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ULTRASTRUCTURAL ORGANIZATION OF THE COMPONENTS OF THE LUNG RESPIRATORY DEPARTMENT IN EXPERIMENTAL ACUTE PANCREATITIS

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Acute pancreatitis is one of the most urgent problems in abdominal surgery. The relevance of this problem is primarily due to a significant increase in the number of patients with acute pancreatitis. The prevalence and incidence of acute pancreatitis has increased dramatically in recent years. Experiments were conducted on male white rats with arginine-induced acute pancreatitis. Conducted electron microscopic studies showed that in the conditions of simulated acute pancreatitis, a violation of the ultrastructural organization of the components of the respiratory department of the lungs was noted already during the first 6 hours of the experiment. It was found that the most pronounced changes of a dystrophic-destructive nature are observed after 12–24 hours of the study. Erythrocyte sludge, adhesion and aggregation of leukocytes and platelets are found in the lumen of the hemocapillaries of the alveolar wall.

Key words: experimental acute pancreatitis, lungs, respiratory part.

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УЛЬТРАСТРУКТУРНА ОРГАНІЗАЦІЯ КОМПОНЕНТІВ РЕСПІРАТОРНОГО ВІДДІЛУ ЛЕГЕНЬ ПРИ ЕКСПЕРИМЕНТАЛЬНОМУ ГОСТРОМУ ПАНКРЕАТИТІ

Гострий панкреатит є однією з найбільш актуальних проблем сьогодення. Актуальність даної проблеми перш за все обумовлена значним збільшенням кількості хворих гострий панкреатит. Поширеність і захворюваність гострим панкреатитом різко зросла протягом останніх років. Експерименти проведено на білих щурах самцях з аргінін-індукованим гострим панкреатитом. Проведені електронно-мікроскопічні дослідження показали, що в умовах змодельованого гострого панкреатиту відмічається порушення ультраструктурної організації компонентів респіраторного відділу легень вже протягом перших 6 год експерименту. Встановлено, що найбільш виражені зміни дистрофічно-деструктивного характеру спостерігаються через 12–24 год дослідження. У просвіті гемокапілярів альвеолярної стінки виявляються еритроцитарні сладжі, адгезія та агрегація лейкоцитів та тромбоцитів.

Ключові слова: експериментальний гострий панкреатит, легені, респіраторний відділ.

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Today, acute pancreatitis (AP) is one of the most urgent problems in abdominal surgery [4, 5, 7, 8]. The urgency of this problem is primarily due to the significant increase in the number of AP patients [6, 11, 14]. A population-based cohort study showed that the global incidence of AP is 33.74 cases per 100,000 people per year with a 95 % confidence interval [3, 8, 10]. AP can be accompanied by both local and systemic damage. Among systemic complications, pulmonary complications occur most often and are potentially the most difficult. Pulmonary complications of AP occur in almost 75 % of cases, ranging from hypoxia to acute respiratory distress syndrome (ARDS). Systemic involvement manifests in the form of organ failure, which occurs in approximately 20 % of AP cases and defines severe AP (SAP) [2]. Organ failure usually develops in the early stages of AP but may develop later and is the most important factor determining the AP outcome. The most common type of organ failure is acute lung injury, which is the most common manifestation of SAP [3, 10]. It has been established that SAP can be complicated by acute lung injury (ALI) in 60–70 % of cases. Moreover, ALI can often be accompanied by ARDS, and the last one is the main cause of death in patients with SAP.

According to literature data [3], pathological reactions and their molecular mechanisms of lung damage are closely related to increased permeability of microvascular endothelial cells, aggregation of the limiting concentration of leukocytes, and abundant expression of ICAM. The accumulation of inflammatory cells and mediators of inflammation in the lungs lead to the development of dystrophic – destructive changes in the constituent components of the lung respiratory department (LRD), such as type I alveolocytes (A-I), type II alveolocytes, (A-II) endotheliocytes of hemocapillaries and alveolar macrophages (AM). Accumulation of a large number of neutrophils in the lungs increases the generation of reactive oxygen species (ROS) and increases the production of pro– inflammatory cytokines, including TNF - α, IL - 1β, IL - 6, which activate various intracellular signaling pathways and adhesion molecules, releasing inflammatory mediators. The generation of inflammatory mediators including cytokines and

chemokines, as well as ROS, is a major cause of AP –induced lung injury. These mediators promote the accumulation of macrophages and neutrophils, triggering a cascade of pathological changes in the pulmonary microcirculation of the lungs, leading to the onset and exacerbation of AP-induced lung injury [3, 8].

