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ULTRASTRUCTURAL CHANGES IN THE LUNGS OF 1-2 MONTHS-OLD RATS IN THE CONDITIONS OF HYPERHOMOCYSTEINEMIA

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A detailed electron microscopic study on structural components in the lungs of 1-2 months-old rats under the conditions of hyperhomocysteinemia was performed. Experiments were performed on 64 white nonlinear male rats. Attention is drawn to the presence of large amount of fibrin and erythrocytes in the lumen of the alveoli surrounded by an electron-dense substrate, which is similar to the blood plasma consistency, which is obviously due to the surfactant film thinning in the alveoli and the increase in fluid transduction from the capillaries placed in the interalveolar septum. Specific features of the study were the presence of transudate in the alveolar space containing blood plasma proteins and fibrin fibers, sludge phenomenon, which prevented blood flow and edema of respiratory alveolocytes. In young animals, changes occur in the blood-air barrier. They are associated with endothelial dysfunction, which in turn is due to local endothelial cells desquamation, changes in the structure of the basement membranes, their loosening, as a result of their layered structure disturbance and obturation of the capillaries' lumen by the blood formed elements. Damage of the endothelial lining integrity leads to permeation of plasma, fibrin and formed elements into the lumen of the alveoli. The process is accompanied by edema of the respiratory epithelium, which is the result of increased vascular permeability and intravascular pressure.

Keywords: homocysteine, hyperhomocysteinemia, lungs, alveolocytes, mitochondria.

The work is a fragment of the research project "The role of exogenous and endogenous sulfur-containing compounds in the mechanisms of affection of internal organs and cytoprotection under various pathological conditions", state registration No. 0119U001142.

Homocysteine is a non-proteinogenic sulfur-containing amino acid formed as a result of the metabolism of an essential amino acid of methionine [11]. Normally, its amount in the human body is ranging from 5 to 15 μmol /L. An elevated homocysteine level in blood plasma above 15 μmol / L is called hyperhomocysteinemia syndrome and is dangerous to the human body [3, 12]. The relationship between disorders in the homocysteine metabolism and numerous pathological conditions remains inadequately studied in our time. Studies of recent years confirm that increased concentration of homocysteine in the blood is an independent risk factor for cardiovascular and cerebrovascular diseases [6, 10]. There is also a strong correlation between hyperhomocysteinemia and neurological disorders, chronic kidney disease, osteoporosis, disorders in the gastrointestinal tract, cancer, congenital defects [5].

However, the relationship between the high concentration of plasma homocysteine and the structure and functions of the respiratory system remains inadequately studied. Existing researches do not fully disclose morphological changes in the structure of the lungs at the optical and electron-microscopic levels.

The **purpose** of the study was to establish ultrastructural changes in the lungs of 1-2 months-old rats under the conditions of hyperhomocysteinemia and to compare the findings to the group of intact rats.

Materials and methods. Experiments were carried out on 22 white nonlinear male rats at the age of 1-2 months. During the experiment animals are divided into two groups – the control (11 animals) and the experimental (11 animals) group [4, 7]. The simulation of the stable hyperhomocysteinemia state was achieved by administering to the rats a research group of thiolactone homocysteine in the dose of 200 mg / kg of body weight intragastrically for 60 days [8]. Animals were sacrificed by decapitation under thiopental anesthesia. The extracted pieces of lungs sizing 0.5-1 mm were fixed in 2.5% glutaraldehyde solution with the pH7.2-7.4 phosphate buffer. It was embedded into a mixture of epon-araldit, according to the generally accepted method [2]. Thinwalled sections were made of the obtained blocks, and then stained with toluidine blue and Hayat. After focusing on the thinwalled sections, ultrathin sections were made using LKB III (Sweden) and Reihart (Austria) ultramicrotomes, which were contrasted with 2% solution of uranyl acetate and lead citrate. The sections were studied and photographed under the PEM-125K electron microscope with magnitudes of 6-20 thousand times.

