REGIONAL CHANGES IN CARDIAC STRUCTURAL PROTEINS IN MYOCARDIAL INFARCTION

Biochemical and histologic correlates

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1. Quantitative analysis of structural proteins from a minute amount of myocardial tissue was performed from 10 μ thick cardiac tissue slices weighing 2 to 5 mg by the extraction in glycerol solution and by sodium dodecyl-sulfate (SDS) gel electrophoresis, and these changes were compared with the histologic alterations in the striated structure of the adjacent cardiac slices in the experimental myocardial infarction in the dog.
2. Approximately 69 μ g of structural proteins were obtained from 1 mg of the normal heart muscle. In the central portions of the myocardial infarction, reductions in myosin heavy chain (HC), light chain (LC) 1 and α -actinin were observed at 12 to 24 hours after the coronary occlusion followed by the decrease in myosin LC 2 at 48 hours. Those changes became intense at 72 hours to 7 days, but restored gradually at 14 to 28 days.
3. Alterations in the striated structure of cardiac muscle fibers of the adjacent tissue slices were found simultaneously with the changes in structural proteins. At 12 to 24 hours after the coronary ligation increase in eosinophilia and overstretch of cross-striation were observed. The findings of coagulation necrosis, loss of striation, fragmentation, swelling of A-band, etc. of the infarcted fibers were markedly observed at 48 hours to 7 days, but the histologic restoration of cardiac fibers was found with the recovery of the infarcted tissue at 14 to 28 days after the coronary ligation.
4. Changes in the compositions of structural proteins corresponded well to the alterations in the striated structure in chronology and in quantity.

Key Words:

Structural proteins
Myocardial infarction
The canine heart
Cardiac tissue slices
SDS gel electrophoresis

RECENT advances in biochemical studies on the muscular structural proteins, both of skeletal and cardiac muscles, have been revealing new knowledges of various neuromuscular and cardiac diseases. In the myocardial infarction, Katagiri¹ reported the useful method to study the alterations in the compositions of cardiac structural proteins from approximately 1g of

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the cardiac tissue. In that paper, the author described the reductions in the relative contents of myosin heavy chain (HC) and α -actinin at 24 to 48 hours after the onset of the myocardial infarction with the relative preservation of actin, tropomyosin and troponin-T, and the recovery of those proteins at 28 days. However, the amount of 1 g is somewhat too large to discuss the precise correlates between the alterations in structural proteins and the histopathologic changes in local cardiac fibers.

Histologically, the infarcted myocardial tissue is not homogeneous²⁻⁴ In myocardial infarction in the dog, necrotic, injured and living myocardial cells are mixed heterogeneously according to the intricate collateral distribution. From this point of view, the authors attempted to analyse structural proteins of the very central portion of the infarction from a minute amount of 10 μ thick tissue slices weighing 2 to 5 mg, comparing those with the histology of the corresponding portion. And the changes in myosin light chains (LC) which were not mentioned in the previous paper were studied as well.

MATERIALS AND METHODS

Animal experiments. The experimental myocardial infarction was produced in dogs in the same manner as described in the previous paper.¹ Briefly, 23 adult mongrel dogs, weighing 12 to 26 Kg, were anesthetized with intravenous injection of pentobarbital sodium (Nembutal®). The anterior descending branch of the left coro-

Fig.1. Contents of structural proteins from cardiac tissue slices in the myocardial infarction in the dog.

nary artery was isolated and ligated with a silk thread just below the diagonal branch and the chest was closed. At 12, 24, 48 and 72 hours, 7, 14 and 28 days after the ligation in 3, 4, 3, 3, 3, 3 and 4 dogs, respectively, the dogs were sacrificed and the beating heart was removed immediately and washed in cold physiological saline solution. The very central portion of the infarcted anterior wall of the left ventricle including the anterior papillary muscle was removed together with the non-infarcted posterior muscle as a control. In all cases the subendocardial layer was included invariably.

The cardiac tissue was cut to 5 mm cubes with scissors and frozen in liquid nitrogen for approximately 15 seconds, and sliced to 10 μ thickness to the longitudinal direction of cardiac fibers

Fig.2. 5 and 10% acrylamide gels of structural proteins from cardiac tissue slices in the myocardial infarction in the dog.

