham group versus MI7d and MI30d groups on 2D gel, we selected proteins that the volume of spot was increased in the MI7d and MI30d groups compared with the sham group. After that, the proteins were identified through liquid chromatography/tandem mass spectrometry (LC-MS/MS) analysis. In result, we could obtain many proteins as follows; albumin, glucose regulated protein 58 KDa, similar to tripartite motif protein 50, ubiquinol-cytochrome c reductase core protein II, sarcomeric mitochondrial creatine kinase, ATP synthetase alpha chain (mitochondrial precursor) and creatine kinase. In conclusion, we suggest many changed proteins shown at chronic ischemia after artificial MI and consider that these proteins play an important role in the function of heart after MI.

Key words : myocardial infarction, chronic ischemia, LC-MS/MS, rat

Introduction

Myocardial infarction (MI) may cause the damage of myocardial cells, therefore acts as a significant factor of acute heart failure and left ventricular dysfunction and affects the physiology and pathology of heart (27). The dysfunction of the cardiovascular system including MI remains the leading cause of death in the Western world and this system is the primary organ attacked by stress. Most studies have been focused on the response of hypertrophied and diabetic heart to myocardial ischemia (4,7,16). And, the experimental animal models for these cardiovascular mechanisms have been reported as diversity of heart failure following ischemia-reperfusion injury after induction of acute MI (3,22,24,31). One emerging experimental approach to the study of cardiac disease, including the ischemia-reperfusion injury known as stunning, is to identify proteins that have been specifically altered or modified in the acute diseased state. Thus, many researches for reversible injury have been performed until now (3,22,24,25,31). However, it is unclear precisely which molecular events are responsible for the onset of stunning and which signaling events, if different, serve to exacerbate the dysfunction.

Many researchers have developed animal models to better understand the pathophysiology and to develop new treatment regimens for heart failure through the imagination of human disease (5,20,24,25). The choice of the animal model is important because the pathophysiology of heart disease in experiments must approximate to the human clinical condition as closely as possible. Therefore, the coronary artery ligation model of heart failure in rats is sufficiently possible as animal model that is clinically relevant (5,6,9,19). The most clinically concerned fact is that the progression to heart failure in these rats is similar to what happens when people sustain a large MI, survive, but go on to develop heart failure without another ischemic insult. However, in both rats and people with large infarctions, the progression to heart failure cannot be prevented sufficiently through the non-infarcted myocardium. Therefore, several models of cardiac hypertrophy, heart failure and disease have been developed in small mammals, especially the rat (14,22,23).

Proteomic techniques based on two-dimensional gel electrophoresis (2-DE) and mass spectrometry (MS) provide a very effective approach to identify proteins that have been specifically altered or modified in the diseased state (2,14,24,25,30,31). The rapid development of proteomic studies has enabled to verify the physiologic and pathologic processes.

[1]Corresponding author.
E-mail : jpark@chonbuk.ac.kr

To date, proteomics has identified changes of many proteins in chronic cardiovascular disease, such as dilated cardiomyopathy, varying degrees of ischemia-reperfusion injury and heart failure, except chronic MI. Therefore, we designed to analyze alterations in the myocardial protein expression following chronic MI using proteome analysis method.

Materials and Methods

Experimental coronary artery ligation

Sprague-Dawley (SD) rats (body weight 293 ± 0.98 g) aged 60 days old were obtained (Bio-Safety Research Institute, Chonbuk National University, Jeonju, Korea) and were conditioned in conformance with the US National Institutes of Health. The rats were given free access to food and tap water, and divided into the control group (sham) and experimental groups (MI). For artificial MI, the rats were anesthetized by ketamine (80.75 mg/kg) and xylazine (5.13 mg/kg) through intraperitoneal injection, and then were provided with artificial ventilation connected to a rodent ventilator with 80 strokes per min at room air by inserting a 16-gauge catheter into the trachea. Thoracotomy was performed through the left fourth intercostal space to expose the heart. To induce MI after exposure of heart, left main descending artery (LMDA) of left coronary artery was ligated just below 2-3 mm of LMDA rising using a 7.0 silk suture in the experimental rats (Fig 1). The sham rats also were treated in same surgical manner except ligation of LMDA. After surgery, all rats received the antibiotics (gentamicin sulfate, 5 mg/kg) treatment for a day. The sham group and MI groups are set according to the elapsed time as follows; sham rats for 7 days (sham, n = 5), MI for 7 days (MI7d, n = 5) and 30 days (MI30d, n = 5). Each rat was sacrificed at day 7 and 30 after surgery.

