

MECHANISM OF STRESS HEART DAMAGE IN THE ANIMALS OF DIFFERENT SENSITIVITY TO HYPOXIA

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Abstract

Hypoxia and resistance to it is the main in today data situation in the world.

The aim of the study was to determine level of myocardium damage and corresponding of it with cytokines level in high- and low-resistance to hypoxic hypoxia rats (HR, LR) in different models of immobilization stress.

Material and methods of investigation. The experiments were performed on 144 outbred HR and LR aged 5.5-6 months, dividing into 3 groups – control and 2 with different model of immobilizing stress. It was determined morphological changes of myocardium and concentration of interleukins 1beta, 2, 4, 6, 10 (IL-1beta, IL-2, IL-4, IL-6, IL-10), tumor necrosis factor alpha (TNF-alpha) in the blood serum.

Results. The morphological investigation showed the bigger damage effect in LR animal, than in HR rats. Damage effects were higher in males, than in females. The stress repeating every 72 hours (stress 2) had higher damage in myocardium, than stress repeating every 24 hours (stress 1). We corresponds the results with cytokines level. High congenital resistance to hypoxia is associated with an increased content of anti-inflammatory cytokines (in males IL-4, in females IL-10). Intact males, compared with females, have a higher production of pro-inflammatory cytokines (IL-6, TNF-alpha).

At stress 1 in HR males, compared with HR females, were lower contents of IL-10, higher level of IL-1beta, IL-4, IL-6, TNF-alpha. At stress 1 in LR males, compared with LR females, were lower contents of IL-10, IL-2. The morphological changes were more in males, that's why higher level of anti-inflammatory cytokines IL-10 really has protection in HR and LR females' animals. At stress 2 in HR male, compared with HR female, were lower contents of IL-1beta and IL-10, higher IL-2 and IL-6. At stress 2 in LR males, compared with LR females, were higher level of IL-10, IL-6, lower contents of IL-2, IL-4. The morphological changes were more in males, that's why pro-inflammatory cytokines IL-6 really take place in damage of myocardium in males with different resistance animals.

Conclusion. High congenital resistance to hypoxia is associated with smaller destroying of the myocardium layer in immobilizing stress, which correspond with increased content of anti-inflammatory cytokines IL-4 and, IL-10. Higher damage of myocardium in immobilizing stress correspond with higher production of pro-inflammatory cytokines (IL-6, TNF-alpha).

Key wards: cytokines; stress; myocardium; hypoxia sensitivity; sex; rats

Introduction. Cytokines play a significant role in stress hormone release [1, 2]. They cause chronic low-grade inflammation [3]. There are data in the literature on the growth of interleukin 6 in chronic stress [4], the increase of which is associated with increased secretion of norepinephrine [5]. Interleukin (IL) -10 is a potent activator of the hypothalamic – pituitary – adrenal axis and immobilization stress may induce an increase in rat cytokine IL-10 [6].

During COVID-19 pandemy increased attention of scientists of the world are study the features of increased resistance to hypoxic hypoxia [7 - 10]. Hypoxia is the bases of that disease. It is known that females are more resistant to hypoxia [11, 12, 13]. COVID-19 lead to stress and limited physical activity also, Stress at that condition can be in the different strength. At that conditions increase development of the inflammation. The main problem can be inflammation condition and local circulatory hypoxia, which leads to necrosis development in the cardiac muscles, more in the males [14].

The aim of the study was to determine level of myocardium damage and corresponding of it with cytokines level in high- and low-resistance to hypoxic hypoxia rats (HR, LR) in different models of immobilization stress.

Material and methods of investigation. The experiments were performed on 144 outbred high- and low-resistance to hypoxia rats (HR, LR) aged 5.5-6 months. Animals were divided into three groups – control and two experimental with immobilization stress. In each

group was 12 males-rats and 12 females-rats. The animals with different resistance to hypoxia was performed according to the of Berezovsky V.Ya. (1978) method [15]. Stress was simulated 4 times by one-hour immobilization of rats on the back down with the difference time between each immobilization epizods: 24 hours (stress 1) and 72 hours (stress 2) [16].

All experiments were performed in the morning in a specially designated room at a temperature of 18-22 °C, a relative humidity of 40-60% and an illumination of 250 lux. All animals kept in one room on a standard diet and vivarium regime. The experiments were performed in compliance with the norms of the Council of Europe Convention on the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (Strasbourg, 18.03.1986), the resolution of the First National Congress on Bioethics (Kyiv, 2001) and the Ministry of Health of Ukraine № 690 of 23.09 .2009 p.

