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Some Functional Measures of the Organism of Rats at Modeling of Ischemic Heart Disease in Two Different Ways

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ABSTRACT

The study was conducted to examine the influence of two methods of modeling of ischemic heart disease on the amount of ischemic fibers in the myocardium parameters, characterizing the cardiovascular system, the coagulation properties of blood, and its electrolyte composition. It was shown that at modeling of IHD by introducing doxorubicin, several functional changes occurred, but the nature of the changes of the studied parameters evidenced a predominantly cardiotoxic effect of doxorubicin, which did not lead to the morphological picture characteristic of IHD. At modeling of ischemic heart disease by co-administering adrenaline and hydrocortisone, morphofunctional disorders were manifested in significant pathological changes in the myocardium of rats, and this method can be used as a model to identify the therapeutic efficacy of potential drugs aimed at preventing and treating the ischemic heart disease.

Keywords: Ischemic Heart Disease, Myocardium, Ischemia, Cardiomyocyte.

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INTRODUCTION

According to the report by the experts of the World Health Organization (WHO), which was confirmed by the statistical analysis, for many years, the main cause of morbidity and mortality in the world (accounting for 55% of the total population) has been cardiovascular diseases caused by atherosclerosis, more than 2/3 of which has been ischemic heart disease (IHD), stroke and peripheral artery disease [1]. Cardiovascular diseases affect life expectancy and damage the quality of life [2]. These chronic diseases have high impacts on patients' quality of life [3].

Diseases related to IHD include a whole group of pathological conditions, united by the same development mechanism - insufficient oxygen supply with blood to the myocardium (heart muscle) or its insufficient oxygen ensuring, and characterized by the absolute or relative disturbance in blood supply to the heart muscle due to the coronary vascular lesions [4]. Coronary heart disease is one of the common diseases in cardiology[5].

The selection of an experimental model of pathology, which allows a researcher to reconstruct the nosological form as closely as possible to the clinic, is one of the most important points in the preclinical study of a polyfunctional drug.

The oxygen requirement of myocardium is determined by heart rate; myocardial

contractility - by the size of the heart and the value of blood pressure. Under normal conditions, there is a sufficient reserve in the dilatation of the coronary arteries, providing the possibility of a fivefold increase in coronary blood flow and, consequently, in the delivery of oxygen to the myocardium. An imbalance between myocardial oxygen requirement and its delivery may occur as a result of the pronounced atherosclerosis of the coronary arteries, their spasm and a combination of atherosclerotic lesion and vasospasm of the coronary arteries, i.e. their organic and dynamic obstruction. The cause of the organic obstruction is atherosclerosis of the coronary arteries. The sharp limitation of the coronary blood flow in these cases can be explained by infiltrating of the wall with atherogenic lipoproteins, the development of fibrosis, the formation of an atherosclerotic plaque, and arterial stenosis. The formation of a thrombus in the coronary artery also has a certain value. Dynamic coronary obstruction is characterized by the development of coronary spasm against the background of atherosclerotic changes in coronary arteries. In accordance with the concept of "dynamic stenosis," the degree of narrowing the lumen of the coronary artery depends both on the degree of organic lesion and the severity of the spasm. Under the influence of spasm, the narrowing of the vessel lumen can increase to a critical level (75%), which leads to the development of effort angina. The sympathetic nervous system and its mediators, peptide-like substance P and neurotensin, and metabolites of arachidonic acid – prostaglandin F2a, leukotrienes – take part in the development of the coronary spasm. An important role in the regulation of coronary blood flow belongs to the local metabolic factors. When the coronary blood flow decreases, the myocardial metabolism switches to the anaerobic pathway that leads to accumulation of metabolites that dilate the coronary arteries (adenosine, lactic acid, inosine, hypoxanthine). In IHD, the coronary arteries changed by the atherosclerotic process cannot adequately expand in accordance with the increased oxygen demand of the myocardium, which leads to the development of ischemia. Endothelial factors are also essential. Endothelins (ET-1, -2, -3) are produced in the endothelium. Endothelin 1 is the most potent vasoconstrictor known. The mechanism of its vasoconstrictor action is associated with an increase in calcium content in smooth muscle cells. The endothelium also stimulates the platelet aggregation. In addition, the substances of pro-coagulant action are produced in the endothelium: tissue thromboplastin, von Willebrand factor, collagen, platelet-activating factor. In IHD, the activity of lipid peroxidation increases, which activates the aggregation of platelets. In addition, lipid peroxidation products exacerbate myocardial ischemia. The decreased production of endogenous opioid peptides (enkephalins and endorphins) contributes to the development and progression of myocardial ischemia [6-10].