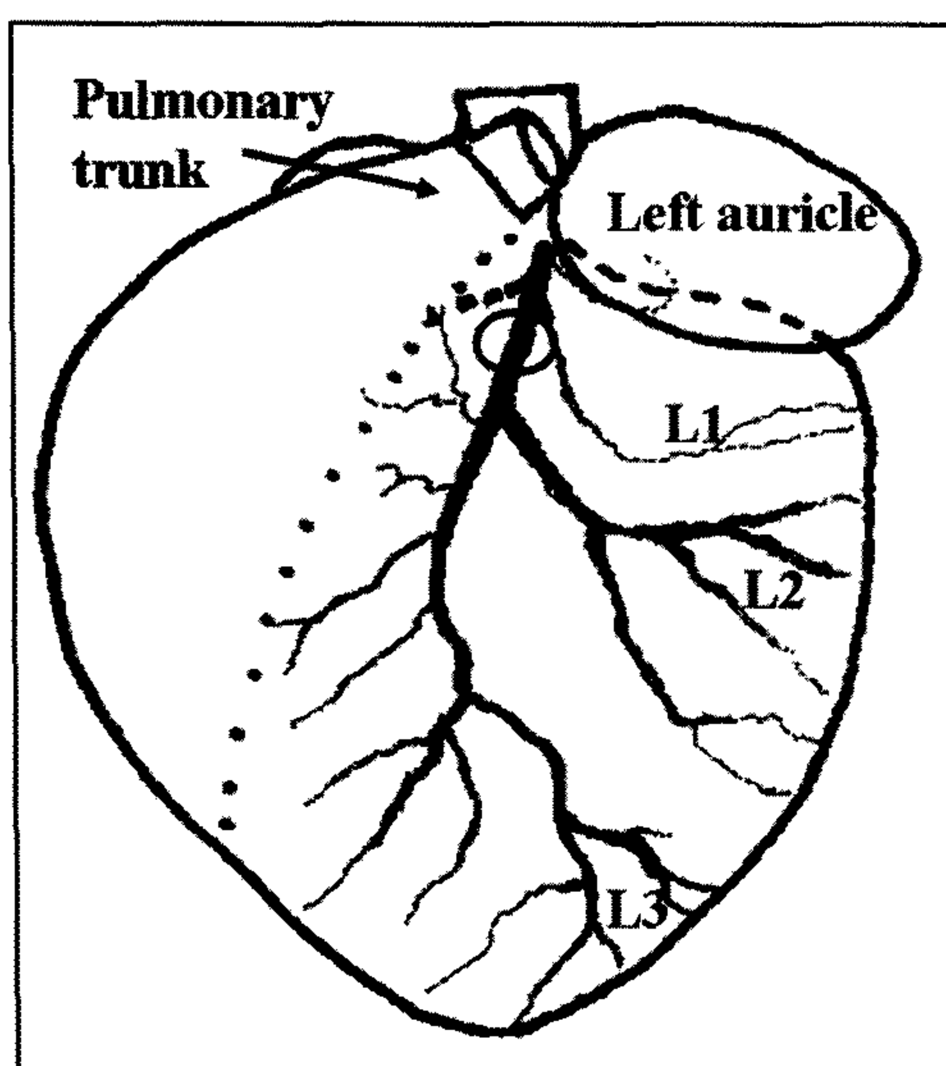

Fig 1. The picture for left main descending artery (LMDA) ligation (circle) of left coronary artery, just 2-3 mm below from its origin, with the experimental groups (both the MI7d and MI30d group). The annotations (L1, L2 and L3) indicate the branches of left coronary arteries.

Protein extraction from infarcted myocardia

To investigate the difference between groups not individual gap, the protein was prepared from three groups (sham, MI7d and MI30d group). After extraction of heart, the necrosis of myocardium was identified macrographically and incised. To extract the protein, the myocardia were treated with 10 nM E2 under serum-free condition for 24 hrs and suspended in 0.5 mL of 50 mM Tris buffer containing 7 M urea, 2 M thiourea, 4% (w/v) CHAPS and 16 μL protease inhibitor cocktail (Roche Molecular Biochemicals, USA). The lysates were homogenized and centrifuged at 15,000 rpm for 15 min. Fifty units of benzonase 250 units/μL (Sigma, USA) was added to the mixture and suitably stored at –80°C until further use after quantification by the Bradford method (Bio-Rad, USA) (17).

One-dimensional (1D) gel electrophoresis

Soluble myocardium extracts were denatured by boiling (5 min) in a sample buffer (125 mM Tris-HCl, pH 6.8, 20% v/v glycerol, 4% w/v sodium dodecyl sulfate (SDS), 10% v/v 2-mercaptoethanol and few crystals of bromphenol blue). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with discontinuous buffer system of pH 6.8 and 8.8 was used to separate proteins; 20 μg of total protein from each pool were loaded in each lane. The gels were run at a constant current of 15 mA in a 4% stacking gel and at 25 mA (3 mA/cm) in 12% resolving gel (with an acrylamide : bisacrylamide = 29 : 1). A standard curve, constructed from their relative migration and known molecular weights, was used to estimate the molecular weight of myocardium proteins by means of computer program table curve. After electrophoresis, the gels were incubated overnight in colloidal Coomassie Brilliant Blue G-250 solution (ProteomeTech Inc., Korea). The gels were recorded as digitalized images using a high resolution scanner (GS-710 Imaging Calibranted Densitometer: Bio-Rad, USA). The scanned images were analyzed by using ImageMaster™ 2D Platinum software (GE healthcare, USA).

