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About Modeling Of Heart Diseases On Wistar Rats.

Areshidze DA*, Makartseva LA, Kozlova MA, and Kucher SA.

Laboratory of experimental biology and biotechnology Moscow State Regional University, 10a Radio st., Moscow, Russia

ABSTRACT

The results of a study of two methods for modeling of ischemic heart disease in Wistar albino rats are presented. It is shown that a number of functional changes occur in the modeling of IHD by the administration of doxorubicin, but the character of the morphological disturbances of the myocardium indicates a predominantly cardiotoxic effect of this substance, which morphological pattern does not match with ischemic heart disease. Modelling of IHD by method of joint administration of adrenaline and hydrocortisone is characterized by pronounced pathomor phological changes in the myocardium of rats, and can be used to define the therapeutic efficacy of potential drugs aimed at preventing and treating of IHD.

Keywords: ischemic heart disease, cardiomyopathy, myocardium, ischemia, cardiomyocyte.

Corresponding author

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INTRODUCTION

Diseases of the cardiovascular system are one of the main causes of mortality. Wherein, ischemic heart disease (IHD) and its complications are among the leading nosological forms of diseases of the cardiovascular system (Shalnova and Deyeva, 2011). The development and implementation of drugs aimed at the prevention and treatment of ischemic heart disease are an actual modern task.

Considering the rather wide range of etiological factors of ischemic heart disease, as well as its complex pathogenesis and a wide range of manifestations, the choice of an experimental model that allows us to recreate the pathological process as closely as possible to the clinic is one of the most important points in the preclinical study of a multifunctional drug (Oshchepkova Y.V. et. al., 2012; Tunstall-Pedoe, 2003).

The main pathophysiological mechanism of ischemic heart disease is the discrepancy between the myocardial oxygen demand and the ability of the coronary blood flow to meet these needs. The main reasons for this discrepancy are:

1) decrease in coronary blood flow due to coronary artery lesion;
2) increased myocardial function due to the increase of its metabolic needs;
3) combination of vascular and metabolic factors (Shanin, 1996; Vaslyayeva et al., 2017; Malygina et al., 2017).

There are several approaches to the question of experimental modeling of IHD. In particular, one of the methods is surgical modeling of coronary insufficiency. This method is quite complicated, since studies are conducted on anesthetized laboratory animals under conditions of an open chest and artificial respiration. The second approach is to model IHD by administering cardiotoxic substances to animals, such as doxorubicin. However, the question of the effective dose of doxorubicin is quite controversial (Lushnikova, 2009; Chatterjee, 2010; Carvalho et. al., 2010). Another method is to simulate IHD by increasing the chrono- and/or inotropic function of the heart (for example, by stimulating the nerve endings of the sympathetic nervous system, its centers or by introducing of sympathomimetics). Based on the above, it seemed relevant to conduct the research of the morphofunctional state of the rat myocardium at various methods of modeling of myocardial pathologies.

MATERIALS AND METHODS

The study was conducted on male Wistar rats kept in a vivarium of SEC MSRU. The age of the animals was 6 months, the weight of the animals was 200-220 g. Before the start of the study, the animals matching the criteria of inclusion into the experiment were divided into 3 groups (control group; I experimental group; II experimental group). Animals without signs of abnormal appearance in exterior were selected and randomized into groups in such a way that each group included 10 animals. The animals were kept in standard conditions in accordance with the rules approved with GOST R 53434-2009 on the organization, equipment and maintenance of experimental biological clinics (vivariums).

The first group served as a control. The method described by D.V. Gaman 2010, was used to model ischemic heart disease in the first experimental group of animals. (). Animals of the first experimental group were daily subcutaneously injected with 0.1 ml of 0.1% adrenaline solution (FSUE “MEP”, Russia) and 1 ml of a 2.5% suspension of hydrocortisone (Farmak, Russia). At modelling of ischemic heart disease in the second experimental group, doxorubicin (Teva, Israel) was introduced intraperitoneally once a week at a dose of 0.4 mg/kg of body weight. Modelling of IHD by the first way lasted for 7 days. On the eighth day of the study, the animals were removed from the experiment. Modelling of ischemic heart disease by the second method lasted for 28 days. On the 29th day of the study, the animals were removed from the experiment. The removal of animals from the experiment was carried out using a carbon dioxide chamber.

The heart was taken for pathological and histological examination, after weighing the organs were fixed in 10% buffered formalin. Blood was collected for hematological and biochemical studies.

Hematological analysis was performed using Abacus Junior VET hematology analyzer (Austria). 18 key parameters were determined and analyzed. To determine the level of alanine aminotransferase (ALT),

aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and creatinine content in the blood of rats, the StatFax 3300 (USA) biochemical analyzer was used. Reagents from Spinreact (Spain) were used for the analysis. To determine the level of glucose in the blood, the SUPER GL compact glucose analyzer (Germany) was used.