Results of the study and their discussion. In the parenchyma of young animals' lungs, under the conditions of hyperhomocysteinemia, changes in both alveoli and blood capillaries were observed. In alveoli, almost all respiratory alveolocytes (pneumocytes I) are swollen. Against the background of blisters

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with edema fluid, in other areas, there was a significant thinning of the cytoplasm (fig. 1 A, B; fig. 2 A) compared to the control group (fig. 2A). Significant thinning led to a decrease in the number of organelles at the metabolic level, in particular, mitochondria lost crysts and were swollen.

Our results are consistent with the data of D.V. Medvedev's et al. study on metabolic changes in the lungs mitochondria with experimental hyperhomocysteinemia in rats. In the conditions of severe hyperhomocysteinemia, homocysteine accumulates in mitochondria and has a direct toxic effect on them. This fact can be due to the presence of a special S-adenosylmethionine transporter on the mitochondria internal membrane, which carries the latter into the matrix of organelles [9].

There was a sharp thinning of epithelial lining, which led to the protrusion of respiratory epithelial cells, their rupture, and facilitated permeation of blood plasma with protein conglomerates of homocysteine into the lumen of the alveoli (fig. 1A, B, C, D). Between respiratory epitheliocytes secretory cells (pneumocytes II) are detected that produce surfactant. We have found that the number of plate cells contained in it (the membrane and the liquid phase, respectively: phospholipids, proteins and glycoproteins) was twice lower than that in the control group rats (experimental animals - 0.2 in 1 μm; control animals - 0.4 in 1 μm). The foregoing facts about the damage of the blood-air barrier in the conditions of hyperhomocysteinemia are described by us for the first time, there are no data about it in the scientific literature.