Thus, the causes of IHD include:

- The atherosclerotic narrowing of the lumen of the coronary arteries;
- The spasm of the coronary arteries;
- The thrombosis and thromboembolism of the coronary arteries;
- The dyslipidemia;
- The tachycardia;
- The myocardial hypertrophy.

There are several approaches to the issue of the experimental modeling of IHD [8]. In particular, one of the methods is surgical modeling of the coronary insufficiency. This method is quite complicated because it requires the surgical intervention. The second approach is to model IHD by administering cardiotoxic substances to animals, for example, Doxorubicin. However, the question of the effective dose of Doxorubicin is very controversial [12, 13]. Another method is to model IHD by increasing the chronotropic and/or inotropic cardiac function (for example, by stimulating the nerve endings of the sympathetic nervous system or its centers, or by the administration of sympathomimetics).

Based on the above, it currently seemed important to study the morphofunctional state of the myocardium of rats at various methods of modeling of IHD.

MATERIALS AND METHODS

Study design

The study was conducted on 60 male Wistar rats, divided into three groups. The first group (n=20) served as a control, in animals of the second group (n=20), ischemic heart disease was modeled by administering adrenaline and hydrocortisone, in animals of the third group (n=20). IHD was modeled by administering doxorubicin.

Modelling of IHD in the first way was continued for 7 days. During this period, in animals, an ECG was registered daily, as well as blood pressure (BP) and heart rate (HR). On the eighth day of the study, the animals were withdrawn from the experiment.

The modelling of the ischemic heart disease by the second method was continued for 28 days. During this period, the ECG was registered as well as blood pressure (BP) and heart rate (HR), weekly. On the 28th day of the study, the animals were withdrawn from the experiment.

For pathomorphological and histological examination, the heart was taken, after weighing, the organ was fixed in 10% buffered formalin. Blood was collected for hematological and biochemical studies, and for determination of the electrolyte composition and coagulation properties.

Animals

The study was conducted on males of the Wistar rats, kept in the vivarium of the Science-Educational Centre of Moscow State Regional University. The age of the animals was 6 months; the weight of the animals was 200-220g.

Selection of animals for research and distribution in groups

Before starting the study, the animals that matched the criteria to be used in the experiment were divided into 3 groups. In the groups, the animals without signs of abnormal appearance were selected. The animals were randomized into groups in such way that each group included 20 animals.

Keeping of 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 animals were placed in polycarbonate cages produced by the company for 3W: with biomedical and laboratory equipment, model M6 with steel lattice lids with a stern recess (Germany-Russia). Each cage housed 5 male rats (the floor area per 1 animal was 400 cm^2). Washing of the cages and bedding change were carried out at least 2 times a week.

The animals were fed the compound feed "Dry polyration feed for rodents" (OOO Aller Petfood, Russia) prepared in accordance with TU 9296-010-70648406-2013, in accordance with the requirements of the Veterinary-Sanitary Norms No. 13-7-2 / 1010-97 from 06.05. 1990. The animals received water corresponding to SOP AB-38v2.

Food and water were given ad libitum to the stern recess of the steel lattice cage lid. The mixed wood shavings, fraction No.3 (Maest, Russia) were used as the bedding. The light mode was 12 hours of light, and 12 hours of darkness. The air temperature was maintained within 18-22 ° C, the relative humidity was 50-70%.

Temperature and humidity were registered daily. No significant deviations of these parameters were noted during the acclimatization period and during the experiment.

Methodology

Induction of pathology

For modelling of ischemic heart disease in the first group of animals, the method described by D.V. Gaman was used [14]. The animals of the 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) for 7 days.