For histological examination, the rats' hearts fixed in neutral formalin were washed, dehydrated and embedded into paraffin blocks according to the standard technique. From paraffin blocks the serial sections of 5 µm thickness were prepared on a rotary microtome MPS-2 in such a way that the right atrium and right ventricle or left atrium and left ventricle appeared on the preparation. In the future, four microscopic specimens of each organ were used for the study. Preparations used for micromorphometry and pathomorphological analysis were stained with hematoxylin-eosin (BioVitrum, Russia). For qualitative staining for the detection of ischemic cardiomyocytes, the HBFP stain (MT Point, Russia) was used. To determine the apoptotic cells, the methylene blue-azure II-basic fuchsin stain was used. Stained sections were embedded in Bio Mount mounting media (Bio Vitrum, Russia). Microscopy of histological specimens was performed on a Nicon Eclipse 80I digital microscope using a Nicon DS-2 digital camera (Japan). For microscopy the eyepieces ×10, ×15 and lenses ×4, ×10, ×20, ×40, ×100 were used; 10 digital images of randomly selected fields of view from each investigated specimen were made at magnification ×400, ×1000. For morphometric studies, ImageJ programs (USA) and related plug-ins were used. Determination of the cross-sectional area of cytoplasm and nuclei was carried out on preparations stained with hematoxylin-eosin using the ImageJ program (USA) and the related plug-ins (Japan). The measurements were carried out in micrometers after preliminary geometric calibration using the object-micrometer scale digitized with the same magnification.

At karyometry, the nuclei in the outer zone of section with a width of 500 µm were not measured, since in this area the nuclei are compressed during histological processing. With use of focusing it was determined whether the nucleus appeared in the section in whole or in part.

Apoptotic index (AI) was calculated by the formula: $AI = N_a / N \times 1000$;

where N_a is the number of apoptotic cells;
N – the total number of cells in the studied population.

For statistical analysis, Graph Pad Prism 6.0 software (USA) was used. The data are given in the form M±SD. Student's criterion was used as a parametric criterion. Statistically significant differences were determined at a confidence level of 0.05.

RESULTS AND DISCUSSION

At modeling of IHD in both ways, the similar changes in the studied hematological parameters were noted. In particular, at a constant number of leukocytes in both groups, there is a decrease in the number of lymphocytes with an increase in the proportion of granulocytes, and P-LCC (platelet large cell count) and P-LCR (platelet large cell ratio) also decrease (Table 1).

Table 1: Some hematological parameters of Wistar rats

Group	Lym, 10^9/l	Gra,10^9/l	Lym, %	Gra, %	P-LCC, 10^9/l	P-LCR, %
Control (n=10)	6.203± 3.39	1.373± 0.38	66.65± 17.46	19.14± 12.27	61.10± 22.94	10.71± 3.14
1 experimental group (n=10)	2.18± 4.55*	5.38± 2.88***	17.47± 26.77***	70.02± 24.29***	37.90± 18.64*	6.79± 1.76**
2 experimental group (n=10)	2.45± 3.05*	6.42± 2.50*	22.82± 14.55**	68.15± 15.60***	40.10± 14.20*	7.20± 1.05*

Note. *P≤0,05; **P≤0,005; ***P≤0,0005 – the statistical significance of differences in comparison with the control group.

By the end of the experiment at the biochemical analysis of blood plasma of rats of the first experimental group we observed a significant increase in the levels of glucose, LDH, AST at a constant level of ALT and a decreased level of creatinine. At that, the De Ritis ratio (AST/ALT ratio) also increases significantly. In

total, this may indicate the development of IHD. At the same time, in the blood plasma of rats of the second experimental group the level of ALT, AST, LDH and De Ritis ratio do not differ from the rats of the control group (Table 2). As in the rats of the first experimental group, an increase in the level of glucose and creatinine occurs.

Table 2: Biochemical parameters of blood of Wistar rats

Group	ALT, U/l	AST, U/l	De Ritis ratio	Glucose, mmol/l	Creatinine, mmol/l	LDH, U/l
Control (n=10)	81.93± 11.02	175.80± 38.72	2.156± 0.220	4.90± 0.41	41.37± 2.94	486.1± 195.4
1 experimental group (n=10)	84.13± 10.00	286.10± 33.40 ***	3.426± 0.324 ***	9.16± 0.91 ***	31.73± 3.37 **	676.3± 195.4 **
2 experimental group (n=10)	86.05± 16.33	164.30± 27.39	1.94± 0.360	7.87± 1.63*	34.83± 1.65*	574.40± 59.8

Note. *P≤0,05; **P≤0,005; ***P≤0,0005 – the statistical significance of differences in comparison with the control group.