The electron microscopic studies performed make it possible to detect changes in the components of LRD at an early stage of the development of AP. Thus, an in-depth study of the pathogenesis of ALI associated with SAP has key importance for improving the prognosis of patients with SAP.

The purpose of the study was to investigate the dynamics of ultrastructural changes in the components of the respiratory system of the lungs in experimental acute pancreatitis.

Materials and methods. The experiments were carried out on 88 white Wistar male rats weighing 180–220 g. The animals were divided into three groups: 1 – intact (n=10); 2 – control (n=40); 3 – experimental with a model of acute pancreatitis (n=38); which was reproduced by intraperitoneal administration of a 20 % solution of L-arginine "Sigma" Chemical Co (USA) at a total dose of 5 g/kg at one-hour interval. The control group of animals was intraperitoneally injected with an equivalent dose of isotonic sodium chloride solution.

Animal husbandry and research were conducted in accordance with the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986), the Law of Ukraine on the "Protection of Animals from Cruelty"(2006) and the "General Ethical Principles of Experiments on Animals" approved by the Fifth NationalCongress on Bioethics (Kyiv, 2013).

The collection of material (lungs) for ultrastructural analysis was carried out and contrasted according to the generally accepted method under thiopental anesthesia after 1, 6, 12, 24 hours [1]. Ultrathin sections were prepared on "Tesla BS-490" ultramicrotome and studied in a PEM-125K electron microscope.

Results of the study and their discussion. The conducted ultrastructural analysis showed that 1 hour after the start of the experiment, the nuclei of A-1, A-II were round or oval with a matrix of medium electron-optical integrity. Chromatin granules are evenly distributed over the entire area of the nucleus. Mitochondria, components of the Golgi apparatus (GA) and the granular endoplasmic reticulum (GER) without any special structural changes. Lamellar bodies (LB) A-II of varying degrees of maturity, size and shape. Basement membranes A-I and A-II throughout retain their characteristic structure.

For the current period of research, violations of the submicroscopic structure of some hemocapillaries of the interalveolar septa of the lungs are already detected. The cytoplasm of individual endothelial cells is characterized by weak electron – optical density. Nuclei are oval with invaginations of the nucleolemma. Mitochondria with a lightened matrix and disorganized cristae are sometimes found. The components of GA and GER are somewhat expanded. Many micropinocytotic vesicles are observed in the peripheral parts of the endothelial cells. Basal membrane of some cells is locally thickened. In the lumen of individual hemocapillaries, an increased number of leukocytes and their adhesion to endothelial cells are determined.

AM in a state of increased functional activity are observed in the alveoli. A characteristic feature of their submicroscopic structure is the presence of a well – defined lysosomal apparatus.

With increasing study time (6 h), individual alveolar cells with swollen mitochondria and single disorganized cristae are observed. The tubules and cisterns of the GA and GER are somewhat dilated. In individual A-I, sail – like protrusions of the peripheral part into the lumen of the alveoli are noted (Fig. 1). Intercellular contacts retain their integrity.

The nuclei of AM are oval with a fine – grained matrix. The cytoplasm contains moderately enlarged components of GA and GER, many lysosomes and phagosomes, as well as individual mitochondria increased in volume.

