ENDO G TRANSLOCATION AND CARDIOMYOCYTE MITOCHONDRIAL POTENTIAL DECREASE AFTER MYOCARDIUM ISCHEMIA

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Apoptosis − a programmed cell death – a process, where it's main purpose is to eliminate any damaged cells or serves as a fundamental function during tissue development.

Most common apoptosis specificities are: cell dehydration compression, loss of transcellular links, blebbing , cytoskeleton destruction, chromatin condensation, nucleus fragmentation and DNA degradation (Häcker, 2000; Bortner, Cidlowski, 2002).

Apoptosis can be caused by external and internal signals, most important of which is DNA damage (Reed, 2000; Roy, Nicholson, 2000; Ghobrial et al., 2005).

EndoG is a nuclear-encoded mitochondrial enzyme that is known to be released from its original location, translocate to nucleus, and degrade nuclear DNA during caspase-independent apoptosis.

EndoG is a nuclease that has a unique site selectivity, initially attacking poly(dG). poly(dC) sequences in double-stranded DNA, as a result of which the enzyme got its name.

The discovery of EndoG inhibitor in drosophila (EndoGI) has been an important discovery, and was conducted his complex crystal structure with EndoG. EndoGI renounces in nucleus as a defender from chromosome damage caused by EndoG.

EndoG crystal structure has not been investigated in mammals, even with detailed research of biochemical qualities and cell function.

The main objective of our research is to analyze cardiomyocyte EndoG cell expression with intervenient ischemic postconditioning, while using immunehystochemical methods of research, because it has been seen that at the ischemia site after short-term occlusion as of the nucleus EndoG translocation.

Methods.

Research was conducted on isolated heart tissue of 16 outbred male rat's. Rat's heats were isolated by Langendorff. We established a value of WHSV coronary flow in rat's heart tissue and review of myocardial contractile activity and perfusion intensity. Into left ventricle, which was connected with blood pressure detector, was injected latex balloon. The heart of small laboratory animals were isolated for heart perfusion. We monitored functional hearts and vessels parameters.

All measuring equipment through analog-to-digital converter was connected with computer, with which we started registration and processing of measured indicators, while using specialized programs.

Heart in the control group was perfusioned with Krebs-Henseleit. Were modulated myocardium heart attacks with coming up series of occlusions/reperfusion to coronary artery in experimental group.

For modeling myocardial infarction animals isolated posterior interventricular branch of the right coronary artery, it is brought under vascular clamp for 5-10 minutes to form an acute ischemia area of myocardium perfused by this artery, after − reduced blood flow (control group). In the main group after removing the wire clip from one to five consecutive short cycles occlusion/reperfusion (10-15 seconds) of the lumen of the coronary artery. After the period of reperfusion in the control and study group received histology and cytological analysis of the heart muscle.

Structural features (semifine sections) ventricular myocardium were studied in transmitted light and fluorescence method. For histological and cytological analysis of half

slices exposed as standard histological staining with hematoxylin-eosin, azure-eosin, azure-2 pikrofuksinom and *gallotsianin-chrome* alum by *Einarsson* and 5,5 ', 1,1 6,6'- tetrahloro-' , 3,3' tetraetilbenzimidazolil-karbotsianin iodide (JC-1), which is able to detect ΔΨm.

For cytochemic analysis were used half-thin slices for Anti-EndoG fluorescence (Anti-Endo G antibody -ab64668, Abcam, UK).

Myocardial slices were fixed with 100% methanol, was carried unmasking antigen permeabilization. Sections were then incubated in 1% solution of bovine serum albumin for 1 h; Incubation with Anti-EndoG antibodies for 12 hours in the dark at 4 ° C. Phycoerythrin-conjugated secondary antibody anti-rabbit IgG were used at a dilution of 1/1000 for 1 hour.

Result.

It has been conducted that rat's myocardium ischemia and reperfusion follows the increase of JC-1 cardiomyocyte cytoplasm. Described changes are an indication of ΔΨm decrease as it could be an evidence of early apoptosis. Cardiomyocytic chromatin at the ischemia/reperfusion models had tendency to compact and optical density increase.

With models of postconditioning in rat's cardiomyocytes the red JC-1 fluorescence was dominant (70% of cell volume), which is a mark of penetration probe in intact mitochondria Chromatin status was similar pre-operation period. Postconditioning, which is used in experimental conditions, causes a decrease of reperfusion damage in myocardium of rat's.

Conclusion.

Research in EndoG translocation of rat's cardiomyocytes conducted that ischemia that was modeled at more intensity in reperfusion and less in acute myocardial infarction as it can start a translocation of Endocnuclease G to cardiomyocyte nucleus. With the model of acute myocardial infarction postconditioning disabled reperfusion EndoG translocation which can be sorted as positive development in acute myocardial infarction. With this said, myocardium of rat's ischemia and reperfusion is bind with cardiomyocytes apoptosis activation.