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Morphological, Immunophysiological And Clinical Features Of Respiration And Blood Circulation Under The Influence Of Combined Stress In The Covid-19 Conditions

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ABSTRACT

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes coronavirus disease 2019 (COVID-19) has reached a pandemic level. There are now numerous indications that the COVID-19 coronavirus can severely affect the heart, blood vessels, nerves, brain, kidneys and skin. Of course, the lungs and airways are the main focus of attention with the COVID-19 respiratory disease. In this article the evaluation of pulmonary circulation indicators in the dynamics of respiratory cycle phases in intact animals was performed. This was done using self-registration method of reopulmonography in rats. In experiments on rats with the coloring of lungs tissue with hematoxylin and eosin were studied morphofunctional changes of pulmonary circulation during the combined influence of immobilization and hypothermia. It was determined that abnormalities run at the level of microvasculature vessels of lungs tissue and are accompanied by blood filling reduction, development of arteriolospasm signs, capillaries and post-capillary venules plethora with sustainable increase of vascular wall permeability.

Keywords: COVID-19, respiration, lung morphology, pulmonary edema, lungs, stress factors, immobilization, hypothermia, pulmonary circulation, cardiorespiratory system, rheogram.

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INTRODUCTION

Currently, in connection with the COVID-19 pandemic, the attention of many researchers is directed to studying the dynamics of respiratory and circulatory changes in organs and tissues, as well as to research ways to prevent this disease [26, 27, 28]. Complications of COVID-19 may include respiratory failure and sudden respiratory distress syndrome, heart failure and heart rhythm disorders, kidney failure, multiple organ failure, and death. COVID-19 is primarily known as a respiratory disease [26, 27, 28, 29, 30].

Of course, the lungs and respiratory tract are the focus of attention for COVID-19 respiratory disease. Since the new pathogen SARS-CoV-2 mainly affects the lower respiratory tract, infected people who experience moderate to severe disease have dry coughing, shortness of breath, and / or pneumonia [27, 28, 29, 30].

Not all parameters of respiration and pulmonary blood flow can be noninvasively determined in humans. The variety of manifestations of respiratory and circulatory changes in simulating the same state makes it necessary to conduct a large number of experiments on small laboratory animals. In the literature, there are hotel data on the normal value of blood pressure, shock and heart index, total peripheral resistance, pressure in the arteries and veins of the small circle of blood circulation of rats [5, 10, 12, 14, 15,16]. However, we have not been able to find works that would assess the indicators of pulmonary circulation in the dynamics of the respiratory cycle phases in intact animals. Environmental factors are often extreme for an organism, and as a rule affect it in a complex [17, 18, 19, 22, 23, 24, 25, 26, 27]. Some literary data testify that the complex of jointly functioning irritants often leads to the complication of the shifts arising in an organism, in comparison with their isolated influence [1,2, 3, 4, 6, 7, 8, 9]. In other works, on the contrary it is found a protective effect of one of the stressors during the complex influence of several ones [9, 10, 11, 13, 15, 16,].

We found out no data on the complex effect of immobilization and hypothermia on lungs morphofunctional changes in available literature.

The aim of the research was to study morphofunctional changes of the lungs and blood circulation in the pulmonary circuit during spontaneous respiration and under the influence of the complex effect of hypothermia and immobilization.

MATERIAL AND METHODS

Experiments were performed on 30 healthy white rats, 10 Guinea pigs and 5 rabbits. Our modified methods of applying rheographic electrodes and the use of a smooth regulator of the output signals of the rheoplethysmograph developed by us made it possible to register simultaneously respiratory and pulse fluctuations of the rheogram in animals [8, 13, 14, 15, 16, 17]. Research were conducted on 20 (there are10 control among them) non-pedigreed, white rats weighing from 160 to 250 g.

The complex effect of hypokinesia and hypothermia was simulated by the placing of experimental rats into the 80 cm^3 camera designed by us, which has connection with the ambient environment within 6 hours for 10 days at the temperature of + 3+4°C [14, 15, 16, 22, 23].

Registration of pulmonary hemodynamic was carried out with the help of rheogram record (RG) on the RPG2 – 02 unit by the technology modified by us [8, 13, 14, 15, 16]. With the aim of studying the pathomorphological changes in the lungs of experimental rats, lungs tissues were taken out during the dissection (by Shore). The study of

morphological changes of lungs was carried out with the help of coloring of histologic cuts of lungs with hematoxylin and eosin.