Endothelial cell nuclei are oval or spherical in shape. The nuclear envelope has sinuous contours and forms shallow invaginations. Chromatin granules are mostly evenly distributed over the entire area of the nucleus. At the same time, some cells with marginal localization of chromatin granules are found. Mitochondria of such endothelial cells are enlarged in volume with a matrix of low electronoptical density and single reduced cristae. GA is represented by moderately expanded cisterns and small vesicles. GER tubules are hypertrophied with a reduced number of ribosomes on the membranes of the latter. The basement membrane is locally thickened. In the lumen of individual hemocapillaries, erythrocyte aggregates and leukocyte adhesion are detected (Fig. 1).

At the 12th hour of the study, it was found that the nuclei of some A-I, A-II with nucleoplasm of low electron – optical density. The perinuclear space is locally expanded. Individual mitochondria of alveolar cells with a clarified matrix and single reduced cristae. The components of GA and GER are expanded and deformed. LB of various sizes and shapes, partially filled with phospholipid material. In the peripheral sections A-I, an increased number of micropinocytotic vesicles is determined, which are tightly adjacent to the apical and basal parts of the cell plasmalemma (Fig. 2).

As in the previous period of study of individual A-I, sail – like protrusions of the peripheral part into the lumen of the alveoli are noted. LB A-II of various sizes and shapes, partially vacuolated.

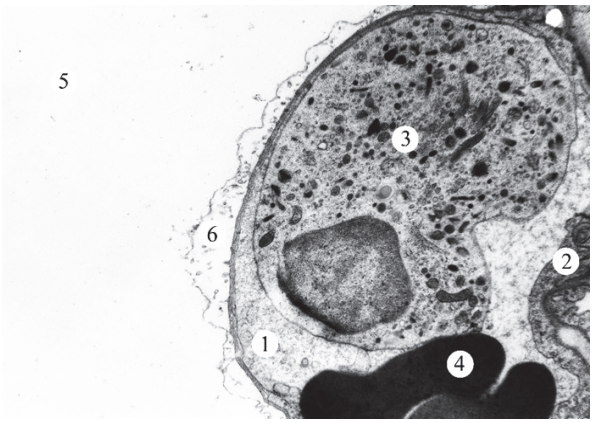

Fig. 1. Ultrastructural organization of the lung respiratory department 6 hours after the start of the experiment. Electron micrograph x 6400. Key: 1 – lumen of the hemocapillary; 2 – peripheral part of the endothelial cell; 3 – leukocyte; 4 – erythrocyte; 5 – lumen of the alveolus; 6 – peripheral part of the type I alveolocyte.

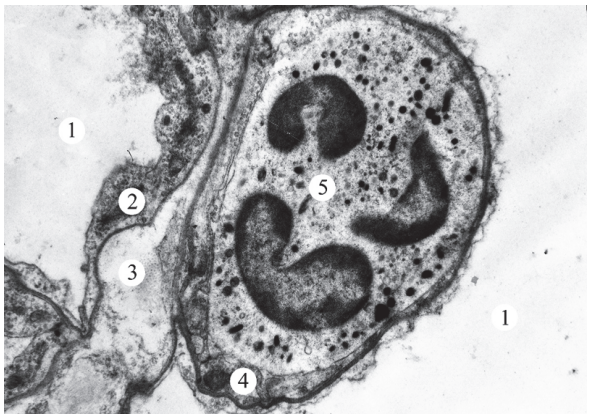

Fig. 2. Respiratory department of the lungs 12 hours after the start of the experiment. Electron micrograph x 6400. Key: 1 – lumen of the alveolus; 2 – peripheral part of the type I alveolocyte; 3 – interstitial tissue; 4 – peripheral part of the endotheliocyte; 5 – leukocyte.

The nuclei of endothelial cells are enlarged in size with marginal localization of chromatin granules. The perinuclear space is expanded. Mitochondria are swollen, of various sizes and shapes with single disorganized cristae. Along with the expanded components of GA, fragmentation of GER membranes is noted. The basement membrane is swollen, of heterogeneous thickness. In the peripheral parts of endothelial cells, an increased number of both small and large micropinocytotic vesicles is observed. Large vesicles often merge with each other, forming vacuoles or transendothelial channels. In the lumen of individual hemocapillaries, leukocyte adhesion is determined.