At modelling of the 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 body weight.

Clinical observations

A clinical observation of each animal was conducted once a day. The detailed examination

of the animals was performed in the cage of animals, and it was done in the hands.

ECG registration

For the registration of ECG, the PhysioBelt animal ECG recording and the analysis system were used (Neurobotics LLC, Russia). Registration was carried out in I and II limb leads.

Measurement of blood pressure and heart rate

For measuring the blood pressure and heart rate, a non-invasive system for measurement of blood pressure of rodents "Systole" (OOO Neurobotics, Russia) was used.

Blood plasma electrolyte analysis.

To determine the electrolyte composition of blood, an E-lyte 5(USA) blood electrolyte analyzer (USA) was used. The content of Na+, K+, Cl-, iCa (calcium ions), nCa (bound calcium), tCa (total calcium), pH was determined.

The study of coagulation properties of blood

To determine the coagulation properties of blood, a TS 4000 coagulometer (USA) was used. Prothrombin time, activated partial thromboplastin time, fibrinogen level and thrombin time were determined.

Histochemical studies

During the autopsy, the heart was removed. A macroscopic description of the organ was performed, then the heart was weighed.

The organs were fixed in neutral buffered formalin, then the dehydration and paraffin embedding were carried out according to the standard procedure.

From paraffin blocks the serial sections with a thickness of 5 μm were prepared on a rotary microtome for the right atrium and right ventricle, or left atrium and left ventricle appeared on the preparation. Then, four microscopic specimens of each organ were used for the study.

For qualitative staining for the detection of ischemic cardiomyocytes, the HBFP stain (MT Point, Russia) was used. Stained sections were embedded in mounting media BioMount (BioVitrum, Russia).

Microscopy of histological specimens was performed on a Nicon Eclipse 80I digital microscope using a Nicon DS-2 digital camera (Japan). For microscopy, eyepieces ×10, ×15, lenses ×4, ×10, ×20, ×40, ×100 were used; 10 digital images of randomly selected fields of view from each investigated specimen were made at the magnification of ×400, ×1000.

For morphometric studies, Image J programs (USA) and related plug-ins were used. [2].

Statistics

For statistical analysis, GraphPad Prism 6.0 software (USA) was used. The data was 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 OF The RESEARCH

Results of pathomorphological research.

At analysis of the results of HBFP stain in the myocardium of the intact animals, ischemic cardiomyocytes were not observed (Figure 1 A, B, C). In the myocardium of rats in the I experimental group, both multiple foci of ischemia and single fuchsinophilic ischemic cardiomyocytes, stained in a purple-red color, have been noted (Figure 1 D, E, F).

