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# Expression of Bax Protein and Morphological Changes in the Myocardium in Experimental Acute Pressure Overload of the Left Ventricle

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The expression of Bax protein, marker of intracellular pathway of apoptosis initiation, in viable left ventricular cardiomyocytes and morphological changes in the myocardium in acute pressure overload of the left ventricle were studied in experiment on male rabbits. The content of Bax protein in the cardiomyocyte cytoplasm decreased, this indicating that the mitochondrial pathway was not involved in the realization of the apoptotic program. This decrease was associated with manifest destructive changes in the left ventricular myocardium.

**Key Words:** *apoptosis; Bax; acute left ventricular overload; cardiomyocyte; myocardium*

The first studies of the cell death as the process aimed at the homeostasis maintenance appeared more than 150 years ago [5]. However, intensive development of this trend of research continued only from the middle of the 20th century with introduction of the notions of "programmed cell death" and apoptosis [11]. According to modern concepts, various types of cell death are referred to one of two groups. One of them unites cases with accidental cell death (ACD) caused by injuries incompatible with life that cannot be corrected by drugs or genetic interventions. The other group represents genetically programmed regulated cell death (RCD). It should be noted that physiological cases of RCD in the absence of cell dysfunctions, *e.g.* in embryonic development or immune response, are usually called programmed cell death (PCD) [6].

The mitochondria play a central role in the mechanisms of integration, activation, and inhibition of cell death. Mitochondrial outer membrane permeabilization (MOMP), often essential for activation of the caspase cascade, appears during realization of the extracellular pathway of apoptosis initiation [7,10]. This mechanism is mediated by proapoptotic factors of protein nature, such as Bax (Bcl-2-associated X protein) and Bak (Bcl-2 antagonist/killer), belonging to Bcl-2 family. The oligomer protein complex Bax/Bak forming pore-like structures in the mitochondrial membrane regulates additional mechanisms of intermembrane proteins (cytochrome C, SMAC/DIABLO, *etc.*) essential for triggering cell structures disintegration and apoptosome formation [6,7,10].

Recent research of apoptosis as one of the most important types of RCD is a prospective trend in the studies of cardiovascular pathologies because of the potentiality to modify this process and spare the functional reserve of the myocardium.

Experimental findings indicate that cardiomyocyte apoptosis plays an important role in the process of morphological changes in the myocardium in chronic left ventricular (LV) pressure overload [1] and in chronic cardiac insufficiency [3]. On the other hand, according to some data, the efficiency of apoptosis inhibition during the development of chronic cardiac insufficiency is doubtful, presumably because of a more substantial contribution of compensatory adaptive mechanisms such as cardiomyocyte hypertrophy and intensification of angiogenesis to myocardial remodeling [9].

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Despite ample data on myocardial programmed cell death, there is still no universal concept on the development of this process in acute injury to the cardiac muscle. The mechanisms of apoptosis regulation in acute LV pressure overload, for example in some forms of hypertensive crisis (with its specific adaptation reactions), remain little studied. Many studies of cardiomyocyte apoptosis, including evaluations of caspase activities, detected higher activities of cell apoptotic death markers in acute overload of LV myocardium [3,4,8]. However, individual molecular mechanisms involved in induction and realization of this process and the relevant phenomena are little studied.

We evaluate the intensity of Bax protein (marker of intracellular mechanism of apoptosis initiation) expression in LV cardiomyocytes and the morphological changes in the myocardium in LV acute pressure overload.

## MATERIALS AND METHODS

The study was carried out on male Chinchilla rabbits (3-3.5 kg) distributed into 4 groups, 4 per group: control (intact animals) and 3 experimental (rabbits with acute LV overload after 1, 3, and 5 days). The animals were kept and handled in accordance with the Order No. 755 of the Ministry of Health of the USSR (August 12, 1977), and the European Convention for protection of Vertebrates Used in Experiments or with Other Research Purposes (Strasbourg, 1986). Pressure overload of LV was created surgically by narrowing the ascending aorta by $^1/_3$ of its initial diameter by means of a metal loop.

Thoracotomy was carried out under total narcosis after 1, 3, or 5 days and extirpation of the heart was carried out. Specimens of LV myocardium were fixed for 72 h in 4% neutral paraformaldehyde. The material was routinely processed and embedded in paraffin. Histological sections (5 μ) were sliced on a Slidt-2003 microtome and applied onto slides coated with poly-L-lysine (for immunohistochemical study) and onto common slides (for morphometric analysis). The sections were deparaffinized in xylene and processed in descending alcohols.

The intensity of Bax protein expression was evaluated using first goat polyclonal antibodies (Santa Cruz Biotechnology). The results of immunohistochemical reaction of cardiomyocytes were evaluated using ImmunoCruz goat LSAB Staining System (Santa Cruz Technology) and poststaining of myocardial preparations with Meyer hematoxylin. The reaction was assumed positive if muscular fiber were stained brown. Thirty visual fields in each myocardial preparation were examined under a Nikon Eclipse E-400 microscope fitted with Watec 221S camera using Avtandilov grid. The proportion of equally distant points occupied by positively stained cardiomyocyte cytoplasm to the total count of points occupied by the cytoplasm was calculated.