Changes from the side of the interstitial tissue are characterized by a decrease in the electron – optical density of the main substance of the connective tissue and the loosening of fibrous structures by edematous fluid.

For the current period of the study, AM with poorly developed lysosomal apparatus is found among macrophage elements. In individual AMs, numerous lipid inclusions are detected. In the cytoplasm of AMs, some mitochondria with a clarified matrix and single reduced cristae are observed. GA is represented by unevenly expanded cisterns and vesicles. The GER tubules are expanded with fine – fibrous contents inside.

The conducted ultrastructural studies showed that the most pronounced changes in A-I are determined 24 hours after the start of AP simulation. A-I nuclei with a matrix of low electron – optical density. Granular components of the nucleoplasm are concentrated near the nuclear envelope. The nucleolemma forms shallow invaginations. The perinuclear space is expanded. Most mitochondria are swollen, of various sizes and shapes with single disorganized crista. GA consists of dilated cisterns and vacuoles. The GER tubules are dilated and fragmented. The number of ribosomes on their membranes is reduced. The basement membrane is thickened with indistinct contours. In the peripheral sections of A-I, the fusion of micropinocytotic vesicles with the formation of large vacuoles is often observed. The increasing edema of individual A-I leads to local ruptures of the apical plasmalemma, especially its peripheral section.

The nuclei of A-II are enlarged (Fig. 3). Chromatin granules are mainly located along the inner surface of the nuclear envelope. The mitochondria of such cells are enlarged in volume with single disoriented cristae. The components of GA and GER are expanded. The number of ribosomes on the membranes of the latter is reduced.

LB are characterized by the appearance of uneven spaces between bimembrane osmiophilic plates. Sometimes LB form cavities with fragments of osmiophilic plates inside. The basement membrane is locally thickened. The number of microvilli on the apical surface of A-II is reduced.

The severity and prevalence of ultrastructural changes in hemocapillaries are significantly greater than in the previous period of study. The nuclei of many endothelial cells with a matrix of low electron – optical density. Chromatin granules in most cells are located along the inner surface of the nuclear membrane or grouped into separate clumps. The perinuclear space is expanded. Sometimes there is partial destruction of mitochondria. GA is represented by expanded cisterns and vacuoles. The GER tubules are vacuolated and fragmented. The basement membrane is thickened with indistinct contours. The peripheral part of many endothelial cells with a clear matrix and many micropinocytotic vesicles and vacuoles (Fig. 4). In some hemocapillaries, areas of lysis of the lumenal plasmolemma are noted, which leads to the release of intracellular contents into the lumen of the microvessel. In the lumen of many hemocapillaries, adhesion and aggregation of platelets are observed. In addition, in some areas, the destruction of inter – endothelial contacts is determined, which leads to the release of formed elements of blood into the interstitial tissue and the lumen of the alveoli.

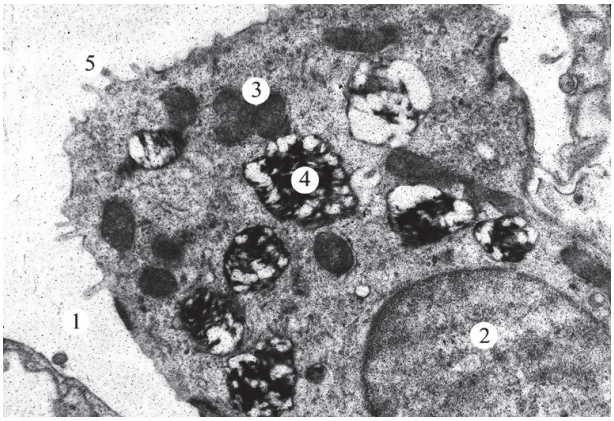

Fig. 3. Fragment of a type II alveolocyte 24 h after the start of the experiment. Electron micrograph x 8000. Key: 1 – alveolar lumen; 2 – nucleus, 3 – mitochondria; 4 – lamellar body; 5 – microvilli.