TABLE 1 COMPOSITIONS OF STRUCTURAL PROTEINS FROM CARDIAC TISSUE SLICES IN MYOCARDIAL INFARCTION IN THE DOG

	Myosin Heavy Chain	α -Actinin	Actin	Troponin-T	Tropo- myosin	Myosin Light Chain 1	Myosin Light Chain 2
Control	2.22 $\pm$ 0.10	0.24 $\pm$ 0.04	1.0	0.10 $\pm$ 0.02	0.31 $\pm$ 0.04	0.31 $\pm$ 0.03	0.19 $\pm$ 0.04
12 hours	2.15 $\pm$ 0.10	0.22 $\pm$ 0.03	1.0	0.11 $\pm$ 0.03	0.30 $\pm$ 0.08	0.17 $\pm$ 0.03*	0.23 $\pm$ 0.05*
24 hours	1.65 $\pm$ 0.16**	0.16 $\pm$ 0.03**	1.0	0.13 $\pm$ 0.04**	0.30 $\pm$ 0.07	0.17 $\pm$ 0.04*	0.23 $\pm$ 0.05*
48 hours	0.86 $\pm$ 0.25**	0.07 $\pm$ 0.04**	1.0	0.14 $\pm$ 0.01**	0.32 $\pm$ 0.07	0.10 $\pm$ 0.03**	0.13 $\pm$ 0.04*
72 hours	0.85 $\pm$ 0.35**	0.12 $\pm$ 0.03**	1.0	0.15 $\pm$ 0.05*	0.23 $\pm$ 0.05**	0.09 $\pm$ 0.04**	0.15 $\pm$ 0.06*
7 days	0.90 $\pm$ 0.28**	0.09 $\pm$ 0.01**	1.0	0.12 $\pm$ 0.03	0.27 $\pm$ 0.09	0.08 $\pm$ 0.05**	0.13 $\pm$ 0.05*
14 days	1.39 $\pm$ 0.38**	0.09 $\pm$ 0.01**	1.0	0.10 $\pm$ 0.01	0.28 $\pm$ 0.05	0.14 $\pm$ 0.03**	0.17 $\pm$ 0.05
28 days	2.24 $\pm$ 0.18	0.25 $\pm$ 0.04	1.0	0.11 $\pm$ 0.02	0.30 $\pm$ 0.02	0.20 $\pm$ 0.10	0.17 $\pm$ 0.09

Values are mean $\pm$ S.D. versus Actin. * $p < 0.05$, ** $p < 0.01$, versus Control.

with a Bright's cryostat.

Preparation of structural proteins from cardiac tissue slices. Eight to 20 pieces of cardiac slices, weighing 2 to 5 mg, were immersed in a solution containing 50% glycerol, 0.1 M NaCl and 5 mM NaHCO₃. The suspension was kept at 0°C (in ice) for 24 hours and at -20°C for another 14 days. Then the suspension was diluted by the addition of 3 volumes of 0.1 M NaCl and 5 mM NaHCO₃ and centrifuged at 10,000 $\times$ G for 10 minutes. The precipitate was suspended again in the same solution and spun at the same speed. This procedure was repeated once again and the so-called protein solution, containing 0.01 M sodium phosphate buffer (pH 7.0), 3% SDS and 3% β -mercaptoethanol, was added directly to the final precipitate and applied to SDS gel electrophoresis.⁵ Twenty adjacent cardiac slices were also treated in the same manner with the additional homogenization with a teflon-glass homogenizer before the first centrifugation for the measurement of protein contents. Protein concentration was determined by the method of Lowry, et al.⁶

Preparation of cardiac myosin. Cardiac myosin was isolated from the left ventricle of the healthy adult mongrel dogs by the method of Katz, et al.⁷ Isolated myosin was used as a marker of myosin light chains among the protein bands of structural proteins on the gel.

SDS polyacrylamide gel electrophoresis. SDS gel electrophoresis was performed principally by the method of Weber and Osborn.⁵ Details were modified according to the description of Katagiri.¹ Samples were applied to 5 and 10% gels. Purified cardiac myosin was run in each series of the phoresis as an authentic marker of myosin

light chains. The compositions of structural proteins were calculated from the densitometric curves.

Histologic studies. Several cardiac tissue slices adjacent to the slices in which structural proteins were analyzed were stained with hematoxylin and eosin for the purpose of histologic examinations.

RESULTS

Contents of structural proteins of the infarcted cardiac tissue slices. Figure 1 shows the contents of structural proteins from non-ischemic and infarcted left ventricular slices. Approximately 69 μ g of structural proteins were obtained from 1 mg of the non-ischemic left ventricular heart tissue. The contents of structural proteins in the infarcted cardiac tissue slices, which were just adjacent to the slices in which the structural proteins were analyzed, were decreased distinctly at 12 to 24 hours after the ligation ($p < 0.02$), falling to less than 30% of that of the non-ischemic myocardium at 72 hours to 14 days. At 28 days the contents recovered to approximately 50% leaving the portion replaced by the connective tissue.