Euthanasia of rats was performed by total bloodletting from the heart after previous thiopental-sodium anesthesia (60 mg/kg of body weight intraperitoneally). For further experimental study in the blood serum were determined the concentration of interleukins 1beta, 2, 4, 6, 10 (IL-1beta, IL-2, IL-4, IL-6, IL-10), tumor necrosis factor alpha (TNF-alpha) [17]. Determination in the blood serum of cytokines levels was performed using by immunopherment method with standard reagent, adapted for white rats «Enzyme-linked Immunosorbent Assay; Kits for Rat: Tumor Necrosis Factor Alpha (TNF α), Interleukin 1 Beta (IL-1 β), Interleukin 6 (IL-6), Interleukin 2 (IL-2), Interleukin4 (IL-4), Interleukin 10 (IL-10)», Uscn, Life Science Inc., E90133Ra, E90563Ra, E90079Ra, CSB-E04628r, CSB-E04635r, CSB-E04595r on the analyzer STAT FAX 303 plus. Also were determine the changes of morphological section of heart, coloring by hematoxilin-eosin.

Statistical processing of digital data was performed using the program "STATISTICA" 6.0 ("Statsoft", USA).

Results and discussion. In the light-optical investigation of cross-sections of the hearts in all groups of animals, which were under immobilization stress, were minor myocardial damage, the degree of which depended on the state and patterns of immobilization stress. In all groups of animals found a small size of cardiomyocytes and stroma, varying degrees of necrobiosis of cardiomyocytes and microcirculation disorders.

Histological investigation of the myocardium of HR-female rats in stress 1 revealed that its general structure was preserved: muscle fibers had an orderly location with well-defined anastomoses between them.

There were minor destructive lesions of muscle fibers, violation of their tinctorial properties, in some places stasis was noted, there was a well-defined blood supply to the vessels in which there was an accumulation of erythrocytes. Cardiomyocyte edema and stroma appeared. Contractile cardiomyocytes retained their typical shape and location. Euchromatic nuclei were mainly located in the center of the cells and well contoured against the background of unevenly illuminated sarcoplasm. These enlightenments had the form of vacuoles of different sizes and color intensities. In the layers of connective tissue between the muscle fibers, fibroblasts with large euchromatic nuclei and fibrocytes of small size with intensely oval-shaped basophilic nuclei were sometimes well distinguished. In some cases, single small lymphocytes were also found. Tinctorial heterogeneity of cardiomyocytes was observed in some parts of the myocardium. Areas were also visible in which the nucleus was not visible in the cells, or it was shifted to the periphery. Some fibers were swollen.

In histological preparations of hearts of LR rats females in immobilization stress 1 expansion of components of a microcirculatory channel were more expressed, they had more diffuse character, gleams were often filled with considerable accumulations of formed elements of blood.

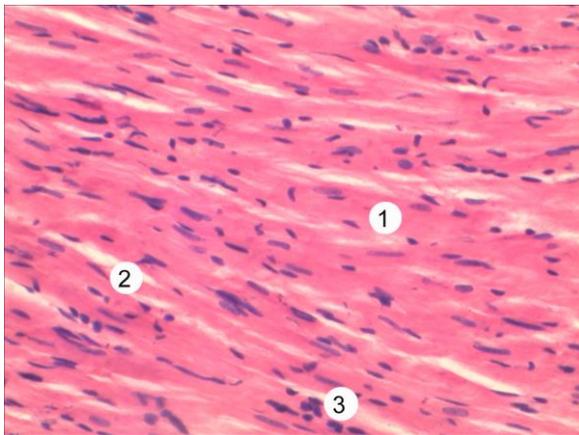
Perivascular spaces were slightly dilated, somewhat illuminated. They had lymphocytes. In animals, altered contractile cardiomyocytes were found, in the basophilic nuclei of which heterochromatin predominated compared to the control. Stratification of muscle fibers was noted. The nuclei of adjacent cells in some areas were placed disorganized. Swelling of myofibrils and stroma was more pronounced. The vessels were dilated and filled with blood, they showed adhesion and aggregation of erythrocytes. It should be noted that in the heart muscle of LR females, the described changes were greater than in HR. When considering myocardial drugs HR male rat, which underwent immobilization stress 1, there were greater morphological changes than in female HR.