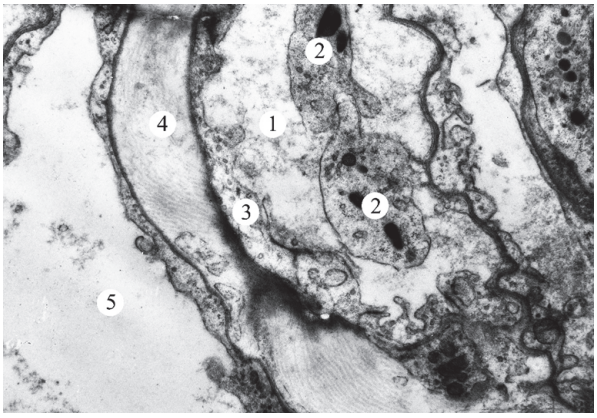

Fig. 4. Respiratory department of the lungs 24 hours after the start of the experiment. Electron micrograph x 8000. Key: 1 – lumen of the hemocapillary; 2 – platelet; 3 – peripheral part of the endotheliocyte; 4 – interstitial tissue; 5 – lumen of the alveoli.

The interstitial tissue is swollen, especially in places adjacent to the area with impaired integrity of the hemocapillary endothelium.

Submicroscopically, a significant heterogeneity of macrophage elements is observed in the alveolar lumen. Along with active phagocytizing, AM with dystrophic – destructive changes are identified. The nuclei of such cells are oval with a matrix of low electron – optical density. The nucleolemma has sinuous contours and forms shallow invaginations. The perinuclear space is expanded. Chromatin granules in many cells are located along the inner surface of the nuclear envelope, in some AM they form separate clumps. Mitochondria of various sizes and shapes with single disorganized cristae. Along with this, vacuolarly transformed mitochondria are found. GA consists of vesicularly expanded cisterns and a small number of vesicles. GER tubules are expanded, deformed. Fragmentation of GER membranes is observed in individual cells. The number of ribosomes on GER membranes is reduced. Lysosomes are represented by single granules. In the cytoplasm of AM, individual phagosomes with polymorphic osmiophilic material are also noted. The progression of submicroscopic changes on the part of the organelles is accompanied by a decrease in the electron – optical density of the cytoplasmic matrix.

Studies have shown that in the early period of development of AP (1 –6 h) in the hemocapillaries of the alveolar wall, a violation of the rheological properties of the blood is detected, as evidenced by excessive accumulation of neutrophils, their adhesion and aggregation. Adhesion of leukocytes to the endothelium is the cause of the formation and release of oxygen radicals, secretory degranulation, which have a direct damaging effect on endothelial cells [2, 3], as indicated by our electron microscopic studies. Increased permeability of endothelial cells of the hemocapillaries of the alveolar wall leads to the release of neutrophils into the interstitial tissue and the lumen of the alveoli. Emigration of neutrophils into the lumen of the alveoli was also observed by us 24 hours after the start of the experiment. The obtained data are consistent with the results of studies by other scientists, who indicate the important role of activated neutrophils in the development of ALI in other pathological conditions [9]. As the electron-microscopic analysis showed, for the current period of the study, submicroscopic changes of A-I, A-II and AM are also noted, which are less pronounced than in the endotheliocytes of hemocapillaries. Ultrastructural analysis performed after 12–24 hours indicated the presence of dystrophic and destructive changes in the

components of the LRD, which leads to the development of interstitial and alveolar edema. A number of other researchers point to changes of a similar nature under the influence of various exo- and endogenous factors [12, 13, 15].

Conclusions

1. Experimental acute pancreatitis is accompanied by submicroscopic changes in types I and II alveolocytes, hemocapillaries of the alveolar wall, and alveolar macrophages.

2. Disturbances in the ultrastructural organization of the components of the respiratory department of the lungs under conditions of simulated acute pancreatitis indicate pronounced changes in the hemocapillaries of the alveolar wall, as evidenced by the presence of erythrocyte sludges and thrombocyte aggregates.

