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Changes of α-B-Crystalline in Cardiac Grafts

L. I. Kovalev*, E. S. Ershova*, L. I. Obel'chuk, D. A. Tsomartova, P. Z. Khasigov, V. V. Severin, E. I. Ryaboshtanova**, I. M. Il'inskii**, and S. S. Shishkin***

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Polypeptide composition of cardiac grafts is studied using two-dimensional electrophoresis. Changes in the content of α-chain of B-crystalline were found, which correlated with remoteness of chronic cardiac graft rejection. In the kidney of patient with chronic cardiac graft rejection, unusual concentrations of α-B-crystalline and products of its degradation (microsequence data) were found.

Key Words: *transplantation; crystalline; man; two-dimensional electrophoresis*

Many problems of practical transplantology and delayed outcomes of heart transplantation are related to the development of chronic graft rejection. Diagnosis of chronic graft rejection is based primarily on clinical manifestations and physiological and biochemical tests. Morphological analysis provides the most precise diagnosis [1,5]. Molecular processes remain poorly studied; however, molecular markers of pathological processes are the most accurate and early diagnostic signs.

Extensive systemic studies of protein spectra with the use of two-dimensional electrophoresis (TDE) and other precise methods [2,8,9] open new prospects in the investigation of molecular mechanisms and screening for marker proteins of various complications in organ transplantation. In the future, these proteins can be used for the development of highly sensitive and inexpensive diagnostic test systems.

I. M. Sechenov Moscow Medical Academy; *Center for Medical Genetic, Russian Academy of Medical Sciences; **Institute of Transplantology and Artificial Organs, Ministry of Health, Moscow

In the present study changes in protein spectrum and signs of cardiac and renal graft rejection were analyzed using TDE and our previous data [2,6,9].

MATERIALS AND METHODS

The study was performed on autopsy samples of human myocardium (n=100) and kidney (n=15) obtained not later than 3 h after death caused by an incident (control group) and on myocardial (n=4) and kidney (n=6) samples from patients died after allograft transplantation.

Myocardial and renal samples were homogenized in a glass-Teflon homogenizer in 5 volumes of 9.5 M urea, containing 2% Triton X-100, 2% 2-mercaptoethanol, and 2% ampholines, pH 3.5-10 [2,6,9].

Preparation of samples for TDE, TDE fractionation according to O'Farrell with some modifications, and staining with Coomassie blue R-250 and silver nitrate were described previously [9].

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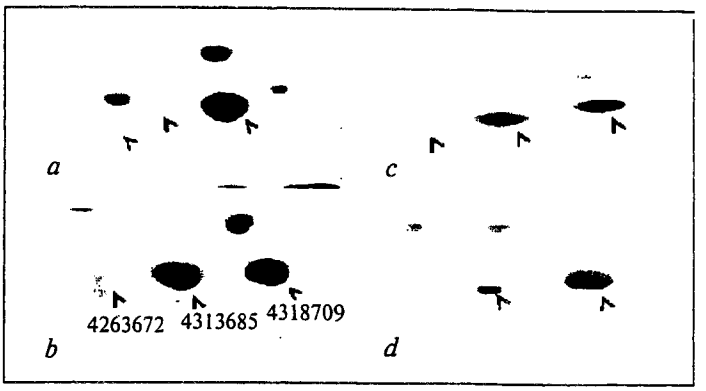

Fig. 1. Crystalline zone fragments of two-dimensional electrophoregrams of myocardial proteins. *a*) norm, *b-d*) myocardial samples from different patients with chronic cardiac graft rejection. Arrows indicate crystalline fractions and enlarged spots.

Protein fractions were collected and used for microsequencing. Microsequencing was performed as described previously [2,9].

RESULTS

The method of TDE allows one to isolate about 250 protein fractions from myocardial samples [9] and about 200 fraction from kidney extracts [4]. All these proteins were enumerated in a standard notation system and each protein was given a personal seven-digit number. Information on muscular and renal proteins collected in computerized databases was used in the study.

Analysis revealed a similar TDE pattern for cardiac allografts and myocardial samples from the control group. In all samples, an increased protein content in 4313685 (20.60 kD, pI 6.85) and 4263672 (18.30 kD, pI 6.72) spots and a decreased protein content in 4318709 (20.80 kD, pI 7.09) spot were found (Fig. 1). Protein 4318709 was previously identified as α-chain of B-crystalline [9].

When comparing electrophoretic patterns of fragments presented in Fig. 1 (and taking into account the decreased protein content in original crystalline spot), it can be hypothesized that fractions 4313685 and 4263672 are products of crystalline modification/degradation. This assumption was confirmed by a correlation between relative increase of 4313685 and 4263672 spots and time after transplantation (Fig. 1, *b-d*).

