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MORPHOLOGICAL CHARACTERISTICS OF RAT BRONCHI AGAINST THE BACKGROUND OF POST-TRAUMATIC STRESS DISORDER AND AFTER QUERCETIN CORRECTION

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War affects the daily lives of the vast majority of Ukrainians. Therefore, post-traumatic stress disorder has become one of the most pressing medical and social problems in Ukraine today. It is characterised by a complex pathogenesis and comorbidity with many other diseases. Studying models of post-traumatic stress disorder in rats, its impact on various organs and systems, as well as finding ways to correct it are crucial for restoring the health of victims. The work presents the results of a morphological study of the effect of post-traumatic stress disorder on the rat bronchi and its correction with quercetin. It has been established that this disorder causes significant destructive changes in the large bronchi of rats, in particular, desquamation of epithelial cells with the formation of cellular detritus in the bronchial lumen, hyperhydration and leukocyte infiltration of the mucosa and submucosa, and haemomicrocirculation disorders. Intraperitoneal injection of water-soluble quercetin complex once a day for seven days in rats significantly reduced the damage to the large bronchi against the background of post-stress disorder, indicating this agent's effectiveness as a stress protector.

Key words: post-traumatic stress disorder, bronchi, lungs, histological changes, morphological changes, correction.

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МОРФОЛОГІЧНА ХАРАКТЕРИСТИКА ВЕЛИКИХ БРОНХІВ ЩУРІВ НА ТЛІ ПОСТТРАВМАТИЧНОГО СТРЕСОВОГО РОЗЛАДУ ТА ПІСЛЯ КОРЕКЦІЇ КВЕРЦЕТИНОМ

Війна впливає на повсякденне життя абсолютної більшості українців. Тому нині в Україні посттравматичний стресовий розлад став однією з найактуальніших медико-соціальних проблем. Він характеризується складним патогенезом та коморбідністю з багатьма іншими захворюваннями. Вивчення моделей посттравматичного стресового розладу на щурах, його впливу на різні органи та системи, а також пошук шляхів корекції мають вирішальне значення для відновлення здоров'я постраждалих. У роботі представлені результати морфологічного дослідження впливу посттравматичного стресового розладу на бронхи щура та його корекції кверцетином. Встановлено, що цей розлад викликає суттєві деструктивні зміни у великих бронхах щурів, зокрема, десквамацію епітеліоцитів з утворенням у просвітах бронхів клітинного детриту, гіпергідратацію та лейкоцитарну інфільтрацію слизової оболонки та підслизової основи, розлади гемомікроциркуляції. Внутрішньоочеревинне введення водорозчинного комплексу кверцетину 1 раз за добу протягом 7-ми діб щурам, значно нівелює ураження великих бронхів на тлі постстресового розладу, що свідчить про ефективність цього засобу як стреспротектора.

Ключові слова: посттравматичний стресовий розлад, бронхи, легені, гістологічні зміни, морфологічні зміни, корекція.

The work is a fragment of the research project "Regularities of the morphogenesis of organs, tissues and vascular and nervous formations in normal, pathological and under the influence of exogenous factors", state registration No. 0118U004457.

The Russian invasion of Ukraine has affected the daily life of all segments of the population of our country [10]. Both members of the Armed Forces of Ukraine and civilians are exposed to constant chronic stress. Military personnel experience combat stress as a result of operating in extreme conditions, which is accompanied by potent external and internal stressors. They threaten a person's life, adversely affect their health, reduce performance or lead to disruption. Combat stress can lead to acute psychological reactions and the development of stress disorders [9]. Combat stress is an integral part of the psychological trauma

of combatants and depends on individual-typological, individual-psychological, situational-environmental, socio-political and socio-psychological factors [7]. In 25 % of cases, combat stress later transforms into post-traumatic stress disorder (PTSD); 98 % of combatants need qualified support and assistance after exposure to combat stressors [2].

PTSD is a non-psychotic delayed reaction to traumatic stress. It occurs in people who have experienced an extreme psychological and/or physical shock and perceive it as particularly painful [3, 12]. PTSD is characterised by a complex pathogenesis and comorbidity with many other diseases and, therefore, poses a serious challenge to modern medicine. The autonomic subsyndrome is one of the four types of body functional states observed against the background of stress. The activity of the autonomic nervous system is not controlled by consciousness, so adaptive autonomic reactions are triggered regardless of the type of stressor. Manifestations of the autonomic subsyndrome of stress include increased cardiovascular tone, activation of phagocytosis and tissue regeneration, thrombocytosis, sweating, vomiting, etc. Under the powerful influence of stress factors mentioned above, local autonomic reactions can lead to the development of "stress diseases" [4]. In particular, significant morphological and functional changes occur in the lungs, where destruction, haemorrhagic events, and haemomicrocirculatory disorders are observed against the background of acute immobilisation stress [5, 15]. Preventing the onset and development of PTSD and finding ways to correct the disorders observed against its background is now crucial for the restoration and preservation of the health of victims. Experimental studies previously conducted in this area are now attracting attention again [13, 14]. There is also an urgent need to conduct new comprehensive studies of PTSD. Due to ethical issues, rat studies are crucial for the experimental research of PTSD models to understand its induction, development and pathogenesis, as well as to test and study the therapeutic properties of pharmaceuticals and other therapeutic agents [1]. Among the natural chemical compounds that can positively affect the course of PTSD (antioxidant, anti-inflammatory and immunomodulatory effects), polyphenols are increasingly attracting the attention of scientists [5, 8].