Compositions and molecular weights of structural proteins of the normal canine heart muscle. As shown in Figure 2, SDS gel electrophoresis of isolated myosin from the canine left ventricle showed two rapid migrating light chain bands and a single slowly migrating heavy chain band. The molecular weights of light chains in SDS gel electrophoresis were on the average 26,000 and 17,000 for LC 1 and LC 2, respectively. Similar patterns and molecular weights have been reported for myosins from rabbit,⁸ calf,⁹ and sheep¹⁰

Fig.3. Photomicrographs of the non-ischemic myocardium and of the early stage of the myocardial infarction in the dog. A bar in the right lower part shows 10 μ . All sections were stained with hematoxylin and eosin.

cardiac muscle.

SDS gel electrophoresis of structural proteins of the normal left ventricle exhibited the same pattern as that in the description of Katagiri,¹ and two bands which were at the positions corresponding to light chains of isolated myosin were also found among the bands of the lower molecular weights of structural proteins on the gel.

Patterns and compositions of structural proteins in the myocardial infarction. Figure 2

shows the patterns of structural proteins obtained from the central portions of the infarcted cardiac tissues. Compositions of structural proteins obtained from the densitometric curves are shown in the Table I. Until at 12 hours after the coronary ligation, no changes were noted in the pattern or compositions of structural proteins. At 24 hours the reductions in the contents of myosin HC and α -actinin were observed accompanying the decrease in LC 1. At 48 hours the decreases in these proteins became intense

Fig.4. Photomicrographs of the chronic stage of the myocardial infarction in the dog. A bar in the right lower part indicates 10 μ . Hematoxylin and eosin.

with the reduction in the content of LC 2. With myosin light chains LC 2 seemed to degrade slowly than HC and LC 1. The components of thin filament, actin, tropomyosin and troponin-T, were relatively preserved. At 72 hours to 7 days, those changes were most prominent. Contents of myosin subunits and α -actinin were decreased to approximately 30% of those of the non-ischemic portion. At 14 to 28 days after the coronary ligation, the restoration of these proteins were observed, showing the complete recovery at 28 days. The changing pattern of structural proteins was quite similar to that in the report of Katagiri¹

Histologic changes. Figures 3 and 4 show photomicrographs of infarcted cardiac tissue slices just adjacent to the slices in which structural proteins were investigated. In this paper the findings of muscle fibers were mainly described with special reference to the striated structure to compare them with the patterns and compositions of structural proteins. Because of frozen sections of unfixed cardiac tissue, the fragmentation and the dissociation of cardiac fibers were often observed, but those were easily differentiated from the histopathologic changes.

At 12 hours after the ligation, the muscle fibers were stained slightly eosinophilic and swollen. The cross-striations were well retained, but sarcomeres were overstretched in places with brighter sarcoplasm. The interstitial edema was noted slightly. At 24 to 48 hours, the necrotic features became quite obvious. Muscle fibers were stretched intensely at the I-band with increasing in eosinophilia. A-bands were swollen. The wave arrangement and the fragmentation of myocardial fibers were observed here and there, and some cells exhibited the complete cellular disorganization with a granularity of the sarcoplasm. Neutrophilic infiltration was observed markedly. At 72 hours to 7 days, the cardiac fibers exhibited the feature of the typical coagulation necrosis. They were stained intensely eosinophilic with swelling of A-band, and the fragmentation and the segmentation were observed abundantly, and the loss of cross-striation was found in places. At 14 days after the coronary ligation, the striated structure was considerably restored in the muscle fibers which had survived in the infarcted tissue together with the regeneration of vessels and the proliferation of granulation tissue. The sarcomere length became narrower. And at 28 days the morphologic features of cardiac muscle fibers remaining in the cicatrized

connective tissue were similar to those of non-ischemic tissue slices. Those morphologic alterations in the striated structure of cardiac fibers corresponded well to the changes in the compositions of structural proteins, mainly reductions in myosin and α -actinin, either in chronology or in quantity.

DISCUSSION

Quantitative analysis of cardiac structural proteins was attempted from a minute amount of the heart tissue, approximately 2 to 5 mg, which was only twice by weight of tissue slices used for histopathologic examination, by glycerination and by SDS gel electrophoresis.