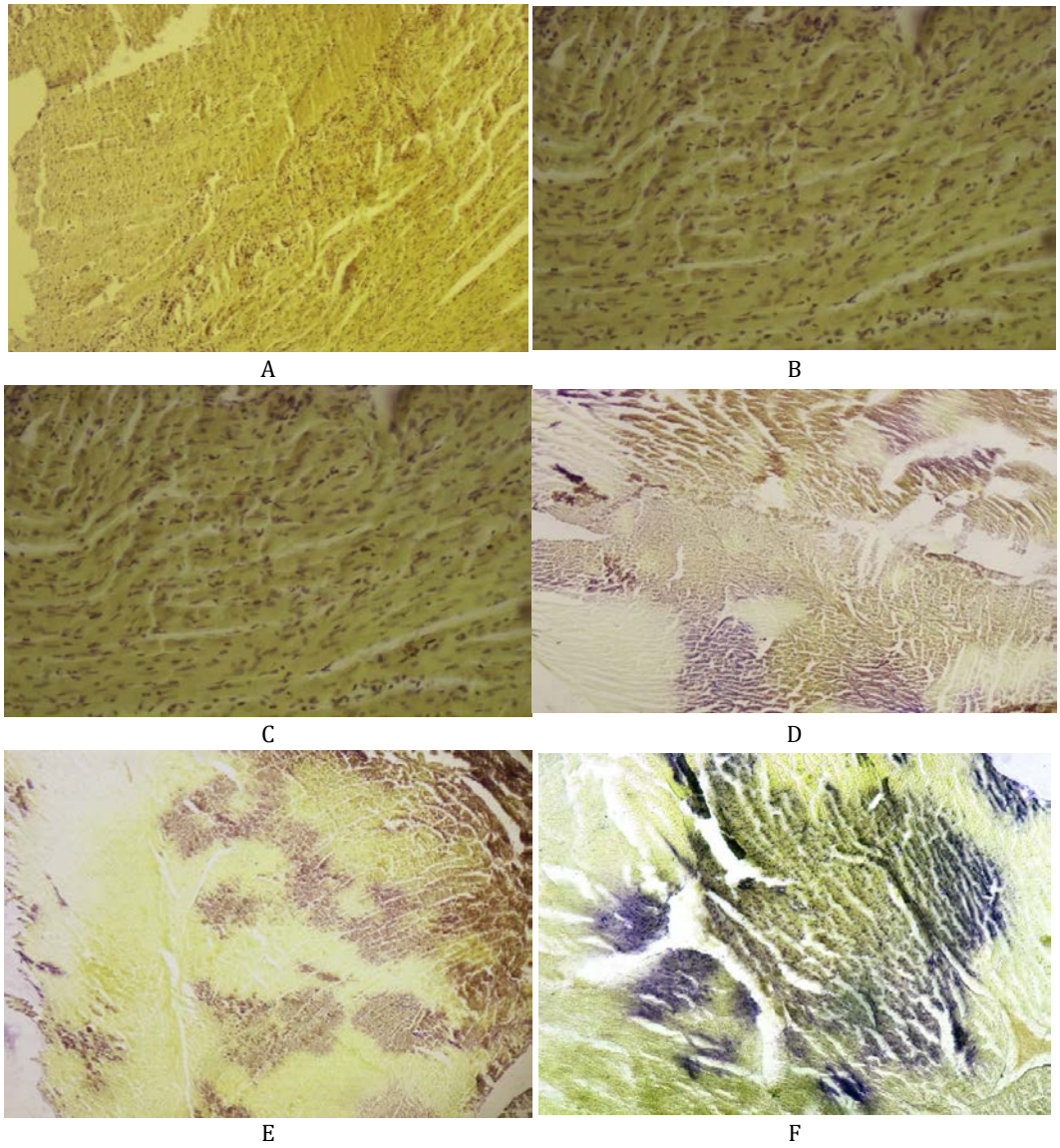

Figure 1. A – Myocardium of rats of control group, HBFP stain by Lie, ×10; B -- Myocardium of rats of control group, HBFP stain by Lie, ×200; C - Myocardium of rats of control group, HBFP stain by Lie, ×400; D and E - Myocardium of rats of I experimental group, HBFP stain by Lie, ×10; F - Myocardium of rats of I experimental group, HBFP stain by Lie, ×100.

For analyzing the results of HBFP stain, ischemic cardiomyocytes were not found in the myocardium of animals of the II experimental group, just as in the control group.

Effect of IHD modeling in various ways on ECG

Initial ECG parameters in animals of the experimental and control groups did not differ significantly, and did not go beyond the physiological norms for rats (Figure 2).

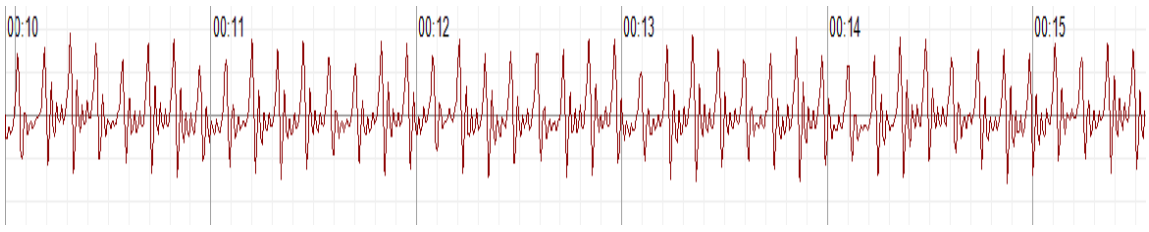

Figure 2: ECG of rats before the introduction of adrenaline and hydrocortisone.