The purpose of the study was to investigate the effect of post-traumatic stress disorder on the rat bronchi and its correction with quercetin at the morphological level.

Materials and methods. The experiment was performed on 30 white, purebred male rats weighing 240–260 grams, aged 8–10 months.

The control group consisted of 10 intact animals kept in standard conditions of the university vivarium. The first experimental group consisted of 10 rats in which the PTSD model was reproduced. Rats of the second experimental group (10 animals) were exposed to a similar model of PTSD against the background of quercetin correction.

The PTSD model [6, 11] combined immobilisation of rats for 2 hours by fixing their limbs with surgical tape with restriction of head movement, forced swimming in fresh water at 24°C, drying with a hairdryer and, after a 15-minute rest, exposure to ether vapour until unconsciousness. Afterwards, all rats were placed in two cages and left alone for seven days. For correction, rats of the second experimental group were injected intraperitoneally with a water-soluble quercetin complex with polyvinylpyrrolidone at a dose of 100 mg/kg (10 mg/kg in terms of quercetin) once a day for seven days.

Fasting animals were euthanised by decapitation under ether anaesthesia.

Microsections for light microscopy were stained with hematoxylin and eosin. Semi-thin sections were stained with 0.1 % toluidine blue solution.

Images of histological specimens were displayed on a computer monitor using a microscope and a Vision CCD camera. Morphometric studies were performed on a personal computer using VideoTest-5.0, KAAPA Image Base and Microsoft Excel. Statistica 10, BiostatPro 6, and Microsoft Excel 2019 software were used to statistically process the data obtained.

The entire experimental part of the study was conducted following the requirements of the international principles of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1985) and the relevant law of Ukraine "On the Protection of Animals from Cruelty" (No. 3446-IV of 21.02.2006, Kyiv).

Results of the study and their discussion. The rat's respiratory system consists of the nose, pharynx, larynx, trachea, bronchi and lungs. The right and left main bronchi begin at the bifurcation of the trachea and head to the respective lungs. The left bronchus enters the lung, where it divides into segmental bronchi. The cranial partial bronchus extends from the right main bronchus immediately at the bifurcation of the trachea. The right middle, caudal, and accessory partial bronchi branch off the main bronchus. Numerous segmental bronchi extend from each partial bronchus; further bronchi division is not of the main type but of the dichotomous type. The set of bronchi and their branches in the lung form the bronchial tree. The wall of the

large bronchi consists of four membranes: mucosa, submucosa, fibrocartilaginous layer and adventitia. As the bronchial calibre decreases in the fibrocartilaginous layer, the cartilage is smaller and less extensive around the circumference of the bronchial walls. The mucous membrane of the large bronchi is lined with a single-layer, multi-row ciliated epithelium consisting of epithelial cells of different structures and functional purposes. Epithelial cells of the mucous membrane have different heights. Their nuclei lie at various levels, forming several rows. In addition to ciliated cells, there are goblet cells, endocrine cells and basal cells among the epithelial cells. Ciliated cells have ciliated cilia that help to remove mucus and dust particles from the bronchial lumen. Between the ciliated cells are goblet cells that secrete mucous secretions. Neuroendocrine cells are few in number, arranged singly, and contain small, dense granules in the cytoplasm. Basal or cambial cells are cubic, with an optically dense nucleus and a small amount of weakly basophilic cytoplasm. They have retained the ability to mitotic division and are located in the basal layer.

The lamina propria, on which the epithelial layer is located, is compacted; the basal membrane is clearly contoured, and the middle part of the lamina propria contains a large number of longitudinal elastic fibres, which provide the bronchi with the ability to stretch.

At the boundary of the mucosa and submucosa lies the muscular lamina, formed by two oblique-circular bundles of smooth muscle cells that descend along the bronchus in the form of two oppositely twisted spirals. As the bronchial calibre decreases, its muscular lamina thickens. In addition, lymphocytes and individual lymphoid nodules are detected in the subepithelial zone of the mucosa.