Contractile cardiomyocytes retained their typical shape and location, the nuclei were located mostly on the periphery of the cell, contoured against the background of unevenly illuminated sarcoplasm. What was special was that not all cardiomyocytes had identical changes: single cells of darker color with better structured sarcoplasm were found between vacuolated cells. Fibroblasts with large euchromatic nuclei and small fibrocytes with intensely oval-shaped basophilic nuclei were seen in the layers of connective tissue between the muscle fibers. Tinctorial heterogeneity of cardiomyocytes was observed in the myocardium. Areas were also visible in which the nucleus was not visible in the cells, or it was shifted to the periphery. Most of the fibers were swollen, there was destruction of cardiomyocytes. In the

lumens of the vessels were seen inhomogeneous accumulations of formed elements of blood, leukocyte infiltration. In LR males rats in stress 1, the changes were most pronounced. There was a significant expansion of the elements of the hemomicrocirculatory tract and their significant blood supply, which was accompanied by the release of formed blood elements outside the vascular bed.

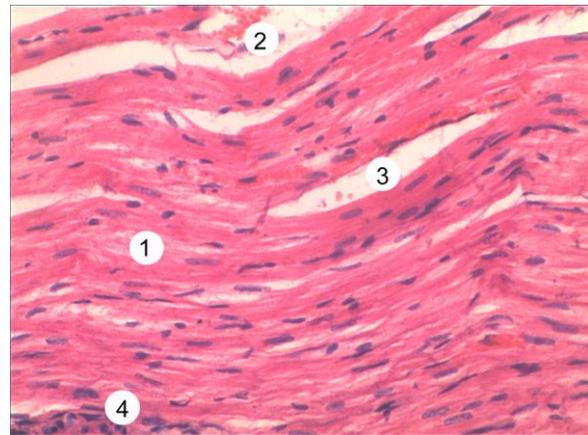
Damage to the myocardium was characterized by small focal areas of altered cells, which in places merged. The color of the sarcoplasm of such cardiomyocytes was uneven. Against the background of the described changes, homogeneous areas of the myocardium of intense oxyphilic color were also detected. The cell nuclei of such areas of the myocardium were not visualized, was deferred cardiomyofibrils, stromal edema. Violations of the tinctorial properties of the myocardium in LR males under stress 1 were higher.

Stress 2 lead to the higher changes in each groups (figures 1 and 2).



1 - edema and homogenization of cardiomyocytes, 2 - edema of the stroma, 3 - leukocyte infiltration.

Figure 1 - Fragment of the LR myocardium of a female rat in immobilization stress 2. Staining with hematoxylin and eosin. x 200



1 - edema and fibrosis of myofibrils, 2 - diapedesis of erythrocytes, 3 - edema of the stroma, 4 - leukocyte infiltration.

Figure 2 - Fragment of the LR myocardium of a male rat in immobilization stress 2. Staining with hematoxylin and eosin. x 200

The determination of cytokines, which can caused that destroying are the next. In control HR rats-males, compared with LR, the lower level of the concentration of IL-1beta in 2.1 times ($p < 0.001$) and higher level of IL-4 on 24.7% ($p < 0.001$) were determined (Tables 1, 2).

At stress 1, compared with the control, in HR male-rats noted significant increase of IL-1beta in 2.3 times ($p < 0.001$), TNF-alpha in 8.2 times ($p < 0.001$), IL-10 on 40.2% ($p < 0.01$), IL-4 on 22.7% ($p < 0.001$). In LR rats-males it was considered significant decrease of IL-1beta on 54.7% ($p < 0.001$), IL-6 on 51.3% ($p < 0.001$), IL-4 on 20.4% ($p < 0.01$), increase of TNF-

alpha in 5.7 times ($p < 0.001$). Compared that two groups with different resistance to hypoxia was determined in HR increase of IL-1beta on 60.4% ($p < 0.001$), IL-6 on 34% ($p < 0.001$), IL-4 on 22.5% ($p < 0.01$).

