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EFFECT OF POST-TRAUMATIC STRESS DISORDER ON THE HEMOMICROCIRCULATORY BED OF RATS' LARGE BRONCHI AND ITS CORRECTION WITH QUERCETIN

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Today, due to the complex political and socio-economic situation, which is burdened by hostilities, the living conditions and health of Ukrainian citizens have become much more complicated, and the number of stressful and traumatic situations has increased, which can lead to the development of post-traumatic stress disorder. It has been experimentally proven that the lungs are an organ that is extremely sensitive to stressful influences, which can lead to haemomicrocirculation disorders and endothelial dysfunction. The paper presents the results of an experimental study of the post-traumatic stress disorder effect on the hemomicrocirculatory bed of the rats' large bronchi and its correction with quercetin. It has been shown that this factor causes significant morphological changes in the vessels of the hemomicrocirculatory bed of the rats' large bronchi, which are manifested by impaired hemomicrocirculation with the development of blood stasis, arteriolar spasm, dilation of metabolic and capacitive hemomicrovessels with the phenomena of red blood cell diapedesis into hyperhydrated and leukocyte-infiltrated perivascular connective tissue. Intraperitoneal administration of water-soluble quercetin complex once a day for seven days significantly reduced the development of structural changes in the vessels of the hemomicrocirculatory bed of rats' large bronchi, which indicates the effectiveness of this agent as a stress protector.

Key words: post-traumatic stress disorder, bronchi, hemomicrocirculatory bed, lungs, morphological changes, structural and metabolic changes, correction.

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ВПЛИВ ПОСТТРАВМАТИЧНОГО СТРЕСОВОГО РОЗЛАДУ НА ГЕМОМІКРОЦИРКУЛЯТОРНЕ РУСЛО ВЕЛИКИХ БРОНХІВ ЩУРІВ ТА ЙОГО КОРЕКЦІЯ КВЕРЦЕТИНОМ

Сьогодні у зв'язку із складними політичною та соціально-економічною ситуаціями, які обтяжені бойовими діями, значно ускладнилися умови життєдіяльності та стан здоров'я громадян України, зросла кількість стресових та травмівних ситуацій, що може спричинити розвиток посттравматичного стресового розладу. Експериментально доведено, що легені є органом, надзвичайно чутливим до стресових впливів, на тлі яких виникають розлади гемомікроциркуляції та прояви ендотеліальної дисфункції. У роботі висвітлені результати експериментального дослідження впливу посттравматичного стресового розладу на гемомікроциркуляторне русло великих бронхів щура та його корекції кверцетином. Показано, що цей чинник викликає суттєві морфологічні зміни у судинах гемомікроциркуляторного русла великих бронхах щурів, які проявляються порушенням гемомікроциркуляції з розвитком явищ стазу крові, спазмуванням артеріол, розширенням обмінних і ємнісних гемомікросудин з явищами діапедезу еритроцитів у гіпергідратовану та інфільтровану лейкоцитами периваскулярну сполучну тканину. Внутрішньоочеревинне введення водорозчинного комплексу кверцетину 1 раз за добу протягом 7-ми діб значно нівелює розвиток структурних змін у судинах гемомікроциркуляторного русла великих бронхів щурів, що свідчить про ефективність цього засобу як стреспротектора.

Ключові слова: посттравматичний стресовий розлад, бронхи, гемомікроциркулярне русло, легені, морфологічні зміни, структурно-метаболічні зміни, корекція.

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In recent decades, society and the world have seen an increase in conflicts and disasters, which undoubtedly have an extreme impact on people, their health and their psyche. For several decades, the development of Ukrainian society has been burdened by large-scale upheavals, reforms, and restructuring of the country's systems. These prolonged transformations have brought about changes and turmoil in all spheres of human activity, constantly causing new stresses that have significantly complicated social and psychological adaptation, reduced the labour activity of the population, and negatively affected the development and well-being of the country. Today, due to the complex political and socio-economic situation, which is burdened by hostilities, the living conditions and health of Ukrainian citizens have become much more complicated, and the number of stressful and traumatic situations has increased [4]. During the full-scale war with the racist invaders, both the military personnel of the Armed Forces of