The submucosal connective tissue base of the middle bronchi contains the terminal sections of mixed mucoprotein glands. The excretory ducts of these glands open on the surface of the epithelium; their secretion moisturises the mucosa and helps to remove foreign particles from the bronchial lumen.

The fibrocartilaginous layer is formed by cartilaginous laminae and islands of elastic cartilaginous tissue. The cartilaginous laminae are crescentic or oval in cross-sectional views of the middle bronchi. The spaces between the cartilage are filled with connective tissue. Cellular elements are few in number, with a predominant fibrous component. The outer adventitial layer is formed by fibrous connective tissue, which passes into the interlobular tissue of the lung (Fig. 1).

In rats with PTSD exposure, histological examination revealed destructive changes in the large bronchi, which were manifested by vacuolation of the epithelial cytoplasm, destruction of intercellular contacts, loss of epithelial layer integrity and epithelial cell desquamation. Foci of bronchial epithelial proliferation were locally detected. In the lumens of the bronchi, there were exfoliated cells in the form of detritus and erythrocytes. The connective tissue of the lamina propria showed signs of hyperhydration and lympho-leukocytic infiltrates. The muscular lamina of the bronchial mucosa was thickened with signs of oedema. Numerous leukocyte clusters were detected in the submucosa, where lymphocytes and plasmocytes predominated (Fig. 2).

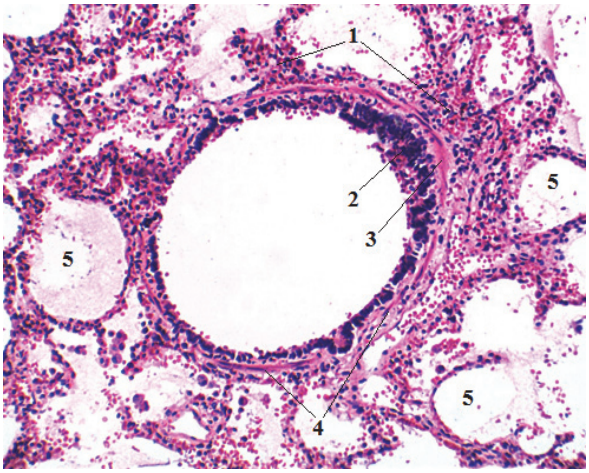

Fig. 1. Morphology of the middle bronchus of rats in the control group. Hematoxylin and eosin staining. Magnification: ×400. 1. Glandular component; 2. Mucosa; 3. Smooth myocytes; 4. Fibrocartilaginous layer; 5. Alveoli.

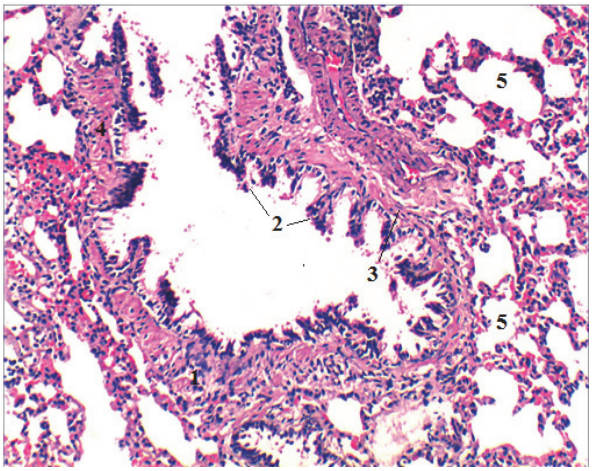

Fig. 2. Remodelling of structural components of the rat lung under the influence of PTSD. Hematoxylin and eosin staining. Magnification: ×400. 1. Glandular component; 2. Mucous membrane; 3. Smooth myocytes; 4. Fibrocartilaginous layer; 5. Alveoli.

In the peribronchial connective tissue, blood stasis was detected in haemomicro-vessels, the lumens of which were densely filled with erythrocytes. The perivascular connective tissue was hyperhydrated, with foci of erythrocyte diapedema, leukocyte infiltrates, and the presence of a large number of interstitial macrophages. The histological specimens showed that the bronchial lumen was filled with optically dense mucus (Fig. 3).

The morphometric studies show that against the background of PTSD, the diameter of the lumen of the large bronchi decreased by 1.75 times, the mucous membrane thickened by 4.35 times, and the overall thickness of the bronchial wall increased by 2.31 times ($p<0.05$).

Against the background of intraperitoneal injection of a water-soluble quercetin complex with polyvinylpyrrolidone, focal destructive changes were also observed in the large bronchi of rats after exposure to the experimental model of PTSD. However, the disruption of the integrity of the epithelial layer was less pronounced, and single exfoliated epithelial cells, erythrocytes, and mucus were found in the bronchial lumen. The manifestations of hyperhydration and lympho-leukocyte infiltration of the bronchial mucosa in rats with pharmacocorrection were less than in those that did not receive quercetin. In the submucosa, there were few foci of minor leukocyte accumulation. In the vessels of the haemomicrocirculatory bed of the peribronchial connective tissue, erythrocyte aggregation was sometimes detected, but these manifestations were less pronounced after correction (Fig. 4).