RESULTS AND CONSIDERATION

The rheographic curve of ventilation had the form of a sinusoid, in which the descending part displayed the inhaling phase, in the ascending - the expiratory phase. Pulse fluctuations of the rheogram, which were layered on the respiratory, in shape resembled the usual curves of pulse pressure or volume pulse. Each segment of the pulsation rheogram, synchronous to the cardiac cycle, consisted of a main systolic prong with a steep anacrot, a slightly rounded top, and a gentle catacrot. At the top of the descending segment of the wave, there was a shallow incisure and sometimes a dicrotic prong. Then the catacrot was followed by a diastolic wave of varying degrees of severity, the amplitude of which in healthy animals was always lower than the amplitude of the systolic one.

Analysis of the rheographic ventilation curve showed that the respiration rate in healthy rats, calculated by rheography, was 86.98 ± 2.24 min^{-1}. The duration of the respiratory cycle was 0.71 ± 0.02 seconds. Moreover, 0.36 ± 0.01 seconds accounted for the inhalation phase, and 0.35 ± 0.01 seconds for the exhalation phase.

From the beginning to the middle of the inhalation (table.1) there was a sharp increase in the rheographic index (ΔRI) of the depth of respiration: from 0.117 ± 0.01 to 1.14 ± 0.06 (46%). At the end of the inhalation, the reverse sequence is marked: from the beginning of the exhalation to the middle. ΔRI decreased slowly – from 1.04 ± 0.06 to 0.83 ± 0.06 (by 21%), by the end of exhalation, a steeper decrease in this indicator was observed from 0.83 ± 0.06 to 0.21±0.02.

Contour analysis of the pulsation rheogram and its quantitative indicators revealed the dynamics of pulse increment of blood to the lungs, the speed of blood flow and changes in vascular tone during different periods of inspiration and expiration.

Time intervals of the rheogram: periods of general (E_0, sec), fast (E_f, sec) and slow (E_s, sec) blood filling, the period of tension (T, sec) did not change during inspiration and expiration. However, significant changes were detected in the amplitude parameters. Thus, the average values of a systolic (As, Ohм) and Ad, Ohм) on inspiration were 1.10±0.06 Ohm and 0.74±0.05 Ohm in Guinea pigs (table. 1) and 0.54 ± 0.03 Ohm and 0.41±0.09 Ohm in rats (table 2), and the output decreased to 0.70 ± 0.06 Ohm in Guinea pigs ($p < 0.001$) and to 0.37 ± 0.02 and 0.25 ± 0.01 Ohm in rats respectively ($p < 0.001$).

Table 1: Rheographic indicators of healthy Guinea pigs' lungs during spontaneous respiration

Indicators	Inhale	Exhale
T, sec x 10^{-2}	4,32 ± 0,13	4,34 ± 0,12
E_0, sec x 10^{-2}	12,40 ± 0,17	12,50 ± 0,16
E_{max}, sec x 10^{-2}	7,95 ± 0,14	7,88 ± 0,15
E_f, sec x 10^{-2}	3,90 ± 0,10	3,80 ± 0,10
E_s, sec x 10^{-2}	4,03 ± 0,12	4,01 ± 0,13
As, Ohм	1,10 ± 0,06	0,70 ± 0,06***
Ad, Ohм	0,74 ± 0,05	0,41 ± 0,04***

Table 2: Rheographic indicators of healthy rats' lungs during spontaneous respiration Indicators

Indicators	Inhale	Exhale
T, sec x 10^{-2}	3,90 ± 0,10	3,92 ± 0,12
E_0, sec x 10^{-2}	8,40 ± 0,45	8,44 ± 0,41
E_{max}, sec x 10^{-2}	5,80 ± 0,17	5,76 ± 0,19
$E_б$, sec x 10^{-2}	2,60 ± 0,07	2,56 ± 0,08
$E_м$, sec x 10^{-2}	2,20 ± 0,10	2,18 ± 0,11
Ac, Ohм	0,54 ± 0,03	0,37 ± 0,02***
Ad, Ohм	0,41 ± 0,09	0,25 ± 0,01***

Note: * - reliability of the difference between the indicators in the first and second columns < 0.05; *** < 0.001

A decrease in the average value of the rheographic wave amplitude on exhalation indicated a decrease in the volume of blood flow and a decrease in the pulse increment of blood to the lungs during this phase of the respiratory cycle.

The ratio between the amplitude and time parameters of the rheographic curve allowed us to estimate the rate of blood flow to the studied lung area.