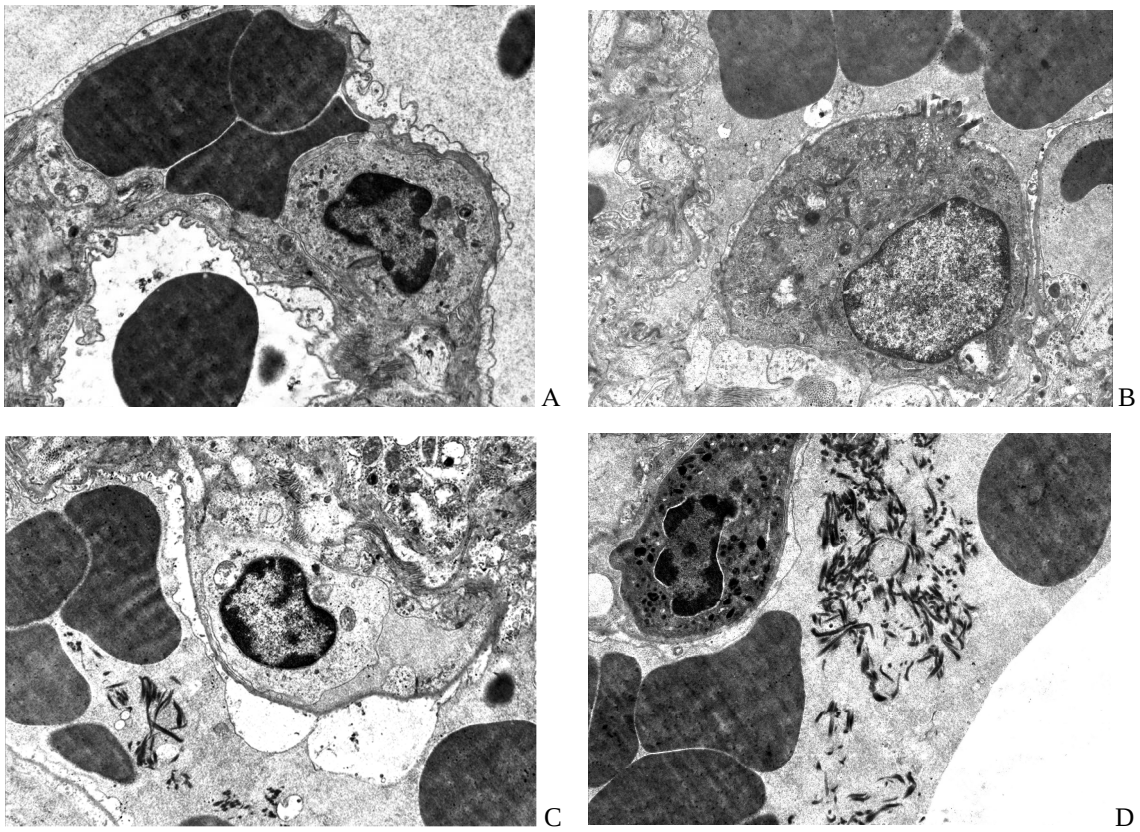

Fig. 1. Respiratory portion of lungs of young rats under conditions of hyperhomocysteinemia: swelling of the cytoplasm of respiratory alveolocytes, red blood cells in the lumen of the alveoli, lymphocytes and neutrophils in the lumen of capillaries; alveolar macrophage, fibrin and blood plasma in the lumen of the alveoli, mitochondria, nucleus. × A- 16000; B - 14000, C-12000; D – 12000 magnification.

In most cells, plate bodies are similar to vacuoles, in which electron-dense substrate is located, formed as a result of the plates disaggregation (fig. 2B). The mitochondria of the secretory cells were observed in small numbers with slight destruction of crysts (fig. 2B). In addition to the respiratory and secretory alveolocytes, there were “wandering” macrophages in the lumen of the alveoli and placed in the wall of the alveoli.The latter, like the secretory cells, had a little amount of small mitochondria with destructively altered crysts and electron-light matrix, microvilli, and protrusion of cytoplasm, which number is significantly increased in experimental animals. The content of these protrusions was electron-dense mass similar to the blood plasma and it was detected in the cytoplasm of the respiratory and secretory cells. (fig. 2A, B). Obviously, transudate from the lumen of capillaries permeates not only through intercellular contacts, but also through the cytoplasm of all cells lining the alveolus.

Attention is drawn to the presence of large amount of fibrin and erythrocytes in the lumen of the alveoli surrounded by electron-dense substrate, which is similar in its consistency to the blood

plasma, which is obviously due to the thinning of the surfactant layer in the alveoli and to the increase in the fluid transduction from the capillaries placed in the interalveolar septum (fig. 1A, B; 2A, B). In this case, in the lumen of capillaries blood plasma has a similar consistency and contains neutrophils, lymphocytes and erythrocytes. Cytoplasm of the endothelial cell is considerably thin, especially in the peripheral zone, which leads to thinning of the blood-air barrier, which can be considered as a manifestation of compensatory and adaptive processes (fig. 1, 2). Lumen of the capillaries, as a rule, is plugged with formed blood cells (erythrocytes, macrophages, lymphocytes, neutrophils). An increase in the content of formed elements and transudate in the alveoli led to the decline of the lumen in some capillaries.