Ukraine and civilians are exposed to constant chronic stress. During combat operations, soldiers experience stress from performing tasks in extreme conditions when exposed to potent external and internal stressors. They have a negative impact on health, reduce efficiency or lead to disruption of military personnel's activities. Combat stress can contribute to acute psychological reactions and the development of stress disorders [6]. In 25 % of cases, combat stress causes the development of post-traumatic stress disorder (PTSD), and 98 % of combatants need professional help after exposure to combat stress [1]. It is now well known that vegetative stress reactions can cause several "stress-related diseases" when the body's adaptive capacities are disrupted [2]. For example, the lungs are susceptible to stress, with significant morphological and functional changes, including haemomicrocirculatory disorders, beginning to occur against the background of activation of lipid peroxidation [15]. Current research suggests a significant link between PTSD and endothelial dysfunction, which contributes to the development and progression of cardiovascular disease [9, 10]. Therefore, given the realities of today, a comprehensive study of the pathogenesis, clinical manifestations, diagnosis, prevention and treatment of PTSD, despite numerous studies in this area, continues to be highly relevant today [5, 7, 9–13].

The purpose of the study was to determine the morphological changes that occur in the hemomicrocirculatory bed of the rats' large bronchi on the background of post-traumatic stress disorder and their correction with quercetin.

Materials and methods. The study included 30 white, purebred male rats weighing 240–260 grams, aged 8–10 months.

The control group comprised ten intact animals housed in the university vivarium under standard conditions. The first experimental group included ten rats in which the PTSD model was induced. The second experimental group, consisting of ten animals, underwent a similar PTSD model induction with quercetin administration.

To model PTSD, we used the effects of several stressors following a multimodal protocol, as described in our previous work and the works of other authors [5, 7, 11]. To administer the correction, rats in the second experimental group received intraperitoneal injections of a water-soluble quercetin complex with polyvinylpyrrolidone, dosed at 100 mg/kg (equivalent to 10 mg/kg of quercetin) once daily for seven consecutive days.

Rats were euthanised under ether anaesthesia by decapitation on an empty stomach.

Histological specimens for light microscopy were stained with haematoxylin and eosin, and semi-thin sections were stained with 0.1 % toluidine blue solution.

The microscopic sections were examined using a microscope and a Vision CCD camera, the images of which were displayed on a computer monitor. Morphometric studies were performed using VideoTest-5.0, KAAPA Image Base, and Microsoft Excel. Digital data were statistically processed using Statistica 10, BiostatPro 6, and Microsoft Excel 2019.

The experimental part of the study adhered to the standards outlined in the international principles of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1985), as well as the applicable legislation in Ukraine, specifically the law "On the Protection of Animals from Cruelty" (№ 3446-IV of 21.02.2006, Kyiv).

Results of the study and their discussion. The haemomicrocirculatory bed of the rats' large bronchi is formed by a system of small vessels: arterioles, capillaries, and venules. Arterioles are the smallest arterial vessels of the muscle type, the walls of which are formed by three poorly defined layers: endothelial, muscular and outer, formed by a thin strip of loose fibrous connective tissue. The results of the morphometric study showed that the average diameter of the arteriolar lumen in the rat bronchi averaged (9.81±0.89) μm.

The inner layer of the arteriole is formed by endothelial cells, a basement membrane, a thin subendothelial layer, and a thin inner elastic membrane. The middle layer consists of 1–2 circular layers of smooth muscle cells; larger arterioles have an outer elastic membrane.

The most numerous vessels of the hemomicrocirculatory bed of the rat bronchi are capillaries, which form dense networks. Red blood cells are arranged in a single row in the bronchial capillaries since their internal diameter is typically 2.51±0.21 μm. The inside of the capillaries is lined with a continuous layer of endothelial cells placed on the basement membrane. Endothelial cells have a flattened shape, mostly rounded or oval nuclei, sometimes with irregular contours. The basement membrane is thin, with pericytes adjacent to its clefts, forming the capillary wall's outer layer.

At their venous end, capillaries pass into postcapillary venules, characterised by an increase in the number of pericytes with increasing diameter. These venules flow into the collecting venules, which, in addition to pericytes, have an outer layer of fibroblasts and collagen fibres. The diameter of the venous lumen usually is 10.23±0.57 μm. The inside of the venules is lined with endothelial cells, which in some

places are tightly adherent, and gaps are formed between their membranes in some areas. Immediately behind the endothelium is the basement membrane, along or around which pericytes are located (Fig. 1).