The rate of systolic inflow to the lungs is determined by the ratio of the amplitude of the systolic wave to the duration of the maximum blood filling period. Given this and the fact that the average height of the rheographic complexes on inspiration was 0.05 ± 0.03 Ohm and 1.1 ± 0.06 Ohm, and on exhalation 0.37 ± 0.02 Ohm and 0.7 ± 0.06 Ohm, while the time intervals remained unchanged, it becomes obvious that the rate of systolic blood flow to the lungs on inspiration is significantly higher than on exhalation.

For normal functioning of the body and adequate saturation of arterial blood with oxygen, one of the necessary conditions is the physiological relationship between alveolar ventilation and capillary blood flow. To study this relationship in the dynamics of inhalation and exhalation, we introduced a coefficient that reflects the amount of blood filling or pulse increment of blood to the lungs, depending on the amount of increment of respiratory volume.

This coefficient (ΔRI/ΔDO) was the highest at the beginning of inspiration and was equal to 10.85 ± 0.87 with the corresponding values of increment of respiratory volume and pulse blood filling of 0.17 ± 0.01 and 1.65 ± 0.09. By mid-breath ΔRI/ΔDO with an increase in ΔDO to 0.78 ± 0.05 and a decrease in ΔRI to 1.21 ± 0.08 decreased 10 times and reached to 1.84 ± 0.19, and by the end of inspiration - 0.86 ± 0.13. In the dynamics of exhalation, despite the reverse changes in the respiratory volume - a decrease to 1.04±0.06, there was a further decrease in the coefficient ΔRI/ΔDO up to 1.25 ± 0.13. With a further reduction of ΔDO, ΔRI/ΔDO slowly increased to 1.52 ± 0.18 by the middle of the exhalation and more significantly (to 8.66 ± 0.92) by the end of it.

According to the rheographic research data during the combined stress determined by hypothermia against the background of immobilization, changes of pulmonary circulation of all animals were unidirectional from first till third hour of the experiment and there were determined the following principles: reduction of blood filling, increase of vessels tonicity of lungs pre - capillary bed and passive congestion in a lesser circulation. The evidence is decrease of As, shortening of E_0, E_{max} and T. From the fourth hour and until the end of the experiment (the 6th hour), these rates of some rats on the rheogram tend to the level of control data, possibly due to the activation of reflex mechanisms of blood redistribution, however, the changes were unreliable. Unreliable reduction of pulmonary vessels tonicity with the increase of local blood filling of pulmonary tissue was noted in 10% of the tests (table 3).

Table. 3 Dynamics of the main rates of RG within six-hour hypothermia against the background of immobilization

Rate	Control	1 hour	2 hour	3 hour	4 hour	5 hour	6 hour
As, mOhm	69,0 ±0,8	56,3 ±2,4*	49,8 ±2,0*º	43,4 ±1,6*º	51,6 ±1,8*º	58,4 ±1,7*º	63,2 ±2,5*
E_{max}, ms	80,7 ±0,5	76,3 ±1,8	74,1 ±1,0*	72,2 ±0,9*	73,9 ±1,5*	75,2 ±1,3*	78,8 ±2,0
E_0, ms	194,7 ±1,0	178,1 ±3,0*	162,2 ±2,7*º	158,7 ±1,5*	167,5 ±2,3*	189,8 ±2,1*º	194,3 ±2,6
T, ms	285,7 ±1,0	255,5 ±3,3*	244,8 ±3,5*	243,2 ±5,9*	247,1 ±5,4*	272,3 ±4,7	285,1 ±7,5

Note: * -distinction reliability of rate with the initial p<0,01 level; - distinction reliability of rates with the previous measurement of p<0,01.

The dynamics of morphological changes of lungs tissue of rats was studied on the 5th and10th days from the beginning of the experiment.

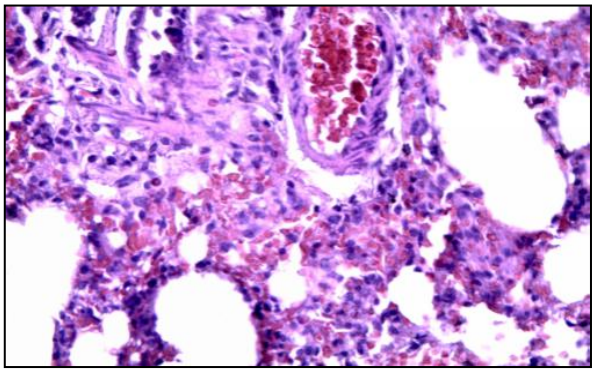

Fig. 1. Lungs tissue of an experimental rat on the 5th day of hypothermal stress against immobilization.
Coloring with hematoxylin and eosin. Enlarging x200.

