

Initiation of Stress Protein Synthesis in the Myocardium of Coronary Patients

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We studied myocardial biopsy specimens from the right atrium of cardiological patients with different degree of cardiac ischemia obtained during surgery. No inducible HSP70 stress proteins were detected in atrial cardiomyocytes of patients with the WPW syndrome without signs of ischemic injuries of the heart. These proteins were detected in the myocardium of coronary patients. Their expression was more intense in patients with coronary disease paralleled by the development of myocardial dyskinesia. Two-dimensional electrophoresis showed only acid HSP70 but no alkaline isoforms in coronary patients even with pronounced dyskinesia. Presumably, alkaline HSP70 isoforms are present in the myocardium directly involved in the dyskinesia zone.

Key Words: *myocardium; stress proteins; coronary disease*

The formation of chronic coronary disease is paralleled by the development of hypoxemic injury in the myocardium and adaptation to these conditions [1,3,4]. Accumulation of stress proteins in cells is a manifestation of universal cellular reaction to extreme exposure in living organisms [6]. These proteins appear in cells after exposure of the cell genome to some initiator factors, *e.g.* intracellular acidosis [10] and LPO metabolites [11]. Synthesis of stress proteins was detected in cardiomyocyte culture exposed to chronic hypoxia [9]. Inducible HSP70 proteins were detected in peripheral blood lymphocytes of patients with acute myocardial infarction [5].

We previously showed that cardiomyocytes from patients with severe CHO are characterized by low content of endogenous antioxidants and high content of lipid peroxides [3]. We assumed that these states can provide conditions for activation of genes determining production of stress proteins in cardiomyocytes.

Here we studied the possibility of appearance of inducible heat shock proteins in the myocardium of coronary patients.

MATERIALS AND METHODS

Biopsy specimens from the right auricle collected during aortocoronary bypass surgery were studied. Resection of the auricula atrii is an obligatory stage of connection to artificial circulation. The study was carried out in 2 groups of patients. The main group consisted of 8 patients, in whom aortocoronary shunting for chronic coronary disease was performed. Control biopsy specimens were collected from 6 patients without ischemic injuries of the heart (according to clinical examinations) and operated for surgical correction of arrhythmia. In coronary patients standard echocardiography was supplemented by Doppler tissue imaging in the following regimens: tissue velocity imaging (TVI), strain rate/strain, and bent strain rate M modes. Echocardiography was carried out in order to detect the hypokinetic, akinetic, and dyskinetic zones (if any) in the ventricular myocardium. The study was carried out on a VIVID7 ultrasonic device (GE Medical Systems).

The resected auricula atrii were washed in cold saline and immediately frozen in liquid nitrogen. Inducible stress proteins were detected in cardiomyocyte cytoplasm by 2D-electrophoresis in polyacrylamide

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gel. To this end, myocardial specimens frozen in liquid nitrogen were ground in a ceramic mortar and put into cold hypotonic buffer (10 mM Tris (pH 7.4), 10 mM KCl, 1 mM PMSF) for 10 min. The suspension was filtered, centrifuged for 15 min at 8000g, and 2D-electrophoresis of supernatant proteins was carried out by the standard O'Farrell method. Isoelectric focusing was carried out at 500 V for 18 h with carbomoyl-treated carbonic anhydrase (LKB Pharmacia) serving as the marker. Electrophoregrams were stained with AgNO_3 . Inducible stress proteins were identified by their molecular weight and isoelectrical points (pI) [2,8]. Immunoblotting was carried out with C 92F3A5 monoclonal antibodies specific to inducible HSP70i proteins.

Hearts of Wistar rats exposed to heat shock [2] served as positive control for the presence of stress proteins in cardiomyocytes.

RESULTS

No inducible stress proteins were detected in myocardial extracts from controls (Fig. 1, 3). This result is in line with the concept on the absence of HSP70i inducible stress proteins in the myocardium of animals not exposed to stress [6]. Electrophoregrams of the main group patients indicated the presence of HSP70i proteins (Fig. 1, 1, 2). Different staining intensity of bands 1 and 2 (Fig. 1) reflects the intensity of inducible stress protein synthesis and accumulation. We hypothesized that this difference was due to specific features of the development of coronary heart disease in

different patients. Ultrasonic examination of the heart low content of stress proteins showed hypokinesia of the left ventricle and the absence of dyskinetic myocardium areas. On the contrary, the dyskinesia zone was clearly seen in specimens from patients with high content of stress proteins (Fig. 2).