In rats, after reproduction of the experimental model of PTSD, blood stasis was determined in all links of the bronchial haemomicrocirculatory bed. Their lumens were filled with erythrocytes. The arterioles were spasmodic, their elastic membrane had an uneven course, and endothelial cells exploded into the lumens of the vessels. The wall of the capacitive vessels was thinned, and the basement membrane was visualized as a continuous thin basophilic strip. The perivascular connective tissue was hyperhydrated and infiltrated with leukocytes, and foci of erythrocyte diapedema from dilated metabolic and capacitive haemomicro-vessels were detected. It was visualized that the lumens of the large bronchi were spasmodic with partial mucosal detachment and destruction of the muscular lamina propria (Fig. 2).

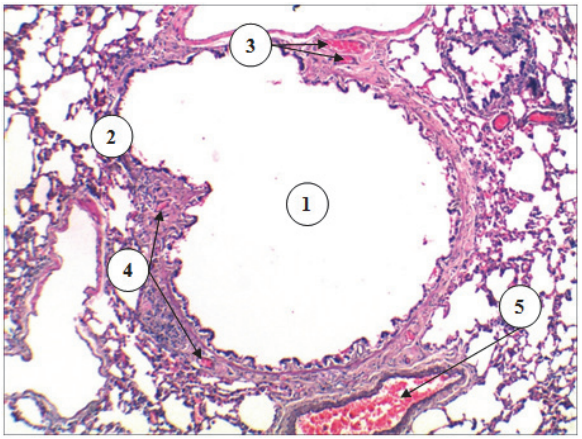

Fig. 1. Hemomicrocirculatory bed of large bronchi of white rats in the control group. Paraffin section. Hematoxylin and eosin staining. Magnification: okh. 10, obh. 10. 1. Lumen of large bronchus; 2. Wall of large bronchus; 3. Bronchial arterioles; 4. Bronchial capillaries; 5. Bronchial venules.

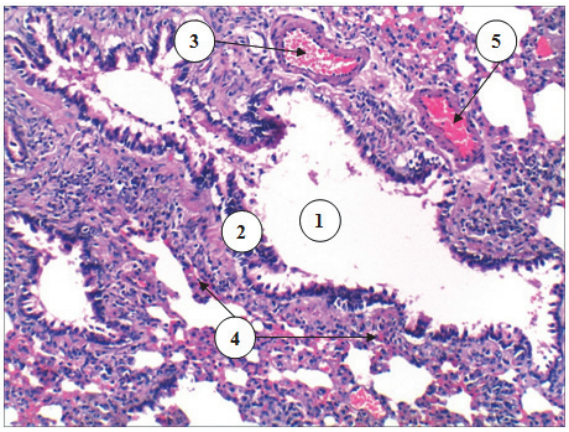

Fig. 2. Morphological and hemodynamic changes in the elements of the hemomicrocirculatory bed of large bronchi of white rats after modeling of experimental PTSD. Paraffin section. Hematoxylin and eosin staining. Magnification: okh. 10; obh. 40. 1. Lumen of large bronchus; 2. Wall of large bronchus with destruction of mucosa; 3. Bronchial arterioles; 4. Bronchial capillaries; 5. Bronchial venules.

The results of the morphometric study on the background of PTSD indicate that the diameter of the lumen of arterioles decreased insignificantly by 10.2 % (from 9.81±0.89 to 8.81±1.47), the average values of capillary lumen diameter increased by 46.6 % (from 2.51±0.21 to 3.68±0.41, $p<0.01$), venular lumen diameter increased by 11.3 % (from 10.23±0.57 to 12.02±0.71, $p<0.01$).