However, already three hours after the introduction of adrenaline and hydrocortisone on the ECG of 30% of the rats of the experimental group, the ST segment merged with the T wave. (Figure 3).

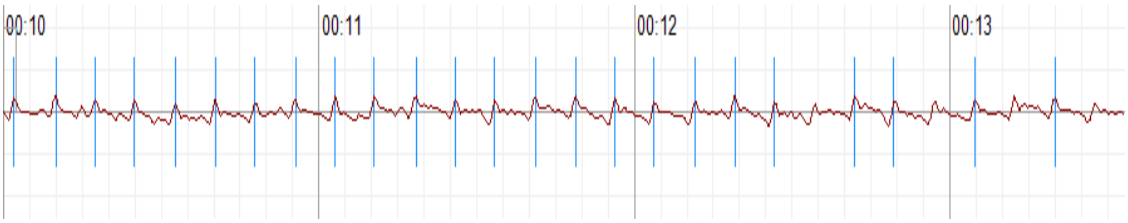

Figure 3: ECG of rats in three hours after the introduction of adrenaline and hydrocortisone.

By the fourth day of the study, the significant ECG changes were observed in all animals of the first experimental group. This picture persisted until the end of the experiment.

An increase in the ST segment above the isoline and its merge with a positive T wave, which indicated a transmural damage of myocardium, so-called "pre-scarring" IHD, has been noted. The development of IHD was also evidenced by the pathological Q wave, the amplitude and duration of which were significantly different from the norm (Figure 4).

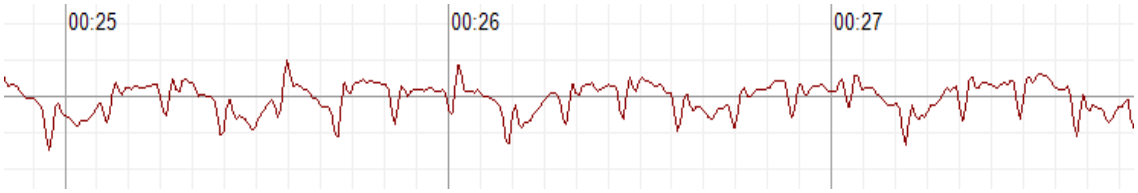

Figure 4: ECG of rats on day 7 after introduction of adrenaline and hydrocortisone.

For analysis of ECG of animals of the second experimental group in the first week of the study, the ST segment merged with the T wave (Figure 5).

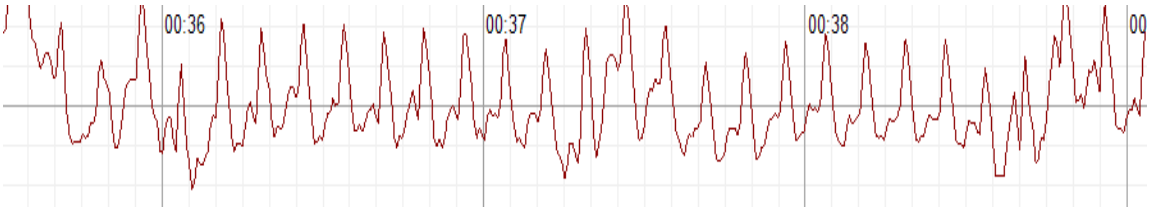

Figure 5 : ECG of rats on day 7 after introduction of doxorubicin.

By the end of the study, ECG of the animals of this group had all the signs of ischemic heart disease, similar to those in rats of the first group on the 8th day of the study, specifically, the ST segment elevation above the isoline, its merging with the positive T wave, and the presence of a pathological Q wave.

Changes in the functional parameters of the cardiovascular system at modeling of IHD in different ways

The study found that blood pressure and heart rate (Figure 6) of animals of all the groups at the beginning of the experiment did not differ from each other. Thus, the value of SBP in rats of the control group was 124.15±5.85 mmHg, the same parameter value in animals of the first experimental group was 125.10±7.71 mmHg, and 127.15 ±8.22 mmHg in the second experimental group. DBP was 84.51±6.35 mmHg in control, 82.47±7.09 and 84.57±6.20 mmHg in the animals of the first and second experimental groups; respectively. The heart rate was 378.60±25.41 beats/min in the control, 370.20±26.22 beats/min and 376.15±24.88 beats/min; respectively.