3. The nature and severity of the changes depend on the duration of acute pancreatitis.

References

1. Doskaliuk BV, Zaiats LM. Structural and functional characteristics of the pulmonary hemomicrocirculatory bed in induced systemic sclerosis: an experimental study. Rheumatology International. 2023;43: 1341–1347. DOI: 10.1007/s00296-023-05328-z.
2. Garg P, Singh VP. Organ Failure due to Systemic Injury in Acute Pancreatitis. Gastroenterology. 2019; 156:2008-2023. DOI: 10.1053/j.gastro.2018.12.041.
3. Ge P, Luo Y, Okoye CS, Chen H, Liu J, Zhang G, et al. Intestinal barrier damage, systemic inflammatory response syndrome, and acute lung injury: a troublesome trio for acute pancreatitis. Biomed Pharmacother.2020;132:110770. DOI: 10.1016/j.biopha.2020.110770.
4. Gukovskaya AS, Gukovsky I, Algul H, Habtezion A. Autophagy, Inflammation, and Immune Dysfunction in the Pathogenesis of Pancreatitis. Gastroenterology. 2017;153(5):1212-1226. DOI: 10.1053/j.gastro.2017.08.071.
5. Kumar P, Gupta P, Ranar S. Thoracic complications of pancreatitis. An open access journal of gastroenterology and hepatology. 2018;3(1):71-79. DOI:10.1002/jgh3.12099.
6. Lankisch PG, Apte M, Banks PA. Acute pancreatitis. Lancet. 2015; 386:85-96. DOI:10.1016/S0140-6736(14)6U649-8.
7. Leppaniemi A, Tolonen M, Tarasconi A, Segovia-Lohse H, Gamberini E, Kirkpatrick AW et al. WSES guidelines for the management of severe acute pancreatitis. World Journal of Emergency Surgery.2019;14:27 DOI: 10.1186/s13017-019-0247-0.
8. Liu D, Wen L, Wang Z, Hai Y, Yang D, Zhang Y, et al. The Mechanism of Lung and Intestinal Injury in Acute Pancreatitis: A Review. Front. Med.2022; 7:9: 904078. DOI:10.3389/fmed.2022.904078.
9. Robb CT, Regan KH, Dorward DA, Rossi AG. Key mechanisms governing resolution of lung inflammation. Semin Immunopathol.2016;38(4):425-48. DOI: 10.1007/s00281-016-0560-6.
10. Schepers NJ, Bakker OJ, Besselink MG, Ali UA, Bollen TL, Gooszenet HG et al. Impact of characteristics of organ failure and infected necrosis on mortality in necrotising pancreatitis. Gut 2019;68(6):1044–1051. DOI:10.1136/gutjnl-2017-314657.
11. Tan JH, Cao RC, Zhou L, Zhou ZT, Chen HJ, Xu J, et al. ATF6 aggravates acinar cell apoptosis and injury by regulating p53/AIFM2 transcription in Severe Acute Pancreatitis. Theranostics 2020;10(18):8298-8314.
12. Wang F, Lu F, Huang H, Huang M, Luo T. Ultrastructural changes in the pulmonary mechanical barriers in a rat model of severe acute pancreatitis-associated acute lung injury. Ultrastruct Pathol.2016;40(1):33-42. DOI: 10.3109/01913123.2015.1088907.
13. Whitsett JA, Weaver TE. Alveolar Development and Disease. Am J Respir Cell Mol Biol. 2015;53(1):1–7. DOI: 10.1165/rcmb.2015-0128PS.
14. Wolbrink DRJ, Kolwijck E, Oever JT, Horvath KD, Bouwense SAW, Schouten JA. Management of infected pancreatic necrosis in the intensive care unit: a narrative review. Clinical Microbiology and Infection. 2020; 26:18-25. DOI: 10.1016/j.cmi.2019.06.017.
15. Zaiats LM, Fedorchenko YuV. Features of lipoperoxidation and morphological changes of the lungs in experimental diabetes mellitus. World of Medicine and Biology. 2022;3(81):214-18. DOI 10.26724/2079-8334-2022-3-81-214-218.

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