Against the background of quercetin correction, post-stress disorders in the haemomicrocirculatory bed of the large bronchi were less defined. In some vessels of the haemomicrocirculatory bed, blood stasis phenomena continued to be determined. Perivascularly, hyperhydration of connective tissue, minor leukocyte infiltrates and foci of erythrocyte diapedema from dilated metabolic and capacitive haemomicro-vessels were detected in some places. There was an increase in the internal diameter of capillaries and venules compared to the control group, but these values were lower compared to animals exposed to PTSD without correction. Thus, the average value of the diameter of the capillary lumen increased by 18.5 % compared to the control group (from 2.51±0.21 to 3.08±0.41, $p<0.01$), but with correction, it was less than without it by 16.3 %, at $p<0.01$. The average value of the diameter of the venous lumen also increased by 8.7 % compared with the control group (from 10.23±0.57 to 11.12±0.37, $p<0.05$), but with correction, it was less than without it by 8.09 %, $p<0.05$. The diameter of the internal lumen of the arterioles was 9.45±0.99 and generally changed insignificantly: compared to the control group, it was smaller, but its value was slightly improved compared to animals that did not receive pharmacocorrection (Fig. 3).

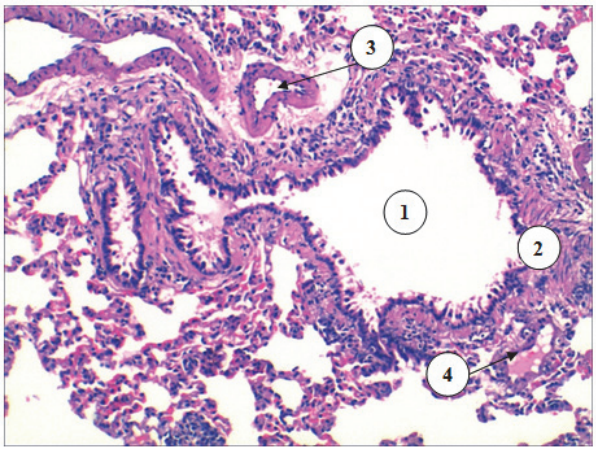

Fig. 3. Morphological and hemodynamic changes in the elements of the hemomicrocirculatory bed of large bronchi of white rats after modeling of experimental PTSD and pharmacocorrection. Paraffin section. Hematoxylin and eosin staining. Magnification: okh. 10, obh. 10. 1. Lumen of the large bronchus; 2. Wall of the large bronchus with restored structure; 3. Bronchial arterioles; 4. Bronchial capillaries.

Thus, intraperitoneal administration of a water-soluble quercetin complex with polyvinylpyrrolidone at a dose of 100 mg/kg (10 mg/kg in terms of quercetin) once daily for seven days has a significant stress-protective effect in rats exposed to an experimental model of PTSD reproduced following a multimodal protocol [5, 7, 11]. PTSD in the hemomicrocirculatory bed of the large bronchi causes significant morphological changes, in particular, blood stasis in all parts of the hemomicrocirculatory bed of the large bronchi, arteriolar spasm, dilation of metabolic and capacitive hemomicrovessels with the phenomena of red blood cell diapedesis into hyperhydrated and leukocyte-infiltrated perivascular connective tissue. Pharmacocorrection significantly eliminates haemodynamic disorders in the vessels of the microcirculatory bed, reducing the manifestations of blood stasis, perivascular hyperhydration of connective tissue, leukocyte infiltration and red blood cell diapedesis from dilated metabolic and capacitive haemomicro-vessels. In comparison with the control group, an increase in the internal diameter of capillaries and venules and arteriolar spasm was also observed, but these changes were less pronounced compared to animals exposed to PTSD without quercetin administration. The results obtained are entirely consistent with the data of other studies [3, 7, 8, 14]. They, therefore, can be used in the development of new methods of prevention and treatment of PTSD, which is currently an urgent medical and social problem [5, 7, 9–13].

Conclusions

1. PTSD causes significant morphological changes in the vessels of the hemomicrocirculatory bed of the rats' large bronchi, which are manifested by impaired hemomicrocirculation with the development of blood stasis, arteriolar spasm, dilation of metabolic and capacitive hemomicrovessels with the phenomena of erythrocyte diapedesis into hyperhydrated and leukocyte-infiltrated perivascular connective tissue.

2. Intraperitoneal administration of water-soluble quercetin complex once a day for seven days to rats exposed to the experimental model of PTSD significantly eliminates morphological changes in the vessels of the hemomicrocirculatory bed of rats' large bronchi, which indicates the effectiveness of this agent as a stress protector.

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