2D-Electrophoresis showed 5 isoforms of HSP70 inducible stress proteins in the area of 62-78 kDa proteins with pI 6.5-5.5 [2,6]. The first three isoforms of these proteins are considered to be acid, the two last ones are alkaline. Heat shock is a universal inducer of acid and alkaline HSP70 stress protein [7], and hence, we compared 2D-electrophoregrams of myocardial extracts from coronary patients and from rats exposed to heat shock for identification of stress proteins (Fig. 3). Electrophoregram of rat myocardium was typical of this exposure [6], and the distribution of proteins in the myocardium of coronary patients was highly reproducible in repeated studies of the same sample and in analyzes of different samples. 2D-Electrophoregrams of the myocardium from coronary patients showed two protein fractions, in addition to actin (43 kDa, pI 5.3), albumin (68 kDa, pI 6.0), and constitutive stress protein (73 kDa, pI 5.6), in the area with interface corresponding to location of inducible stress proteins. By their characteristics (72 kDa, pI 5.7-5.9) these proteins corresponded to acid isoforms of stress proteins. No alkaline fractions of HSP70 (pI 6.1 and 6.2) were detected.

Hence, these results indicate that acid isoforms of inducible stress proteins can accumulate in the myocardium of patients with chronic coronary disease.

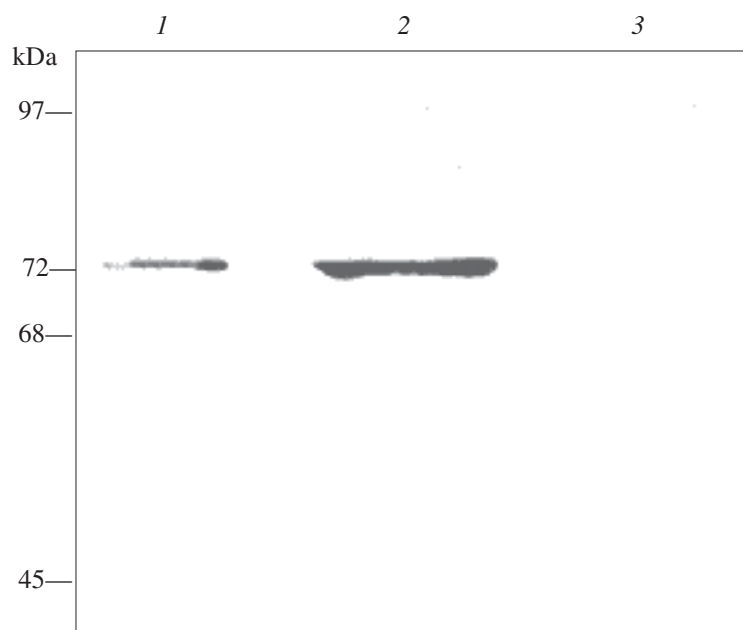


Fig. 1. Immunoblotting of HSP70 stress proteins. 1) myocardium of a coronary patient (functional class II); 2) myocardium of a coronary patient (functional class III-IV); 3) myocardium of a patient without ischemic injury.

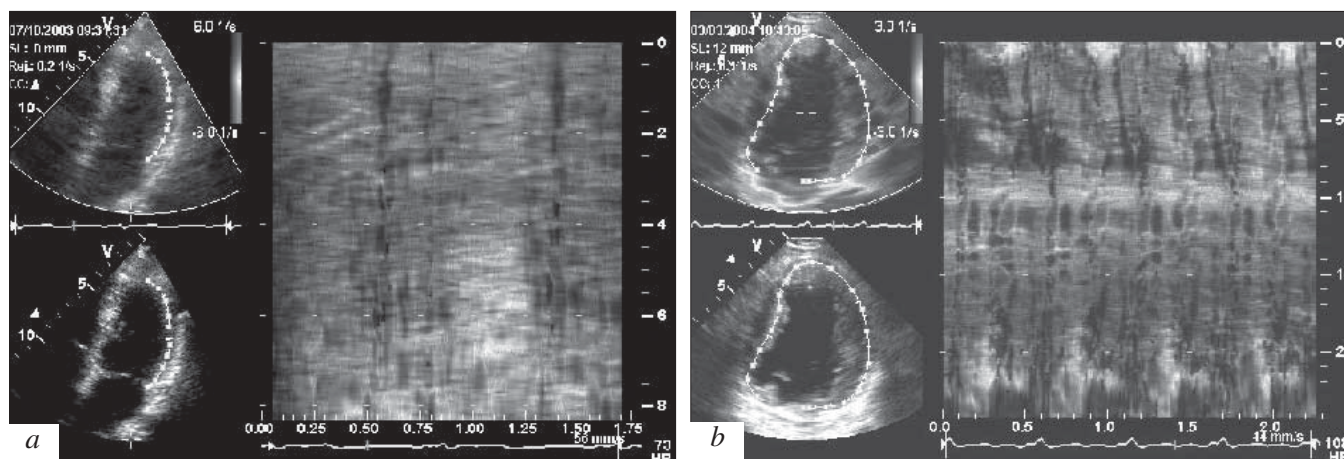


Fig. 2. Echocardiographic (bent strain rate M-mode) examination of the hearts of coronary patients with different content of HSP70 inducible stress proteins. *a*) hypokinesia of the basal, middle, and apical segments on the lateral wall of the left ventricle; *b*) pronounced dyskinesia of the basal, middle and apical segments on the lateral wall of the left ventricle.