At the pathomorphological study it was established that the structure of the myocardium of animals in the control group corresponds to the norm. In particular, the myocardium is a network of muscle fibers with narrow gaps between them. The cross-striation is well visualized, cardiomyocytes have predominantly elongated nuclei, endothelium of blood vessels is intact. Blood filling of vessels is normal. In the myocardium of rats of the first experimental group a number of significant pathomorphological changes occur. Ischemic areas of the organ are clearly visible. Swelling of the sarcoplasm of cardiomyocytes, indistinct boundaries of cells and nuclei, heterogeneity of the color of muscle fibers are noted. The striation is significantly less noticeable than in the myocardium of control animals. The nuclei of cardiomyocytes are located less frequently than in intact animals, a greater number of round nuclei appears, and the presence of single large, probably polyploid nuclei is noted. Pycnotic nuclei are also found.

Macrophage and lymphocytic infiltrates are noted, mainly perivascular. Some thickening of the walls of blood vessels, necrotic cells, both single and foci of micronecrosis are also visualized.

Analyzing the myocardial preparations of rats of the second experimental group, we identified changes of a slightly different nature. In particular, at low magnification no ischemic myocardial regions were detected. At the same time, as in the case of the first experimental group, swelling of the sarcoplasm of cardiomyocytes, the disappearance of the cell and nucleus boundaries is detected, but the striation of the fibers is clearly visible in the unaltered parts of the myocardium. There are also pycnotic nuclei in cells of animals of this group.

There are no changes in the blood vessels, no hemorrhages and infiltrates. At the same time necrotic cells were found, but they are located individually, without forming of foci of micronecrosis.

At analysis of the results of HBFP staining in the myocardium of intact animals ischemic cardiomyocytes were not found.

In the myocardium of rats of the first experimental group both multiple foci of ischemia and single fuchsinophilic ischemic cardiomyocytes, acquiring a purple-red color, are noted. When analyzing the results of HBFP staining in the myocardium of animals of the second experimental group, ischemic cardiomyocytes were not encountered.

The results of micro morphometric studies showed that in the myocardium of animals of the first experimental group there was a significant decrease in the area of the nuclei of cardiomyocytes due to a decrease in the length of the long diameter (Table 3). At the same time, the thickness of cardiomyocytes also decreases from 14.56± 0.19 μm in the control to 13.64±0.14 μm in the myocardium of rats of the first experimental group.

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Table 3: Some karyometric parameters of rat myocardium at the modeling of ischemic heart disease in various ways

Group	Cardiomyocyte nucleus length, µm	Cardiomyocyte nucleus width, µm	Cardiomyocyte nucleus area, μm^2
Control (n=10)	13.5±0.65	2.35±0.24	21.95±1.67
1 experimental group (n=10)	9.44±0.48*	2.17±0.21	17.79±1.23*
2 experimental group (n=10)	10.01±0.51*	2.30±0.26	18.14±1.35*

Note. *P≤0,0005 – - the statistical significance of differences in comparison with the control group.

In the myocardium of rats of the second experimental group the similar changes in the nuclei of cardiomyocytes are also observed.

The apoptotic index in the myocardium of intact animals made 5.04±0.35‰. In the myocardium of animals of the first experimental group, this parameter is significantly higher, 7.89±0.49‰, in the myocardium of animals of the second experimental group it is 6.28±0.59‰, which is also higher than in the control.

CONCLUSION

According to the results of the study, methods of modeling IHD both by the joint administration of adrenaline and hydrocortisone, and by the administration of doxorubicin, cause significant changes in an organism of experimental animals. These changes are both morphological and functional.

At modeling of IHD by the joint administration of adrenaline and hydrocortisone, the significant pathomorphological changes in the myocardium of rats, characteristic of this pathology, were revealed. Functional changes also reflect ischemic changes in the heart of animals of the first experimental group. The investigated functional parameters that directly characterize the state of the cardiovascular system also confirm the success of modeling of IHD in this way.

At modeling of myocardial pathology by administering of doxorubicin, a number of similar functional changes occur, but the nature of the morphological myocardial disorders indicates the predominantly cardiotoxic effect of doxorubicin, which does not lead to the morphological picture characteristic for IHD.

Based on the foregoing, the method of modeling of IHD through the joint administration of adrenaline and hydrocortisone is characterized by high reproducibility, involvement of the leading patho genetic mechanisms of IHD formation, natural genesis of IHD and can be used as a model of IHD in the testing of drugs aimed at prevention and treatment of pathology. In turn, the use of doxorubicin, at least in the dosage used in the study, leads to a complex of morpho functional changes in the myocardium of rats, which is characteristic for the previously described cardiotoxic effect of this substance, but is unsuitable for experimental modeling of IHD.

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