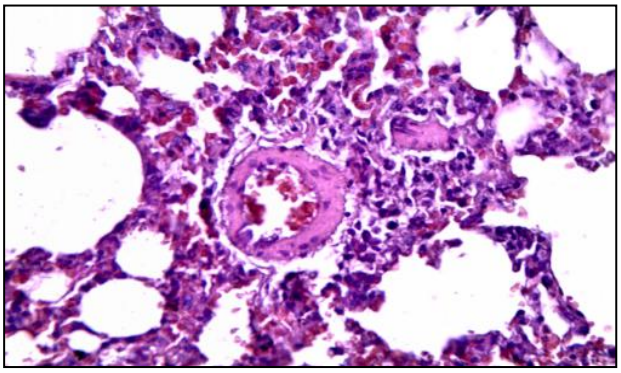

Fig. 2. Lungstissue of an experimental rat on the 5th day of a hypothermal stress against immobilization.
Coloring with hematoxylin and eosin. Enlarging x200.

In the dynamics of experimental observations there were determined characteristics of the progressive increase of morphological features of blood circulation disturbance at the level of the lung's microvasculature of rats from experimental groups. Thus, on the 5th day of the research, in lungs tissue of experimental rats were noted the progression of sharp plethora of capillaries and postcapillary venules with erythrocytes stasis. Also it was noted interalveolar septum edema and diapedetic hemorrhages from vessels of capillary type (Fig. 1).

Against the background of plethora of the microvasculature venous sector was noted a partial spasm and blood deficiency of alveoli, and fibroid swelling- in vascular walls (Fig. 2). Imbalance of microhemocirculation was followed by paretic capillaries

congestion and erythrocytes sludge in vessels bore, progressing of tissue hypoxia (Fig. 3).

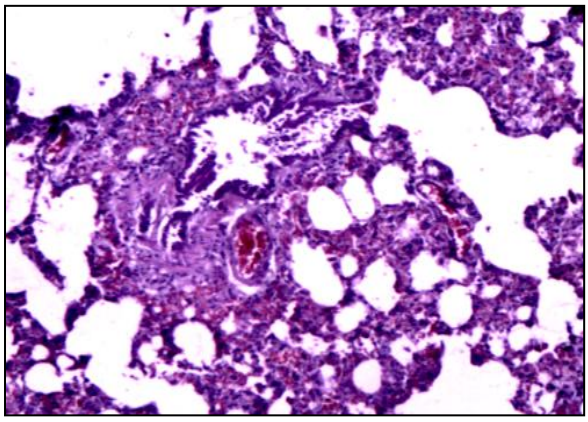

Fig. 3. Lungs tissue of an experimental rat on the 5th day of a hypothermal stress against immobilization. Coloring with hematoxylin and eosin. Enlarging x200.

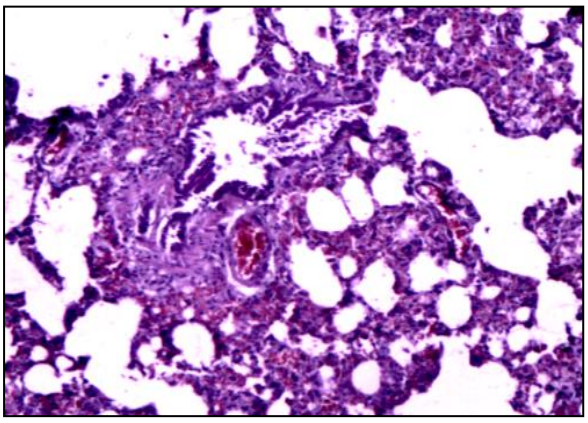

Fig. 4 –Lungs tissue of a rat on the 10th day of hypothermal stress against immobilization. Coloring with hematoxylin and eosin. Enlarging x200

In 10 days from the beginning of experimental observation there remained congestion of vessels microvasculature venous part with hypostasis of interalveolar septum in lungs tissue of the tested rats (Fig. 4).

Along with the pulmonary emphysema, there were observed dystelectasis of pulmonary parenchyma where bores of alveolus had a form of the slit-like alveolar canals, in bores of which were determined desquamated alveolocytes and erythrocytes (Fig. 5).