Two-dimensional (2D) gel electrophoresis

For 2D analysis, 18 cm, pH 3-10 IPG strips (GE healthcare, USA) were rehydrated in swelling buffer containing 7 M urea, 2 M thiourea, 0.4% (w/v) DTT, and 4% (w/v) CHAPS. Five hundred micrograms of the protein lysates were cup-loaded into the rehydrated IPG strips using a Multiphor II apparatus (GE healthcare, USA) for a total of 57 kVh. The 2D separation was performed on 8-16% (v/v) linear gradient SDS-PAGE gels. Following fixation of the gels for 1 hr in a solution of 40% (v/v) methanol containing 5% (v/v) phosphoric acid, the gels were stained with colloidal Coomassie Brilliant Blue G-250 solution (ProteomeTech Inc., Korea) for 5 hrs. The gels were destained in 1% (v/v) acetic acid for 4 hrs

and then imaged using a GS-710 imaging calibrated densitometer (Bio-Rad, USA)(17).

Protein spot detection and 2D pattern matching were carried out using ImageMasterTM 2D Platinum software (GE healthcare, USA). For comparison of protein spot densities between control and treated samples, more than 20 spots throughout all gels were correspondingly landmarked and normalized. The quantified spots of candidate proteins were compared with the aid of histograms. For ensuring the reproducibility of 2D experiments, each sample was analyzed in duplicate.

In-gel protein digestion

Protein bands of interest were excised and digested in-gel with sequencing grade, modified trypsin (Promega, USA)(1). In brief, each protein spot was excised from the gel, placed in a polypropylene (Eppendorf) tube, and washed 4-5 times (until the gel was clear) with 150 μL of 1 : 1 acetonitrile/25 mM ammonium bicarbonate, pH 7.8. The gel slices were dried in a Speedvac concentrator (Hanil, Korea), and then rehydrated in 30 μL of 25 mM ammonium bicarbonate, pH 7.8, containing 20 ng of trypsin. After incubation at 37°C for 20 hrs, the liquid was transferred to a new tube. Tryptic peptides re-maining in the gel matrix were extracted for 40 min at 30°C with 20 μL of 50% (v/v) aqueous acetonitrile containing 0.1% (v/v) formic acid. The combined supernatants were evaporated in a Speedvac concentrator and dissolved in 8 μL of 5% (v/v) aqueous acetonitrile solution containing 0.1% (v/v) formic acid for mass spectrometric analysis.

Identification of proteins by LC-MS/MS

The resulting tryptic peptides were separated and analyzed using reversed phase capillary HPLC directly coupled to a Finnigan LCQ ion trap mass spectrometer (LC-MS/MS)(32). Both of a 0.1 × 20 mm trapping and a 0.075 × 130 mm resolving column were packed with Vydac 218MS low trifluoroactic acid C18 beads (5 μm in size, 300Å in pore size; Vydac, USA) and placed in-line. Following the peptides were bound to the trapping column for 10 min at with 5% (v/v) aqueous acetonitrile containing 0.1% (v/v) formic acid, then the bound peptides were eluted with a 50 min gradient of 5-80% (v/v) acetonitrile containing 0.1% (v/v) formic acid at a flow rate of 0.2 μL/min. For tandem mass spectrometry, a full mass scan range mode was m/z = 450-2000 Da. After determination of the charge states of an ion on zoom scans, product ion spectra were acquired in MS/MS mode with relative collision energy of 55%.

The individual spectra from MS/MS were processed using the TurboSEQUEST software (Thermo Quest, USA). The generated peak list files were used to query either MSDB database or NCBI using the MASCOT program (http://www.matrixscience.com). Modifications of methionine and cysteine, peptide mass tolerance at 2 Da, MS/MS ion mass tolerance at 0.8 Da, allowance of missed cleavage at 2, and