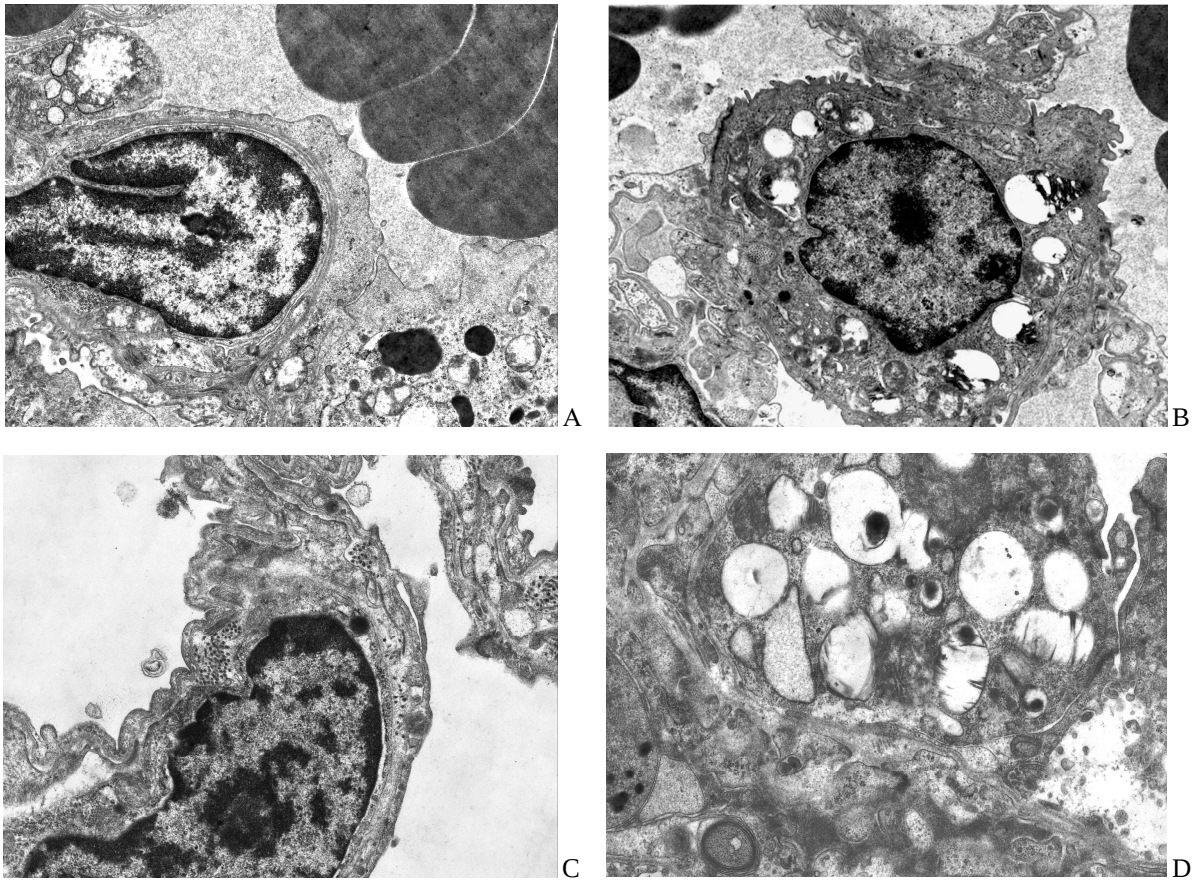

Fig. 2. The respiratory portion of the lungs of young rats under conditions of hyperhomocysteinemia: blood plasma in the lumen of the alveoli, red blood cells in the lumen of the alveoli, secretory alveolocyte, lumbar corpuscles, mitochondria, nucleus cytoplasm. B, G – Respiratory portion of lungs of young control rats. × A-16000; B - 14000; C - 12000; D -16000 magnification.

The foregoing results of our work coincide with the studies of L. P. Voroninet al: significant changes in the vascular epithelium in patients with bronchial asthma under the conditions of hyperhomocysteinemia are determined, while the severity of endothelial dysfunction strongly correlates with the level of homocysteine in the blood plasma [1].

Specific features for this term of the study were the presence of transudates in the alveolar space containing blood plasma proteins and fibrin fibers, slag phenomenon, which prevented blood flow and edema of respiratory alveolocytes.

Conclusion

In young animals, primarily, changes occur in the blood-air barrier. They are associated with endothelial dysfunction, which in turn is due to local desquamation of the endothelial cells, changes in the structure of the basement membranes, their loosening, as a result of their layered structure disturbance and obturation of the capillaries' lumen by the formed elements. Damage to the integrity of endothelial lining leads to permeation of plasma, fibrin and formed elements into the lumen of the alveoli. Secondly, the process is accompanied by edema of the respiratory epithelium, which is the result of the increased vascular permeability and intravascular pressure.

