An experimental investigation and visualization of the programmed cell death of cardiomycites

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Abstract

The main purpose of a project was a research on the topic of apoptosis as well as a visualization of apoptosis process on the base of experiments the group has made. It has been first time when the scientific methods of visualization of apoptosis mechanism in cardiomycytes was modeled. Results of the study revealed the basics for the visualization of pathogenetic mechanisms of coronary heart desease. The project shows visualization of new information about the apoptosis in the development of coronary heart disease, describes the visualization methods of apoptosis in the cardiovascular system, the effect of nitric oxide donator on apoptosis, shows anti-apoptotic function of hsp70 protein.

Keywords: apoptosis, cardiomyocytes, experiment, visualization

1 Introduction

The processes of apoptosis of myocardial cells in animals which suffer from coronary insufficiency syndrome have not been adequately studied. The aim of the study was to study and model apoptosis in cardiomyocytes of people with coronary insufficiency. The methods which were applied include experimental, histological, biochemical and computer modelling. As a result of the work, apoptosis of cardiomyocytes was studied in the modelling of coronary insufficiency and modelling of the process was carried out.

The aim of the research was to investigate and visualize the process of apoptosis in the heart cells of animals during coronary insufficiency.

Materials of the study. For the experimental part of the study, 300 non-breed male rats weighing 200-250 g were used. Coronary insufficiency syndrome was developed in the group including 20 animals. The control group consisted of 20 male rats with a mass of 200-250 g, contained on a single diet, at room temperature, in aerated cells, under conditions similar to experimental animals that were not under any stress. The experiments were conducted in the period of autumn and winter. Experimental work on laboratory animals was carried out in accordance with the European Convention for the Protection of Vertebrates.

According to M. Paltsev's Histological studies of myocardial tissue - staining with hematoxylin - eosin, Sudan III, Schick reaction on glycogen, reaction to succinate dehydrogenase. [4]. The studies were performed on a microscope "Leica DM4000B" with a semi-chromatic / Fluotar lens, a digital video camera "Leica DFC 320" and a resolution of 7.2 Mpx. Leica Microsystems. Apoptosis in cardiomyocytes was determined using the TUNEL method. The apoptotic index of myocardial tissue is calculated as the ratio of the number of positively stained nuclei to the total number of cardiomyocytes. Calculations were carried out in 20 randomly selected fields of view with increasing 400□. In animals, ECG studies were performed in I, II, III standard leads on the EC1T-3M2 device. "Aloka 1700" with a zoom of 100% in the "Teicholc" program. The levels of total and free blood corticosterone were calculated by the method of Pankov Yu.A., Cherkasova OR. [5]. The products of free radical reactions were determined on an EPR spectrometer of the firm "Bruker" (Japan) by the method of Agip Ya.I. [6]. To study the dynamics of the development of coronary insufficiency for 3, 7, 11, 14 days in animals, the histological parameters of the heart were studied according to the methods described above. Simulation of the apoptosis process had been provided using computer programs.

It is known that inducers of apoptosis can be steroid hormones that affect the nucleus of the cell and lead to the initiation of apoptosis of cells [2]. The results of the study showed (Table 1) that the level of both free and total blood corticosterone in animals with coronary insufficiency was 1.3 times and 1.5 times, respectively, as compared to the analogous data of the animals in the control group, $p < 0.05$.

An increase in the level of the steroid hormone of blood in animals with coronary insufficiency testified to the presence of trigger activation factor for apoptosis of cells.

TABLE 1 - Indices of free and total blood corticosterone in animals with coronary insufficiency (M ± m, ng / L)

Indicator	Control group n = 40	Group with coronary insufficiency syndrome n = 56	Reliability of differences
Ceruloplasmin	2,57 ± 0,21	4,32 ± 0,38	P < 0,05
Nitric oxide	35,47 ± 3,14	7,44 ± 0,68	P < 0,001
Superoxide dismutase	15,64 ± 0,29	7,93 ± 0,44	P < 0,05
Superperoxidanion	7,86 ± 0,13	19,56 ± 0,12	P < 0,001
Hydroxyl radical	2,82 ± 0,16	6,91 ± 0,10	P < 0,001
Xanthine oxidase	0,59 ± 0,05	1,23 ± 0,07	P < 0,001
Peroxynitrite	0,96 ± 0,02	4,31 ± 0,07	P < 0,001

NOTE: P-reliability of differences between the groups

Another universal mechanism for triggering the process of apoptosis of cells is the violation of the balance of free radical reactions occurring in the body under various pathologies [7]

Table 2 reflects the indices of free radical reactions of blood of animals with coronary insufficiency caused by immobilization stress. In the blood of animals with coronary insufficiency, the concentrations of superoxidanion, hydroxyl radical (2.5 times), xanthine oxidase (2.1 times), peroxynitrite (4.5 times), as well as lower levels of nitric oxide (in 4.8 times) Times) and superoxide dismutase (in 2 times) in comparison with the analogous data of animals of the control group.