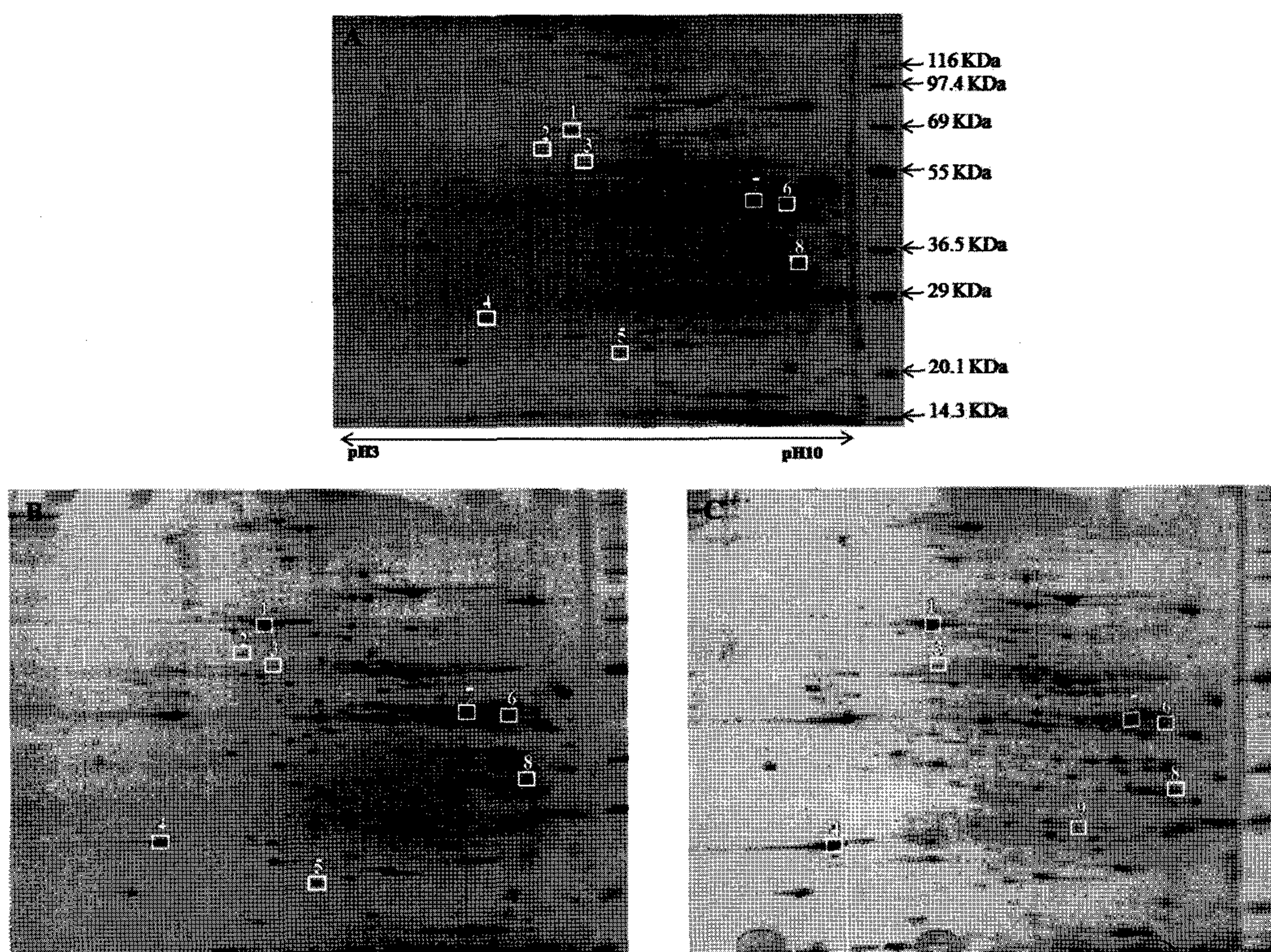

Fig 2. The comparison of proteins shown the increase in volume between the sham (A), MI7d (B) and MI30d (C) group on 2D gel. Protein identity for spot number 1~9 is identical to Table 1.

Table 1. Identification of the increased proteins in spot volume on 2D gels after myocardial infarction

Spot no.	Protein identity	Accession no.	Top score	Matched peptides/ Sequence coverage (%)	Theoretical Mr / pI	Observed Mr / pI
1	Albumin	NP_599153	882	19/38	71.16/6.09	68.00/6.00
2	Glucose regulated protein 58 kDa	AAH62393	851	49/46	56.59/5.88	62.00/5.60
3	Albumin	NP_599153	98	2/3	71.16/6.09	57.00/6.20
4	Similar to tripartite motif protein 50	XP_344968	312	6/12	54.01/5.88	26.50/5.10
5	Similar to tripartite motif protein 50	XP_344968	191	5/17	22.28/5.03	22.50/6.70
6	Ubiquinol-cytochrome c reductase core protein II	NP_001006971	926	50/41	48.37/9.16	48.00/9.15
7	Sarcomeric mitochondrial creatine kinase	CAA42414	629	51/33	47.36/8.76	49.00/8.50
8	ATP synthase alpha chain, mitochondrial precursor	P15999	428	10/18	58.93/9.22	34.00/9.25
9	Creatine kinase	AAA40936	139	2/10	33.25/7.88	28.50/8.00

charge states (+1, +2 and +3) were taken into account. Only significant hits as defined by MASCOT probability analysis were considered initially.

Results

Three representative examples of Coomassie blue stained gels for protein alterations following MI are shown in Fig 2. Two-dimensional image analysis was detected protein spots which were differently exhibited between the sham group and experimental groups (MI7d and MI30d). In each gel, 448 protein spots were located on sham gel (A and C), 592 spots on MI7d gel (B) and 454 spots on MI30d gel (D), respectively. Total number of matched spots between the sham and MI7d group was 32, and in between the sham and MI30d group was 20. The number of changed spots in volume between the sham and MI7d group was 35 and in between the sham and MI30d group was 21. Most spots detected on 2D gels of both the MI7d and MI30d group showed the increase in volume when was compared with the sham group. Among these spots, we selected the largely increased spots in volume and identified the protein using LC-MS/MS.