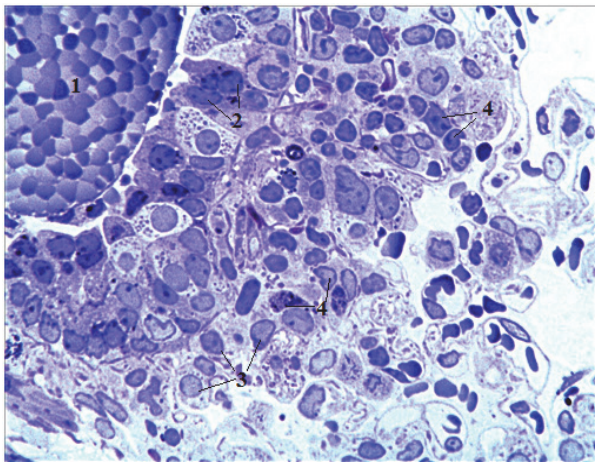

Fig. 3. Remodelling of structural components of peribronchial connective tissue in rat lungs under the influence of PTSD. Toluidine blue staining. Magnification: ×1000. 1. Optically dense mucus. 2. Goblet cells. 3. Leukocyte cells. 4. Interstitial macrophages.

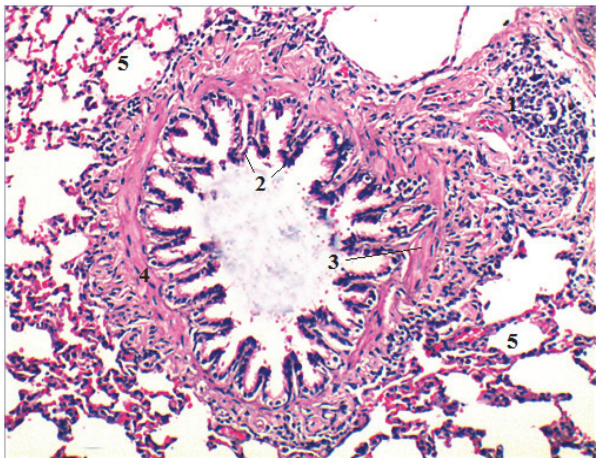

Fig. 4. Remodelling of lung structural components in rats under the influence of PTSD and administration of water-soluble quercetin complex with polyvinylpyrrolidone. Hematoxylin and eosin staining. Magnification: ×400. 1. Glandular component; 2. Mucous membrane; 3. Smooth myocytes; 4. Fibrocartilaginous layer; 5. Alveoli.

The morphometric studies show that against the background of PTSD correction by the introduction of a water-soluble quercetin complex with polyvinylpyrrolidone, the diameter of the lumen of large bronchi decreased only 1.37 times, the mucous membrane thickened 2.5 times, and the overall thickness of the bronchial wall increased 1.42 times ($p<0.05$).

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 in rats exposed to an experimental model of PTSD reproduced following the multimodal protocol [6, 11] has a significant stress-protective effect. PTSD at the morphological level in large bronchi causes significant destructive changes, in particular, destruction of intercellular contacts, loss of integrity of the epithelial layer, desquamation of epithelial cells with the formation of cellular detritus and accumulations of erythrocytes in the bronchial lumen, hyperhydration and leukocyte infiltration occur in the mucosa and submucosa. Blood stasis is detected in the vessels of the haemomicrocirculatory bed. Morphometric studies confirm that against the background of PTSD, the diameter of the lumen of large bronchi decreased by 42.86 %, the thickness of the mucous membrane increased by 335.39 %, and the bronchial wall thickened by 130.56 % ($p<0.05$). Against the background of pharmacocorrection with quercetin, the manifestations of PTSD in the bronchi of rats are reduced significantly (focal destructive changes, haemomicrocirculation disorders, hyperhydration and lymph-leukocyte infiltration of the mucous membrane are less defined, according to morphometry, the diameter of the lumen of the large bronchi decreased by only 27 %, the thickness of the mucous membrane increased by only 150.03 %, and the bronchial wall thickened by 42.01 % ($p<0.05$)), which indicates the effectiveness of this product as a stress protector. The obtained results are entirely consistent with the data of other studies [5, 8]. 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 [3, 4, 12].

Conclusions

1. PTSD causes significant destructive changes in the large bronchi of rats, which are manifested by desquamation of epithelial cells with the formation of cellular detritus in the bronchial lumen, hyperhydration and leukocyte infiltration of the mucosa and submucosa, and blood stasis in the vessels of the haemomicrocirculatory bed.

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

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