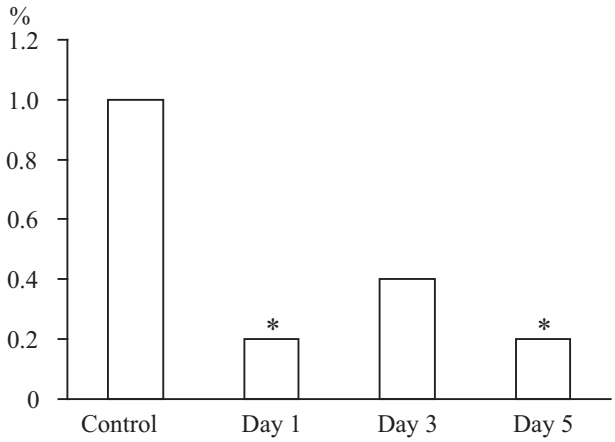


**Fig. 1.** Content of Bax protein in LV cardiomyocytes of rabbits on days 1, 3, and 5 of acute LV pressure overload. $p \leq 0.05$ in comparison with the control.

Morphometric analysis of the myocardium was performed on histological sections stained with hematoxylin and eosin. The content (vol%) of muscular fiber, cardiomyocyte nuclei, foci of destruction and infiltration, and volume of extracellular space were measured under a microscope at ×400 in 30 visual fields using Avtandilov grid. The nucleus/cytoplasm ratio, *i.e.* the ratio of cardiomyocyte nuclei to muscular fiber was calculated (in %).

The significance of differences was evaluated by Student's *t* test, the differences were considered significant at $p \leq 0.05$.

## RESULTS

On day 1 after modeling of acute LV overload, a significant decrease in Bax protein content in cardiomyocyte cytoplasm was observed. On day 3, this parameter slightly increased in comparison with day 1 and the difference became negligible (trend). On day 5, Bax expression somewhat decreased in comparison with the previous term and its level was again below the control (Fig. 1). Hence, the data in general indicate lower Bax expression in LV myocardium.

Qualitative evaluation of myocardial sections after immunohistochemical reaction for Bax in acute LV overload demonstrated mosaic uneven staining and very low density of positive staining of cardiomyocytes. On the other hand, a trend to an increase in the number of positively stained cardiomyocytes was found, with more intense reaction in myocardial zones adjacent to the foci of destructive and infiltrative changes and in areas with denser vascular network.

**TABLE 1.** Morphometric Values of Histological Sections of LV Myocardium on Days 1, 3, and 5 of Acute LV Pressure Overload (vol%; *M*±*m*)

| Structural elements | Control | Day of experiment | | |
|---|---|---|---|---|
| | | 1 | 3 | 5 |
| Muscular fiber | 88.04±0.37 | 84.60±1.02* | 82.72±0.96* | 81.79±2.06* |
| Nuclei | 5.48±0.21 | 3.31±0.20* | 5.08±0.24$^{+}$ | 4.46±0.23* |
| Nucleus/cytoplasm ratio | 0.06±0.003 | 0.04±0.003* | 0.06±0.003$^{+}$ | 0.06±0.003 |
| Vessels | 1.01±0.77 | 0.73±0.20 | 0.42±0.17 | 0.14±0.09 |
| Destruction foci | 0.29±0.06 | 0.30±0.07 | 0.36±0.16 | 2.58±1.24 |
| Extracellular space | 6.19±0.32 | 10.31±0.79* | 10.69±0.83* | 9.50±1.26* |

**Note.** $p \leq 0.05$ in comparison with *control, $^{+}$previous period.

Slight structural changes in LV myocardium were detected on day 1 of the experiment in comparison with the control: cross-striation was more homogeneous and cardiomyocytes were more oxyphilic, there were signs of congestion in the vascular bed, and hemorrhagic foci. The volume of muscular fiber decreased significantly in comparison with the control and the extracellular space was larger, presumably because of extracellular edema and lower number of viable muscular fiber. The number of cardiomyocyte nuclei in LV decreased significantly.

In 3 days after aortal stenosis modeling, myocardial changes progressed in comparison with day 1: they were more significant and less homogenous. Intact myocardial areas alternated with pathological areas, muscular fiber borders were sometimes blurred, there were foci of muscular fiber hypercontraction and hemorrhagic foci; histiocytic infiltration foci appeared and signs of intra- and extracellular infiltration augmented. According to morphometry data, the content of muscular fiber decreased significantly in comparison with day 1 and the number of destruction foci increased. The number of cardiomyocyte nuclei continued to decrease. The volume of extracellular space remained unchanged in comparison with day 1.

After 5 days, the pathological changes in the myocardium remained heterogeneous and progressed. Homogenization of muscular fiber augmented, infiltration zones grew larger. A trend to a decrease in the muscular fiber content persisted. The total number of nuclei did not change in comparison with day 3. The area of tissue destruction was morphologically larger, though remained statistically negligible throughout the experiment.

The nucleus/cytoplasm ratio slightly decreased, the difference from the control was significant only on day 1.

Our previous study of LV cardiomyocyte apoptosis on an identical experimental model showed an increase in activities of effector caspase-3 and initiatory caspase-8 mediating realization of apoptotic cell death by the external pathway [2]. The decrease in Bax protein expression detected in the present study indicated that initiation of apoptotic processes in LV cardiomyocytes in acute LV overload did not depend on the mitochondrial pathway and was caused mainly by the receptor-mediated signal mechanisms.

Hence, acute LV pressure overload is associated with lesser Bax expression protein in LV cardiomyocytes combined with manifest morphological changes in the myocardium most pronounced on day 5 of the process.

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