Among changed spots, 9 spots were continuously increased until 30 days after MI, and identified the protein using LC-MS/MS method (Table 1, Fig 2). The protein identity, accession no. and matched peptides are summarized in Table 1. The proteins and their changes in expression were as follows: (1) albumin was increased 3.5412-fold in the MI7d group and 6.2327-fold in the MI30d group; (2) glucose regulated protein 58 KDa was increased 8.8402-fold in the MI7d group and gradually decreased, then disappeared in the MI30d group (Fig 3); (3) albumin was increased 4.9494-fold in the MI7d group and 7.8341-fold in the MI30d group; (4) similar to tripartite motif protein 50 was increased 2.6927-fold in the MI7d group and 6.1119-fold in the MI30d group (Fig 3); (5) similar to tripartite motif protein 50 was increased 11.1009-fold and gradually decreased, then disappeared in the MI30d group; (6) ubiquinol-cytochrome c reductase core protein II (MI7d-IN20) was increased 5.6777-fold in the MI7d group and 9.6485-fold in the MI30d group (Fig 3); (7) sarcomeric mitochondrial creatine kinase (sMtCK) was increased 11.5577-fold in the MI7d group and 8.8813-fold in the MI30d group (Fig 3); (8) ATP synthase alpha chain, mitochondrial precursor was increased 2.5586-fold in the MI7d group and 3.5074-fold in the MI30d group (Fig 3); (9) creatine kinase (CK) was absent on the MI7d group and presented 6.9315-fold increase in the MI30d group (Fig 3).

Discussion

This study was designed to investigate the differences of protein expression following chronic MI in rats. In chronic MI (for 7 and 30 days after ischemia), the changed proteins in volume were analyzed and identified using 2D and LC-MS/MS method.

As an experimental ischemia was progressed, glucose regulated protein 58 KDa (GRP58) was gradually increased until 7 day after MI, and then it was disappeared during long ischemia (for 30 days). GRP58, known as endoplasmic reticulum (ER)-60, ERp57 and ERp61, is present within the lumen of the ER and is induced in response to various stresses including glucose deprivation (11). It also plays a pivotal role in folding of glycoproteins in the ER (8) and overexpression of this protein reduces the frequency of Ca^{2+} oscillations (13). It was suggested that phosphorylation of GRP58 was induced by glucose insufficiency in rat liver (11) and dephosphorylation of this protein was occurred during ischemia and reperfusion in rat heart (22). Also, Thuerauf *et al.*