The heart tissue contains approximately 60 mg per gram of structural proteins.¹¹ Therefore it should be possible to prepare 60 μ g of proteins from 1 mg of the heart tissue which is enough to be applied to SDS gel electrophoresis, with the most efficient method of the extraction. The treatment of the cardiac muscle in glycerine solution, i.e., glycerination, was adopted for this purpose. And it is needless to say the usefulness of SDS gel electrophoresis for the analysis of structural proteins.^{1,8,9,12}

Concerning another analytical method of tissue proteins from a smaller amount of the muscular tissue, Mittelbach and Hamburger¹³ reported the determination of muscle proteins by applying skeletal muscle slices directly to polyacrylamide gels detecting the protein bands by specific enzymic stains. The proteins in soluble fraction were mainly determined with their method, but the actomyosin system would have been in the form of the aggregation and stayed on the top of polyacrylamide gels.

With the normal myocardium, the pattern and the composition of structural proteins were quite identical to those in the previous report¹ in which the extraction of structural proteins was performed by the repeated procedure of suspension and precipitation by the centrifugation of heart tissue homogenate in low ionic strength. The molecular weights and the compositions of isolated myosin subunits were also identical to those of previous reports.⁸⁻¹⁰

In the myocardial infarction the degrading pattern of structural proteins was the same as those in the previous paper,¹ the reductions in myosin and α -actinin with relative preservation of actin, tropomyosin and troponin-T. However, the degradation of these proteins was more rapid in the very central portion of the infarction com-

pared to the data in the previous report in which structural proteins were studied from 1 gram mass of the infarcted tissue. This means that the ischemic changes do not homogeneously spread through the myocardium and the central portion of the infarction, especially its subendocardial portion, is affected most rapidly.¹⁴ With myosin light chains, LC 1 was decreased together with the degradation of HC. Whereas LC 2 was reduced somewhat later. However, the mechanism of this different degradation is not defined yet, some differences in turnover rates and in amino acid compositions, especially contents of the thiol groups, will be suspected in speculations.

The observed histologic changes in cardiac muscle fibers were similar to those of many previous articles^{2-4,15-18}. Briefly summarizing, the necrotic features of infarcted cardiac muscle fibers become evident at 6 to 12 hours after coronary occlusion; hyaline degeneration of myocardial fibers, increased eosinophilia, agglomeration of swollen anisotropic substance, fragmentation of anisotropic substance and diminution of the isotropic substance, etc. The cross-striation is stretched at the I-band. After 24 to 48 hours these changes become more extensive. Hyaline muscle shows considerable fragmentation, and the cross-striation of the cardiac fibers is lost. With the infiltration of polymorphonuclear leukocytes, phagocytotic figures of the fragmented fibers are apparent.

These changes are also described in the papers with the electron microscope. Bryant and the co-workers¹⁹ and Denker and the associates²⁰ described increased density, disruption and distortion of Z-bands and A-bands at around 24 hours after the coronary ligation. These morphologic changes are compatible to the reductions in myosin and α -actinin, which are the main constituents of the A-band and the Z-band, respectively. These histologic and biochemical alterations in cardiac muscle fibers and their constituents were in good correspondence both in chronology and in quantity.

The contents of structural proteins in the chronic stage of myocardial infarction were also somewhat different from the previous report.¹ Previously, the almost complete recovery of the content of structural proteins at 28 days was described together with the normalization of the compositions of structural proteins. However, in this report in which the very central portion of the infarction was studied, the recovery of the

protein content was not completed; only 50% of structural proteins was obtained with the remaining cicatrized connective tissue. The reason of this difference is suspected that the amount of the connective tissue would be negligible in 1 gram mass of the infarcted tissue which may contain the peri-infarctional non-ischemic heart muscle at 28 days after the coronary ligation. The remaining structural proteins were in the normal composition, and the cross-striation structure of the remaining cardiac fibers showed the restored features. Concerning these improvements of the composition of structural proteins and of the histologic findings in the chronic stage of myocardial infarction, it is still not clear, as discussed in the previous report,¹ whether the ischemically injured cardiac fibers had recovered in the time course of infarction or the non-ischemic myocardium in the infarcted tissue was taken as a sample after the absorption of necrotic fibers. Schwartz, et al.²¹ described the presence of viable cells in the infarcted tissue at around 15 days after the ligation and the thickened Z-bands in them suggestive of new contractile filaments. With this point the more precise investigations should be done in the future. Anyhow it should be said that the restoration of cardiac fibers in the infarcted tissue is accomplished at 14 to 28 days after the coronary ligation even though the cicatrized connective tissue is still remaining in the infarction.

The extraction and analytical method of cardiac structural proteins described in this article would be expressed as histologic biochemistry of cardiac structural proteins and would be truly useful for the detection of changes in structural proteins of locally affected heart tissue not only in the pathologic but also in the clinical fields utilizing cardiac biopsy.

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