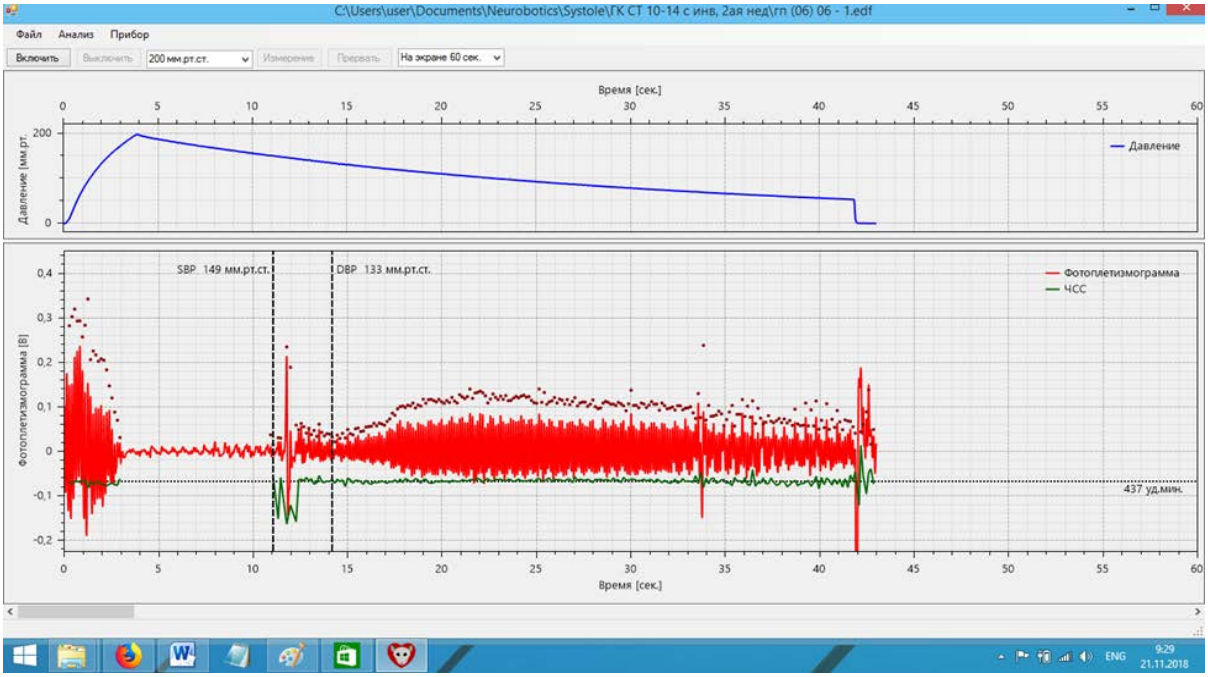

Figure 6: Measurement of blood pressure and heart rate in rats.

The values of the studied parameters, characterizing the cardiovascular system of the rats of the control group, did not change significantly during the experiment. At the same time, in animals of the experimental group already after three hours after the introduction of adrenaline and hydrocortisone, a tendency towards an increase in heart rate began to be noted. On the 4th day of the study, this value already significantly exceeded the initial values and continued to increase, amounting to 419.70±51.67 beats/min by the end of the experiment (Figure 7).