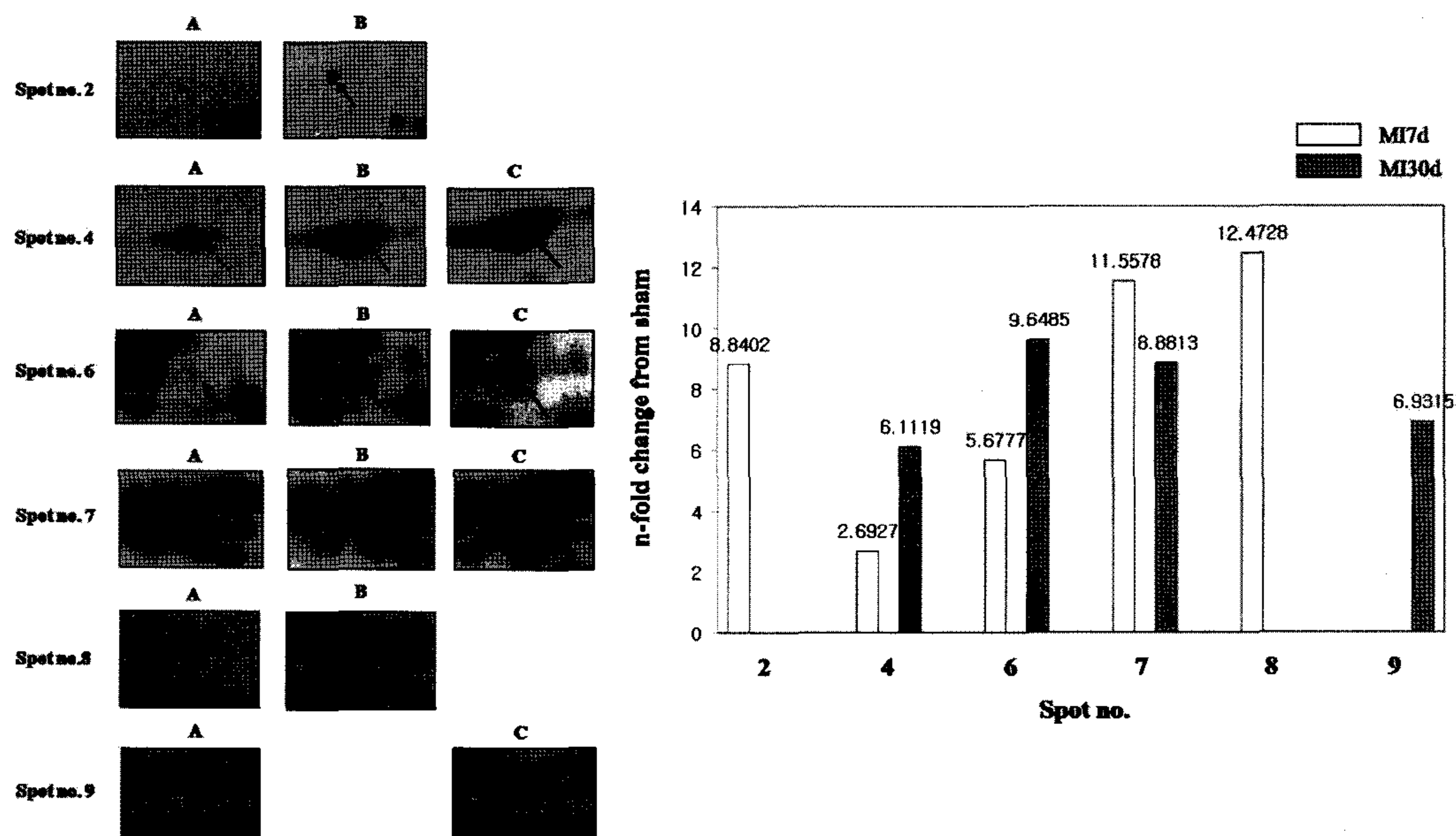

Fig 3. Densitometric analysis of 2D gels from the sham, MI7d and MI30d group show the level of proteins shown the change in the MI7d and MI30d group compared with the sham group

(28) reported that mouse hearts subjected to in vivo MI exhibited increased GRP78 expression. In our study, this protein was gone in myocardia suffered a long-term ischemia. Therefore, we can suppose that a long-term ischemia may induce the exhaustion of GRP58 in myocardium, because a continuous stress like chronic ischemia in this study affects the induction of GRP58.

Ubiquinol-cytochrome c reductase, complex III of the mitochondrial electron transport chain, was shown to be reduced in activity in rat hearts due to ischemia-reperfusion injury (12,18). These reductions in activity are routinely attributed to modification of the constituent electron transport complex proteins. One among subunit of complex III, core II, was shown in partial cleavage of complex III of bovine heart mitochondria (15). Core protein I was decreased over half of volume in bovine dilated cardiomyopathy (30) and increased after ischemia-reperfusion injury in rabbit myocardium (31). But, ubiquinol-cytochrome c reductase core II protein was little known in diseased heart. In result, we confirmed that ubiquinol-cytochrome c reductase core II protein was constantly increased until day 30 after MI. Lesnefsky *et al.* (12) and Petrosillo *et al.* (18) reported that the activity of ubiquinol-cytochrome c reductase was reduced in rat hearts, not ubiquinol-cytochrome c reductase core II protein. Thus, we could know the alteration of ubiquinol-cytochrome c reductase core II protein in chronic ischemia, though unknown well in diseased heart until now.

We observed proteins involved in myocardial energy metabolism, as a result of long ischemia. CK, sarcomeric mitochondrial precursor, is considered as a biomarker of long-term ischemic insult to the myocardium, and increased levels of CK in the plasma or serum are an indicator of the severity of myocardial injury following acute MI. The CK pathway during ATP deficiency is considered as an important complementary pathway for ATP deficiency. Liu *et al.* (14) reported that activation of CK was increased in chronic stressed rat. This report indicates that the energy supply was damaged after chronic stress and the complementary mechanism must be operated to protect cardiomyocytes from stress-induced injury. On the contrary, CK was decreased in ischemic injury for dog and ischemia-reperfusion for rabbit (24,31) and inhibition of CK function was associated with impaired recovery of contractility after a period of brief ischemia-reperfusion. In our results, CK was largely increased after a long-term ischemia, similarly with increase in chronic stressed rat. These increased activities may be indicated as a complementary reaction for insufficient energy supplement due to a long-term ischemia of myocardium. Therefore, we need an additional investigation for the difference from the increased activation of CK in dog and rabbit.

sMtCK, CK isoenzyme, is expressed in heart and skeletal muscle (20,21) and is primarily responsible for cellular energy homeostasis in the heart (21,26). But, in ischemia-reperfusion injury of rabbit, sMtCK volume was decreased (31). We observed an explosively increased volume of this protein in the MI7d group and a little decrease was present as ischemia is passed, but its volume was increased as ever in the MI30d group when was compared with the sham group. In our result, an increased sMtCK activity is thought to maintain energy homeostasis following energy deficiency due to ischemia.