Prospects of further research *will encompass the study of the morphological changes in the structural components of the lungs in mature and old rats under the conditions of hyperhomocysteinemia.*

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Реферати

УЛЬТРАСТРУКТУРНІ ЗМІНИ В ЛЕГЕНЯХ ЩУРІВ ВІКОМ 1-2 МІСЯЦІ В УМОВАХ ГІПЕРГОМОЦИСТЕЇНЕМІЇ

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Проведене детальне електронномікроскопічне дослідження структурних компонентів легень щурів віком 1-2 місяці в умовах гіпергомоцистеїнемії. Звертає на себе увагу, наявність у просвітах альвеол великої кількості фібрину та еритроцитів у оточенні електроннощільного субстрату, по консистенції аналогічного плазмі крові, що, очевидно, пов'язано зі зменшенням сурфактантної плівки у альвеолах та підвищенню транссудації рідини з капілярів, що містяться у міжальвеолярних перегородках. Специфічними ознаками для цього терміну є наявність у альвеолярному просторі транссудату, який містив білки плазми крові та фібринові волокна, сладж-феномен, що перешкоджає кровотоку та набряк респіраторних альвеолоцитів. У молодих тварин, перш за все, зміни відбуваються у аерогематичному бар'єрі. Вони пов'язані з ендотеліальною дисфункцією, яка в свою чергу обумовлена локальною десквамацією ендотеліоцитів, змінами у структурі базальних мембран, їх розпушуванням, у результаті порушення їх пошаровості та обтурації просвітів капілярів форменими елементами крові. Ушкодження цілісності ендотеліального вистелення призводить до просякнення плазми, фібрину і формених елементів крові у просвіт альвеол. По-друге, процес супроводжується набряком респіраторного епітелію, що є результатом підвищення проникності стінки судин та підвищенням внутрішньокапілярного тиску.

Ключові слова: гомоцистеїн, гіпергомоцистеїнемія, легені, альвеолоцити, мітохондрії.

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УЛЬТРАСТРУКТУРНЫЕ ИЗМЕНЕНИЯ В ЛЕГКИХ КРЫС В ВОЗРАСТЕ 1-2 МЕСЯЦА В УСЛОВИЯХ ГИПЕРГОМОЦИСТЕИНЕМИИ

Самборская І.А., Маєвский А.Е., Агафонов К.М., Ковальчук А.И.

Проведено детальное электронномикроскопическое исследования структурных компонентов легких крыс в возрасте 1-2 месяца в условиях гипергомоцистеинемии. Обращает на себя внимание, наличие в просветах альвеол большого количества фибрина и эритроцитов в окружении электронноплотного субстрата, по консистенции аналогичного плазме крови, что, очевидно, связано с уменьшением сурфактантной пленки в альвеолах и повышению транссудации жидкости из капилляров, расположенных в межальвеолярных перегородках. Специфическими признаками для этого срока – наличие в альвеолярном пространстве транссудата, содержащий белки плазмы крови и фибриновые волокна, сладж-феномен, который препятствует кровотоку и отек респираторных альвеолоцитов. У молодых животных, прежде всего, изменения происходят в аэрогематический барьере. Они связаны с эндотелиальной дисфункцией, которая в свою очередь обусловлена локальной десквамацией эндотелиоцитов, изменениями в структуре базальных мембран, их разрыхлением, в результате нарушения послойности их строения и обтурации просветов капилляров форменными элементами крови. Повреждения целостности эндотелиального слоя приводит к пропотеванию плазмы, фибрина и форменных элементов крови в просвет альвеол. Во-вторых, процесс сопровождается отеком респираторного эпителия, является результатом повышения проницаемости стенки сосудов и повышением внутрикапиллярного давления.

Ключевые слова: гомоцистеин, гипергомоцистеинемия, легкие, альвеолоциты, митохондрии.

Рецензент Єрошенко Г.А.