It has been previously shown that similar changes of the crystalline zone accompany autolytic processes in human myocardium [6]. Figure 2 shows TDE pattern of cardiac protein in the crystalline zone at different stages of autolytic degradation. However, these changes in grafts are directly induced by usual autolytic process. First, the time when the samples were obtained (3 h after death) far preceded the appearance of autolytic degradation signs in the crystalline zone (more than 1-2 days after death). Second, TDE analysis revealed no other changes in the crystalline zone typical of autolytic degradation: additional polypeptides in the zone of light myosin chains (day 1 after death) and changes in the amount of myosin light chain 2 and ATP synthase (day 2 after death) [6]. Thus, changes of TDE pattern in the crystalline zone are most likely related to specific processes in cardiac grafts involving these particular proteins.

Surprisingly, parallel analysis of renal proteins in patients with chronic cardiac graft rejection revealed some changes in TDE pattern of the crystalline zone. Figure 3 shows manyfold accumulation of two proteins; one of these proteins corresponded to crystalline by electrophoretic properties.

For more precise identification semipreparative amounts of these proteins were obtained and subjected to trypsinolysis followed by microsequencing of individual fragments. Amino acid sequences of two fragments of renal 4318709 protein (20.8 kD, pI 7.09, which corresponded by electrophoretic properties of crystalline) were VLGDV and VKHFSPEEL. This completely coincided with the known amino acid sequence of human α-B-crystalline within 93-97

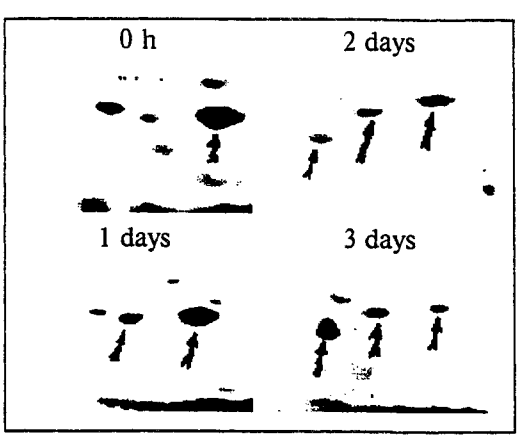

Fig. 2. Changed protein spots in the α-crystalline zone in autolysis at different times of incubation at 25°C. Crystalline and changeable spots are indicated by arrows.

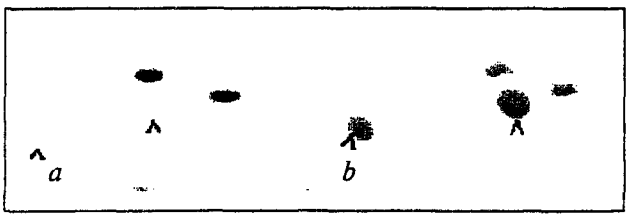

Fig. 3. α-B-Crystalline zone fragments of two-dimensional electrophoregrams of renal proteins. *a*) norm, *b*) chronic cardiac graft rejection. Arrows indicate spots enlarged in rejection.

and 81-89 residues. Among tryptic fragments of another protein (4295677, 19,80 kD, pI 6.77), a fragment YRIPADVDPL was found. This sequence also coincided with that of a-B-crystalline within 122-131 residues. Thus, renal 4295677 protein is a shortened α-B-crystalline molecule. In other words, our findings indicate accumulation of α-B-crystalline and products of its modification in the kidneys of patients with chronic kidney graft rejection.

On the other hand, analysis of renal proteins from patients with chronic renal graft (n=3) rejection revealed no changes in the content of α-B-crystalline in the kidney graft. This suggests that crystalline accumulation in the kidney is specifically related to chronic cardiac graft rejection (Fig. 3).

The function of α-B-crystalline in the muscular tissues remains unknown. It has been hypothesized that α-B-crystalline exhibiting properties of molecular chaperone [7,10] participates in protein folding and/or protein translocation through mitochondrial membranes and membranes of the endoplasmic reticulum and assists to other types of protein transport [3]. In light of this, it can be assumed that in chronic graft rejection and ischemia accompanying this pathology α-B-crystalline participate in translocation of myocardial proteins from cardiomyocytes and therefore appeared in the circulation. Finally, crystalline molecules are accumulated in the kidneys. Experimental verification of this assumption will provide an approach to the understanding of the role of B-crystalline in muscular cells and to the use of this protein as a molecular marker in the diagnosis of cardiac graft pathology.

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