It is well known that the tissue level of ATP as a result of increased energy demands is decreased during acute ischemia or ischemia-reperfusion. Jugdutt *et al.* (10) reported that a

concurrent decrease in tissue ATP during reperfused MI explains the changes in the two components of the ATP synthase protein complex (decrease in the ATP synthase α-subunit and increase in the ATP synthase δ chain). Ischemia affects the components of the mitochondrial ATP synthase complex in rat hearts (29). The ATP synthase α-subunit in reperfused ischemic myocardium was decreased in dog (10,24), and was increased or decreased in rabbit (31). Similarly or reversely to these results, we could acquire an increase of mitochondrial ATP synthase α-chain by 12.47-fold in the MI7d group. Therefore, we suppose that ischemia affects the mitochondrial ATP synthase α-subunit in rat hearts like the result of Van Eyk *et al.* (29) and cause the increased activity of this protein.

We also obtained additional proteins, albumin and similar to tripartite motif protein 50. It was well-known that albumin is abundantly present in plasma. The similar to tripartite motif protein 50 increased after ischemia is lack to formulate the hypothesis that the role of this protein in myocardium is important through this study.

In conclusion, this study presents the alterations in myocardial protein expression after chronic ischemia. These changes might be explained by activity of altered proteins in chronic MI which could exacerbate the dysfunction in the failing heart. The change of these multiple proteins in infarcted myocardia will substantively affect molecular function, rather than a result of damage to a single important protein. Therefore, we suggest that these proteins affect organically to heart function in chronic ischemia.

Acknowledgements

This work was supported by grant No. R01-2005-000-10369-0 from the Basic Research Program of the Korea Science & Engineering Foundation.

References

1. Bahk YY, Kim SA, Kim JS, Euh HJ, Bai GH, Cho SN, Kim YS. Antigens secreted from Mycobacterium tuberculosis: identification by proteomics approach and test for diagnostic marker. Proteomics 2004; 4: 3299-3307.
2. Corbett JM, Why HJ, Wheeler CH, Richardson PJ, Archard LC, Yacoub MH, Dunn MJ. Cardiac protein abnormalities in dilated cardiomyopathy detected by two-dimensional polyacrylamide gel electrophoresis. Electrophoresis 1998; 19: 2031-2042.
3. De Celle T, Vanrobaeys F, Lijnen P, Blankesteijin WM, Heeneman S, Van Beeumen J, Devreese B, Smits JFM, Janssen BJA. Alterations in mouse cardiac proteome after in vivo myocardial infarction: permanent ischaemia versus ischaemia-reperfusion. Exp Physiol 2005; 90: 593-606.
4. Feuvray D, Lopaschuk GD. Controversies on the sensitivity of the diabetic heart to ischemic injury: the sensitivity of the diabetic heart to ischemic injury is decreased. Cardiovasc Res 1997; 34: 113-120.
5. Fishbein MC, Maclean D, Maroko PR. Experimental myocardial infarction in the rat: qualitative and quantitative changes during pathologic evolution. Am J Pathol 1978; 90: 57-70.
6. Fletcher PJ, Pfeffer JM, Pfeffer MA, Braunwald E. Left ventricular diastolic pressure-volume relations in rats with healed myocardial infarction: Effects on systolic function. Circ Res 1981; 49: 618-626.
7. Friehs I, del Nido PJ. Increased susceptibility of hypertrophied hearts to ischemic injury. Ann Thorac Surg 2003; 75: 678-684.
8. High S, Lecomte FJL, Russell SJ, Abell BM, Oliver JD. Glycoprotein folding in the endoplasmic reticulum: a tale of three chaperones?. FEBS Letters 2000; 476: 38-41.
9. Johns TPN, Olson BJ. Experimental myocardial infarction. I. A method of coronary occlusion in small animals. Ann Surg 1954; 140: 675-682.
10. Jugdutt BI, Sawicki G. AT1 receptor blockade alters metabolic, functional and structural proteins after reperfused myocardial infarction: Detection using proteomics. Mol Cell Biochem 2004; 263: 179-188.
11. Kita K, Okumura N, Takao T, Watanabe M, Matsubara T, Nishimura O, Nagai K. Evidence for phosphorylation of rat liver glucose-regulated protein 58, GRP58/ERp57/ER-60, induced by fasting and leptin. FEBS Letters 2006; 580: 199-205.
12. Lesnefsky EJ, Gudz TI, Migita CT, Ikeda-Saito M, Hassan MO, Turkaly PJ, Hoppel CL. Ischemic injury to mitochondrial electron transport in the aging heart: Damage to the iron-sulfur protein subunit of electron transport complex III. Arch Biochem Biophys 2001; 385: 117-128.
13. Li Y, Camacho P. Ca2+-dependent redox modulation of SERCA 2b by ERp57. J Cell Biol 2004; 164: 35-46.
14. Liu X, Qian L, Gong J, Shen J, Zhang X, Qian X. Proteomic analysis of mitochondrial proteins in cardiomyocytes from chronic stressed rat. Proteomics 2004; 4: 3167-3176.
15. Lorusso M, Gatti D, Marzo M, Papa S. Effect of papain digestion on redox-linked proton translocation in b-c1 complex from beef heart reconstituted into liposomes. FEBS 1985; 182: 370-374.
16. Ooie T, Takahashi N, Nawata T, Arikawa M, Yamanaka K, Kajimoto M, Shinohara T, Shigematsu S, Hara M, Yoshimatsu H, Saikawa T. Ischemia-induced translocation of protein kinase C-epsilon mediates cardioprotection in the streptozotocin-induced diabetic rat. Circ J 2003; 67: 955-961.
17. Park YD, Kim SY, Jang HS, Seo EY, Namkung JH, Park HS, Cho SY, Paik YK, Yang JM. Towards a proteomic analysis of atopic dermatitis: A two-dimensional-polyacrylamide gel electrophoresis mass spectrometric analysis of cultured patient-derived fibroblasts. Proteomics 2004; 4: 3446-3455.
18. Petrosillo G, Ruggiero FM, Venosa ND, Paradies G. Decreased complex III activity in mitochondria isolated from rat heart subjected to ischemia and reperfusion: role of reactive oxygen species and cardiolipin. FASEB J 2003; 17: 714-716.
19. Pfeffer MA, Pfeffer JM, Fishbein MC, Fletcher PJ, Spadaro J, Kloner RA, Braunwald E. Myocardial infarct size and ventricular function in rats. Circ Res 1979; 44: 503-512.
20. Qin W, Khuchua Z, Boero J, Payne RM, Strauss AW.