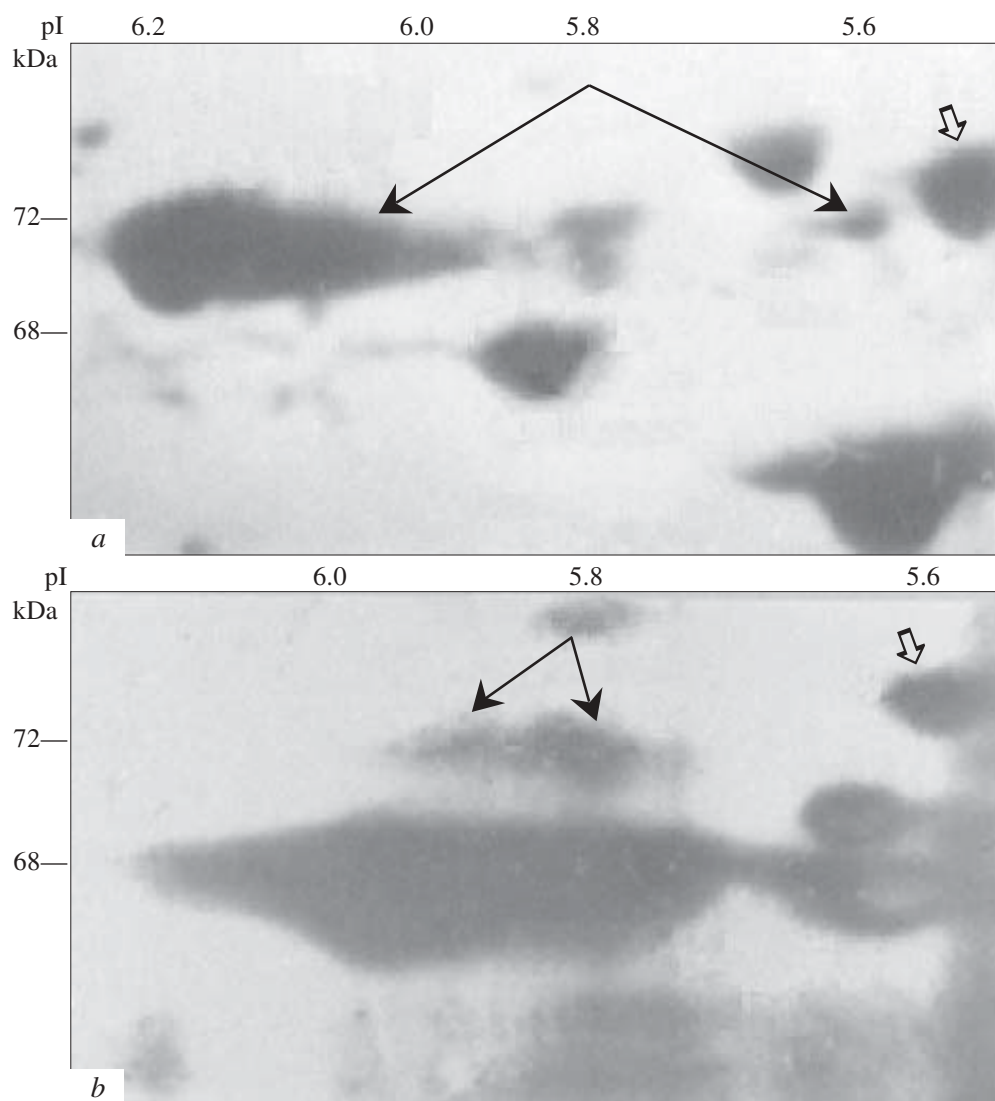


Fig. 3. Fragments of gels corresponding to location of HSP70 stress proteins after 2D-electrophoresis of cytoplasmic myocardial proteins. *a*) myocardium of a rat exposed to heat shock; *b*) biopsy specimen of the atrium from a coronary patient with pronounced dyskinesia of the heart. Arrow shows inducible stress proteins; double arrow shows constitutive stress proteins.

Their appearance is due to the initiating effect of metabolic disorders in intracellular homeostasis on the cardiomyocyte genome and can indicate that the myocardium is exposed to metabolic stress. Alkaline HSP70 isoforms are not produced in human atrial cardiomyocytes under conditions of chronic coronary insufficiency. It seems that initiation of alkaline HSP70 synthesis at the expense of metabolic shifts is possible in cardiomyocytes maximally close to the focus of infarction. Appearance of these isoforms can be a factor determining viability of stunned and hibernated myocardium.

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