In the peribronchial spaces were traced the signs of vessels plethora with diapedetic hemorrhages, into peribronchial tissue as well as into bronchial tubes (Fig. 6).

Tissue hypoxia caused on the one hand by microhemocirculation imbalance, and on the other - a bronchospasm with development of dystelectasis and focal emphysema of pulmonary parenchyma, contributed to the progressing increase of permeability of microvessels vascular wall with the following migration of lymphocytes in paravasal spaces from a lymphocytic infiltration of bronchial tubes wall and interalveolar septum (Fig. 7).

CONCLUSIONS

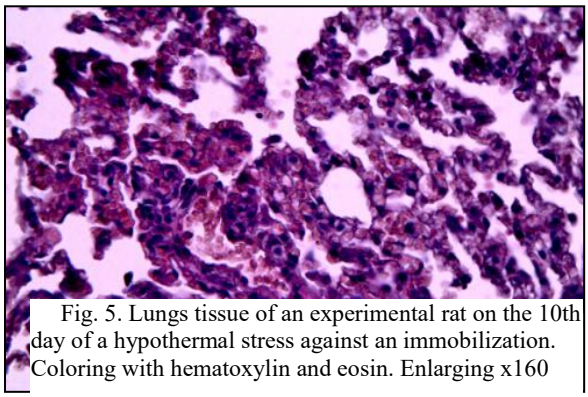

Fig. 5. Lungs tissue of an experimental rat on the 10th day of a hypothermal stress against an immobilization. Coloring with hematoxylin and eosin. Enlarging x160

Thus, with spontaneous respiration in healthy animals, the pulse increment of blood, blood filling, the ratio of blood flow, ventilation and the speed of systolic blood flow to the lungs are maximum at the beginning of inspiration, and then, by the middle of inspiration, these indicators significantly decrease and their slower decline continues until the maximum of inspiration. In the dynamics of exhalation, they begin to slowly increase again, but even at the end of expiration, they remain below those at the beginning of inspiration.

Complex six-hour impact of plethora and immobilization on rats cause unidirectional changes of pulmonary circulation of rats: reduction of blood filling, increase of vessels tonicity of lungs precapillary bed and venous blood congestion in a lesser circulation. Morphological imbalances progress at the level of the microvasculature

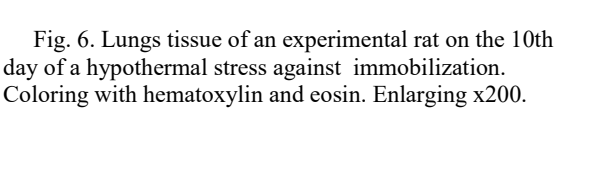

Fig. 6. Lungs tissue of an experimental rat on the 10th day of a hypothermal stress against immobilization. Coloring with hematoxylin and eosin. Enlarging x200.

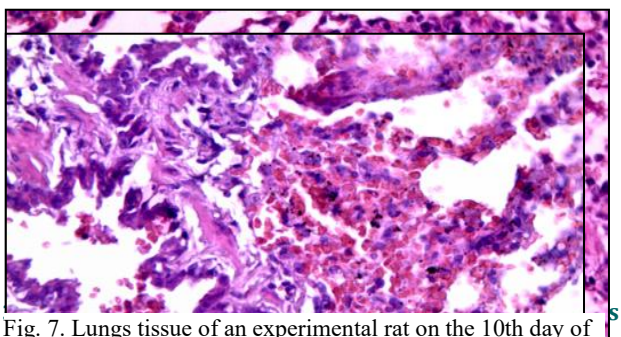

Fig. 7. Lungs tissue of an experimental rat on the 10th day of a hypothermal stress against immobilization. Coloring with hematoxylin and eosin. Enlarging x200.

vessels of lungs tissue and are followed by the appearance of arteriolo spasm signs, plethora of capillaries and post-capillary venules with the sustainable increase of vascular wall permeability. Reactive bronchospasm, by the end of the experiment, leads to the progression of focal emphysema and dystelectasis of pulmonary parenchyma.

The results obtained in this work will help theorists and doctors better understand the mechanisms of changes occurring in organs and tissues under the influence of combined stress during the COVID-19 pandemic. It also will be beneficial to identify the pattern of cardiovascular complications, to develop a risk model for cardiac complications, and to identify and/or predict response to various treatment modalities.

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