Elements regulating cardiomyocyte expression of the human sarcomeric mitochondrial creatine kinase gene in transgenic mice. Histochem J 1999; 31: 357-365.
21. Qin W, Khuchua Z, Klein SC, Strauss AW. Elements regulating cardiomyocyte expression of the human sarcomeric mitochondrial creatine kinase gene in transgenic mice. J Biol Chem 1997; 272: 25210-25216.
22. Sakai J, Ishikawa H, Kojima S, Satoh H, Yamamoto S, Kanaoka M. Proteomic analysis of rat heart in ischemia and ischemia-reperfusion using fluorescence two-dimensional difference gel electrophoresis. Proteomics 2003; 3: 1318-1324.
23. Sakai J, Ishikawa H, Satoh H, Yamamoto S, Kojima S, Kanaoka M. Two-dimensional differential gel electrophoresis of rat heart proteins in ischemia and ischemia-reperfusion. Methods Mol Biol 2007; 357: 33-43.
24. Sawichi G, Jugdutt BI. Detection of regional changes in protein levels in the in vivo canine model of acute heart failure following ischemia-reperfusion injury: functional proteomics studies. Proteomics 2004; 4: 2195-2202.
25. Schwertz H, Langin T, Platsch H, Richert J, Bomm S, Schmidt M, Hillen H, Blaschke G, Meyer J, Darius H, Buerke M. Two-dimensional analysis of myocardial protein expression following myocardial ischemia and reperfusion in rabbits. Proteomics 2002; 2: 988-995.
26. Spindler M, Niebler R, Remkes H, Horn M, Lanz T, Neubauer S. Mitochondrial creatine kinase is critically necessary for normal myocardial high-energy phosphate metabolism. Am J Physiol 2002; 283: 680-687.
27. Stephenson RB. Overview of cardiovascular function. In: Cunningham JG, Klein BG. Textbook of veterinary physiology. 4th ed. Philadelphia, PA: Saunders. 2007: 178-191.
28. Thuerauf DJ, Marcinko M, Gude N, Rubio M, Sussman MA, Glembotski CC. Activation of the unfolded protein response in infarcted mouse heart and hypoxic cultured cardiac myocytes. Circ Res 2006; 99: 275-282.
29. Van Eyk JE, Powers F, Law W, Larue C, Hodges RS, John Solaro R. Breakdown and release of myofilament proteins during ischemia and ischemia/reperfusion in rat hearts: Identification of degradation products and effects on the pCa-force relation. Circ Res 1998; 82: 261-271.
30. Weekes J, Wheeler CH, Yan JX, Weil J, Eschenhagen T, Scholtysik G, Dunn MJ. Bovine dilated cardiomyopathy: Proteomic analysis of an animal model of human dilated cardiomyopathy. Electrophoresis 1999; 20: 898-906.
31. White MY, Cordwell SJ, McCarron HCK, Prasan AM, Craft G, Hambly BD, Jeremy RW. Proteomics of ischemia/reperfusion injury in rabbit myocardium reveals alterations to proteins of essential functional systems. Proteomics 2005; 5: 1395-1410.
32. Zuo X, Echan L, Hembach P, Tang HY, Speicher KD, Santoli D, Speicher DW. Towards global analysis of mammalian proteomes using sample prefractionation prior to narrow pH range two-dimensional gels and using one-dimensional gels for insoluble and large proteins. Electrophoresis 2001; 22: 1603-1615.