TABLE 2 - Parameters of free radical reactions in blood of animals with coronary insufficiency (M ± m, nmol / ml)

Indicator	Control group n = 20	Group with coronary insufficiency syndrome n =20	Reliability of differences
Free corticosterone	40439,5 ± 2612,7	52681,3 ± 1768,1	P < 0,05
Total corticosterone	132584,2 ±11612,4	196475,8 ± 12535,7	P < 0,05

NOTE: P is the reliability of the differences between the groups.

The analysis of the obtained results showed that in animals with coronary insufficiency, the balance of free radical reactions changes in the direction of increasing the formation of toxic free radicals, which are inducers of apoptosis of cells.

In the myocardium tissue of animals with coronary insufficiency, the concentrations of superoxide anion were significantly increased (by 2.7 times), hydroxyl radical (2.4 times), xanthine oxidase (2.6 times), peroxynitrite (3.5 times), and the levels of nitric oxide (2.7 times), superoxide dismutase (1.8 times) are lower compared with the analogous data of the animals in the control group.

The results obtained in animals with coronary insufficiency showed a change in the balance of free radical reactions in myocardial tissue and an increased level of toxic forms of free radicals, which is a universal starting program for activating the processes of apoptosis of cells.

Histological examination of myocardial tissue of animals with coronary insufficiency showed that, in contrast to myocardial tissue of intact animals, zones of myocardial ischemia, small foci of necrosis of muscle fibers with swelling of interstitium were recorded. In the tissue of the myocardium, multiple foci of proliferation of connective tissue elements around the dead groups of muscle fibers were found. At the same time, in the near-necrotic zones of the myocardium of animals with coronary insufficiency, vasoconstriction was revealed and there were no clear destructive changes in muscle cells. When studying the myocardial tissue of animals in the control group, no pathological changes were observed.

The results of immunohistochemical study showed that in the group of animals with coronary insufficiency, the number of specifically stained cardiomyocyte nuclei significantly increased in comparison with the analogous index of the animals in the control group. Thus, the apoptosis index of cells of myocardial tissue of animals with coronary insufficiency was 21.4 ± 3.5%, which was 3.4 times higher than that of the control group animals, p <0.001.

Immunohistochemical study of myocardial tissue of animals with coronary insufficiency during 3, 7, 11, and 14 days showed not only the presence of a variation in the apoptosis index from 18% to 61%, but also a statistically significant increase from 3 to 14 days.

Thus, the study of the dynamics of changes in myocardial tissue of animals with coronary insufficiency from 3 to 14 days showed that there is a gradual increase in the number of cardiomyocytes that have entered the path of apoptosis. Simulation has shown that with time, the processes of apoptosis of cells in pathology gradually increase.

2 Conclusions

1. The apoptotic index of myocardial tissue of animals on the 3rd day of development of coronary insufficiency was 21.4 ± 3.5%, which was 3.4 times higher than that of healthy animals, p <0.001.

2. In the blood and tissue of the myocardium of animals with coronary insufficiency, a significant increase in the level of superoxidanion (2.5 and 2.7 times, respectively), hydroxyl radical (2.5 and 2.4 times, respectively), xanthine oxidase (in 2, 1 and 2.6 times, respectively), peroxynitrite (4.5 and 3.5 times, respectively) compared with similar data of healthy animals. In the blood and in the myocardium tissue of animals with coronary insufficiency, the content of nitric oxide significantly decreases (by 4.8 and 2.7 times, respectively), superoxide dismutase (by 2 and 1.8 times, respectively) in comparison with the same data of healthy animals. The disturbed balance of free-radical reactions leads to an increase in the apoptosis process of cells.

3. Study of the dynamics of changes in myocardial tissue of animals with coronary insufficiency from 3 to 14 days showed that there is a gradual increase in the number of cardiomyocytes that have entered the path of apoptosis.

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