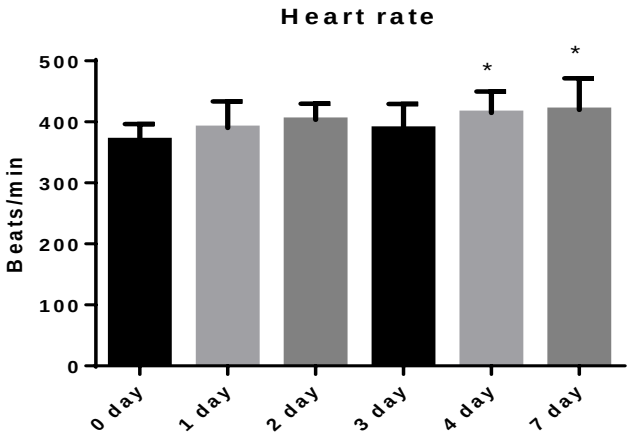

Figure 7: Dynamics of the heart rate of rats of I experimental group during the experiment. Note. Hereinafter * (P≤0,05); ** (P≤0,005); *** (P≤0,0005) - statistical significance of differences in comparison with the control group.

The magnitude of the SBP during the investigated period was fluctuating, but by the end of the experiment its value was 142.1±12.4 mmHg, which was higher than the baseline (Figure 8).

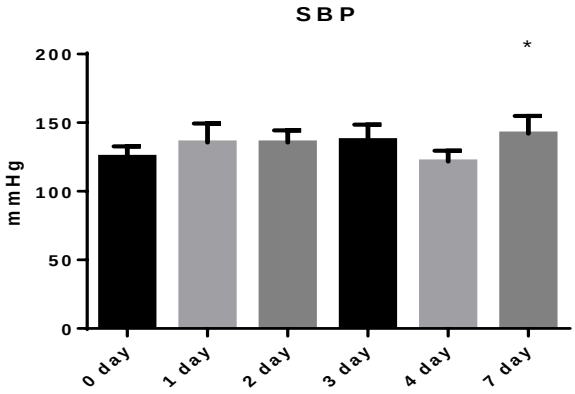

Figure 8: Dynamics of the SBP of rats of I experimental group during the experiment.

The magnitude of DBP underwent less pronounced fluctuations, increasing substantially only on the first day of the study, amounting to 96.90±16.95 mmHg at the end of the study (Figure 9).

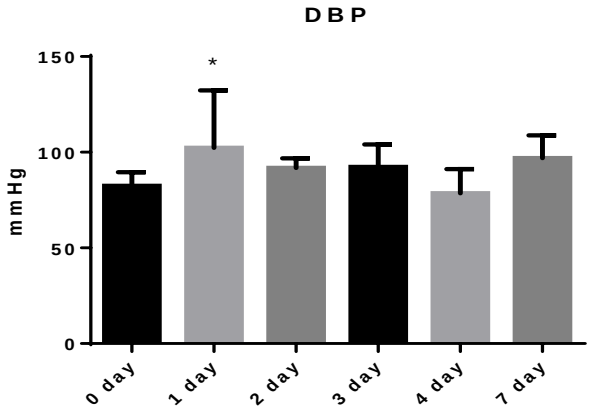

Figure 9: Dynamics of DBP in rats of I experimental group during the experiment.

In rats of the second experimental group, a significant increase in the heart rate was observed at 2nd week of the experiment, and continued to increase until the end of the study (Figure 10).

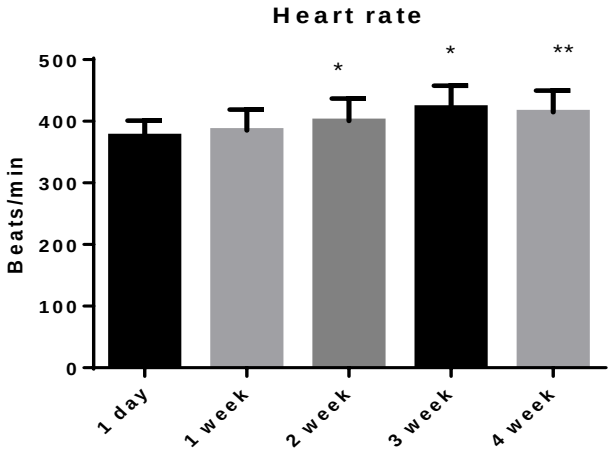

Figure 10: Dynamics of heart rate in rats of the II experimental group during the experiment.

Significant fluctuations in SBP and DBP in the animals of the second experimental group were not observed. SBP by the fourth week was 138.55±8.96 mmHg, DBP was 89.17±8.80 mmHg.