Table 1 - Changes of proinflammatory cytokines in the serum of high- and low-resistance to hypoxia rats of different sex caused by stress, $M \pm m$ (n=12)

Group	Index			
	IL-1beta, pg/ml	IL-2, pg/ml	IL-6, pg/ml	TNF-alpha, pg/ml
High-resistance to hypoxia male-rats				
Control	40.45 ± 3.83	6.92 ± 1.11	3.33 ± 0.57	6.30 ± 0.22
Stress 1	95.11 ± 2.77*	6.00 ± 0.61	2.47 ± 0.08	51.69 ± 8.85*
Stress 2	31.25 ± 0.62*	7.75 ± 0.82	3.73 ± 0.46	18.12 ± 3.67*
Low-resistance to hypoxia male-rats				
Control	83.26 ± 5.23**	8.25 ± 1.42	3.35 ± 0.39	6.76 ± 0.22
Stress 1	37.69 ± 5.38*,**	5.00 ± 0.88	1.63 ± 0.11*,**	39.62 ± 5.60*
Stress 2	50.34 ± 3.71**,**	6.67 ± 0.80	1.86 ± 0.10**,**	35.03 ± 8.46*
High-resistance to hypoxia female-rats				
Control	55.79 ± 5.04 [#]	8.08 ± 0.84	1.67 ± 0.24 [#]	5.62 ± 0.13 [#]
Stress 1	39.07 ± 3.10*, [#]	7.17 ± 0.50	0.67 ± 0.05*, [#]	18.92 ± 4.80*, [#]
Stress 2	70.25 ± 3.91*, [#]	5.00 ± 0.68*, [#]	0.62 ± 0.07*, [#]	18.28 ± 4.75*
Low-resistance to hypoxia female-rats				
Control	47.48 ± 2.28 [#]	9.50 ± 0.73	1.53 ± 0.11 [#]	5.65 ± 0.28 [#]
Stress 1	42.38 ± 4.74	11.08 ± 0.72**, [#]	1.23 ± 0.19**	41.12 ± 9.26*,**
Stress 2	60.24 ± 3.85*	9.83 ± 1.20**, [#]	1.19 ± 0.15**, [#]	32.36 ± 8.4*

Notes: 1. * – indexes are reliable, compared to control; 2. ** – indexes are reliable, compared to HR rats; 3. # – indexes are reliable, compared to male-rats.

High levels of serum TNF-alpha are a marker of the risk of heart damage. It stimulates the activity of leukocytes, the production of cells IL-1 beta, IL-6 and has a destructive effect on tissues. The increase of TNF-alpha and IL-1 beta was obtained under stress 1 in HR male-rats.

IL-10 is the anti-inflammatory cytokine and is an important endogenous regulator of immune and inflammatory processes, able to inhibit the activation and function of T cells, NK cells, macrophages, their production of proinflammatory cytokines. Simultaneously with the activation of pro-inflammatory cytokines under stress 1, only in HR male increased IL-10. Under this stress, the indicators of IL-1 beta and IL-10 did not exceed the values of control LR males-rats.

At stress 2, compared with the control, in male HR there was a significant decrease in IL-1 beta on 22.7% ($p < 0.02$) and IL-4 on 32% ($p < 0.001$). In this kind of stress in HR there was an increase of TNF-alpha in 2.9 times ($p < 0.002$), IL-10 on 25.6% ($p < 0.02$). In LR males-

rats there was a significant decrease of IL-1 beta on 39.5% (p<0.001), IL-6 on 44.5% (p<0.001), IL-4 on 25.9% (p<0.001), increase of TNF-alpha in 5.2 times (p<0.001), IL-10 on 46.9% (p<0.001). In HR males-rats, compared with LR, were lower level of IL-1beta in 1.6 times (p<0.001), IL-10 on 37.6% (p<0.001), higher level of IL-6 on 50.2% (p<0.001), IL-4 on 18% (p<0.001).

Table 2 - Changes in the concentration of anti-inflammatory cytokines caused by stress in the serum of high- and low-resistant to hypoxia rats of different sex, M ± m (n=12)

Group	Index	
	IL-4, pg/ml	IL-10, pg/ml
High-resistance to hypoxia male-rats		
Control	1.74 ± 0.08	7.79 ± 0.67
Stress 1	1.35 ± 0.03*	10.92 ± 0.85*
Stress 2	1.18 ± 0.01*	9.79 ± 0.49*
Low-resistance to hypoxia male-rats		
Control	1.31 ± 0.03**	9.17 ± 0.61
Stress 1	1.04 ± 0.100*,**	9.82 ± 0.62
Stress 2	0.97 ± 0.03*,**	13.47 ± 0.43*,**
High-resistance to hypoxia female-rats		
Control	1.15 ± 0.07#	15.04 ± 1.00#
Stress 1	1.14 ± 0.06#	15.61 ± 1.35#
Stress 2	1.13 ± 0.03	16.66 ± 1.45#
Low-resistance to hypoxia female-rats		
Control	1.21 ± 0.06	9.42 ± 0.86**
Stress 1	1.07 ± 0.06	12.97 ± 0.50*,#
Stress 2	1.34 ± 0.03*,**,#	10.31 ± 0.48**,#

Notes: 1. * – indexes are reliable, compared to control; 2. ** – indexes are reliable, compared to HR rats; 3. # – indexes are reliable, compared to male-rats.

In control HR females, compared with LR, found a higher on 37.4% (p<0,001) concentration of IL-10. At stress 1, compared with the control, in HR rats there was an increase in TNF-alpha in 3.4 times (p<0,01), a decrease in IL-1beta on 30% (p<0,001), IL-6 on 59.9% (p<0.01). In LR rats there was a significant increase of IL-10 on 37.7% (p<0.001), TNF-alpha in 7.3 times (p<0.001). Moreover, in HR females, compared with LR females, were lower IL-2 on 54.7% (p<0.001), IL-6 on 83.6% (p<0.001), TNF-alpha in 2.2 times (p<0.05).

In stress 2, compared with the control, in HR female there was an increase of IL-1beta on 25.9% (p<0.05), TNF-alpha in 3.3 times (p<0.01), a decrease of IL-2 on 38.1% (p<0.01), IL-6 on 62.7% (p<0.001). In LR there was a significant increase in the concentration of IL-1 beta on 26.9% (p<0.05), IL-4 on 10.8% (p<0.001), TNF-alpha in 5.7 times (p<0,01). In HR

female, compared with LR, were lower content of IL-2 on 96.7% ($p<0.001$), IL-6 on 91.8% ($p<0.001$), IL-4 on 18.5% ($p<0.001$), higher level of IL-10 on 38.1% ($p<0.001$).

In control HR males, compared with HR females, were lower content of IL-1beta on 37.9% ($p<0.02$) and IL-10 on 93.1% ($p<0.001$), higher level of IL-4 on 33.9% ($p<0.001$), IL-6 on 49.9% ($p<0.01$), TNF-alpha on 10.9% ($p<0.01$). In control LR males, compared with LR females, IL-1beta was higher on 43% ($p<0.001$), IL-6 on 54.3% ($p<0.001$), TNF-alpha on 16.4% ($p<0.002$).

At stress 1 in HR males, compared with HR females, were lower contents of IL-10 on 42.9% ($p<0.02$), higher level of IL-1beta on 58.9% ($p<0.001$), IL-4 on 15.3% ($p<0.02$), IL-6 on 72.9% ($p<0.001$), TNF-alpha on 63.4% ($p<0.002$). At stress 1 in LR males, compared with LR females, were lower contents of IL-10 on 32.1% ($p<0.001$), IL-2 in 2.2 times ($p<0.001$). The morphological changes were more in males, that's why higher level of anti-inflammatory cytokines IL-10 really has protection in HR and LR females' animals.

At stress 2 in HR male, compared with HR female, were lower contents of IL-1beta in 2.3 times ($p<0.001$) and IL-10 on 70.3% ($p<0.001$), higher IL-2 on 35.5% ($p<0.01$), IL-6 on 83.3% ($p<0.001$). At stress 2 in LR males, compared with LR females, were higher level of IL-10 on 23.4% ($p<0,001$), IL-6 on 35.9% ($p<0,001$), lower contents of IL-2 on 47.5% ($p<0.05$), IL-4 on 38.07% ($p<0.001$). The morphological changes were more in males, that's why pro-inflammatory cytokines IL-6 really take place in damage of myocardium in males with different resistance animals.

Conclusion. High congenital resistance to hypoxia is associated with smaller destroying of the myocardium layer in immobilizing stress, which correspond with increased content of anti-inflammatory cytokines IL-4 and, IL-10. Higher damage of myocardium in immobilizing stress correspond with higher production of pro-inflammatory cytokines (IL-6, TNF-alpha).

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