Changes in the coagulation properties of blood in the modeling of ischemic heart disease in different ways

As a result of the research, it was found that at modeling of IHD by the introduction of adrenaline and hydrocortisone, changes in blood coagulation properties occurred, manifested a significant increase in the concentration of fibrinogen (FIB) and a decrease in thrombin time (TT) with unchanged activated partial thromboplastin time (APTT) and prothrombin time (PT) (Table 1).

Table 1. The studied coagulation parameters of blood in rats.

Group	Fib, g/l	TT, sec	PT, sec	APTT, sec
Control (n=20)	1,459±1,291	28,65±4,44	17,42±1,79	26,61±1,79
I experimental group (n=20)	3,130±1,757 *	22,38±3,33 **	16,18±2,07	21,65±9,57
II experimental group (n=20)	1,31±0,58	20,38±6,53	14,52±4,40	17,63±4,11 **

At the same time, the use of doxorubicin for modeling IHD led to less significant changes in the coagulogram of animals. In rats of the second experimental group, only a decrease of the activated partial thromboplastin time was observed.

Changes in the composition of blood electrolytes in rats when modeling IHD in different ways.

In the plasma of rats of the first experimental group, the level of Cl-, iCa, nCa decreased and the pH of the blood plasma increased (Table 2). At analysis of the electrolyte composition of blood in rats of the second experimental group, an increase in the concentration of all types of calcium ions was found, and the other studied parameters remained unchanged.

Table 2. The level of some electrolytes and the pH of the blood plasma of rats at modelling of IHD in different ways.

	pH	Cl, mmol/l	iCa, mmol/l	nCa, mmol/l	TCa, mmol/l
Control (n=20)	7,51±0,04	125,90±2,698	0,9720±0,0418	1,002±0,0388	1,892±0,240
I experimental group (n=20)	7,59±0,03 ***	121,10±2,143 **	0,90±0,0223 ***	0,9571±0,0236 *	1,869±0,042
II experimental group (n=20)	7,496±0,05	123,60±4,83	1,049±0,047 **	1,073±0,039 **	2,097±0,076 *

CONCLUSION

According to the results of the study, the modeling of ischemic heart disease, both by the joint introduction of adrenaline and hydrocortisone, as well as by the introduction of doxorubicin, caused significant changes in an organism of the experimental animals.

In both cases, the ECG results testified the development of IHD.

The dynamics of the studied functional parameters, which directly characterized the state of the cardiovascular system (heart rate, SBP, and DBP), also confirmed the success of IHD modeling in these ways.

These changes had both morphological and functional characters. At modeling of IHD by the joint introduction of adrenaline and hydrocortisone, the morphological changes were manifested in significant pathological changes in the myocardium of rats, characteristic for the modeled pathology. At modeling of IHD through the use of doxorubicin, significant morphological changes were not observed.

Functional changes (coagulation properties of blood, its electrolyte composition) also demonstrated ischemic changes in the heart of the animals of the first experimental group.

At modeling of IHD by introduction of doxorubicin, a number of similar functional changes occurred, but the nature of the morphological myocardial disorders indicated a predominantly cardiotoxic effect of doxorubicin, which did not lead to the morphological picture characteristic for IHD.

Based on the foregoing, the method of modeling of IHD by the joint introduction of adrenaline and hydrocortisone was characterized with:

- high reproducibility;
- involvement of the leading pathogenetic mechanisms of IHD formation;
- natural genesis of IHD;
- having potential to be used as a model of IHD when testing drugs aimed at the prevention and treatment of pathology.

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AUTHOR'S CONTRIBUTION

The article was written at the expense of the authors.

CONFLICT OF INTEREST

In the article, there has been no information capable of provoking conflict of interests, with the exception of the information contained in previously published articles by Areshidze, David Alexandrovich, Mischenko Denis Valerievich, Makartseva Lyudmila Andreevna, Kucher Sergey Alexandrovich, Kozlova Maria Alexandovna, Timchenko Lyudmila Dmitrievna, Rzhepakovsky Igor Vladimirovich**,** Nagdalian Andrey Ashotovich, Pushkin Sergey Viktorovich.

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