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Ultrastructural changes in type 2 alveolocytes in young rats on the background of chronic hyperglycemia

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ABSTRACT

Introduction: Diabetes mellitus (DM) is considered as a group of metabolic diseases with a global distribution and severe complications. It is caused by insulin deficiency, which with time leads to development of pathological changes in the cardiovascular, respiratory, and other systems. Several studies have shown some features and the connection of structural changes of the lungs with DM, however very little is known regarding ultrastructural changes of type 2 alveolocytes (AT2).

Materials and methods: The study involved 24 white non-linear male laboratory rats which were divided into two groups (experimental and intact). The experimental group was further divided into two subgroups depending on the duration of study: the first group with hyperglycemia for 30 days, and the second with hyperglycemia for 60 days. For the experimental modeling of hyperglycemia, the rats were injected once subcutaneously with solution of alloxan monohydrate hyperglycemia.

Results: AT2 of the intact group had a high degree of differentiation with plates of high electron density. In AT2 of rats with hyperglycemia for 30 days, there were signs of vacuolation, mass accumulation of primary and secondary lysosomes, and lamellar bodies were grouped as conglomerates. In AT2 of the rats with 60 days of hyperglycemia, nuclei with scalloped contour, karyoplasmic outgrowths and intussusception, and condensation of heterochromatin were observed.

Conclusion: Under conditions of experimental chronic hyperglycemia, proliferation and destruction of AT2 are observed, which is the morphological basis for the violation of surfactant synthesis and immunocompetent properties in lung tissues of young rats.

1. Introduction

Diabetes mellitus (DM), which has hyperglycemia as its main symptom, is now considered as a group of metabolic diseases with a global distribution and severe complications (Tomic et al., 2022). According to World Health Organization (WHO) estimates, the number of people with diabetes in the world will increase to 570 million by 2030, with almost half of them having a high risk of developing severe complications (*Recommendations to strengthen and monitor diabetes responses within national noncommunicable disease programmes*, n.d.).

Complications of diabetes can be divided into three categories: microvascular (neuropathy, nephropathy, retinopathy), macrovascular (cardiovascular diseases, including diseases of peripheral arteries, and impaired cerebral blood circulation), as well as a rather extensive category of other complications, which includes malignant neoplasms of various localization, infectious pathology, non-alcoholic fatty liver disease, fibrosis, diseases of the skeletal system, respiratory system, affective, cognitive and other disorders (Tomic et al., 2022; Papatheodorou et al., 2018; Cole and Florez, 2020). The problem with diseases of the respiratory organs, particularly the lungs, in the context of complications with DM is currently being actively studied, and its solution requires the accumulation of more data that can be obtained in the course

Abbreviations: DM, diabetes mellitus; AT2, type 2 alveolocytes; HbA1c, glycosylated hemoglobin; AKM, nm, alveolar-capillary membrane thickness; TE, endotheliocyte thickness; TA, thickness of alveolocytes; TI, thickness of interstitial space; GER, granular endoplasmic reticulum; UPR, unfolded protein response; ER, endoplasmic reticulum.

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of morphological, physiological, genetic and epidemiological studies.

A number of reviews, experimental and clinical studies have shown some features and the connection of structural changes in the lungs with diabetes, in particular, epithelial-mesenchymal transformation and the development of fibrosis, infiltration of the lung interstitium by inflammatory cells, and dilation of pulmonary capillaries (McCloud et al., 2004; Forgiarini et al., 2009; Eren et al., 2010; Hu et al., 2014; Talakatta et al., 2018; Chen et al., 2018; Rajasurya et al., 2020). However, very little is known today regarding ultrastructural changes of the lung in DM, especially type 2 alveolocytes (AT2), which synthesize, secrete and transform surfactant, act as immunoregulatory cells, regulate alveolar fluid balance, proliferate, dedifferentiate, acquire stem cell identity and act as a source of type 1 alveolocytes in the process of lung tissue regeneration (Fehrenbach, 2001). The results of a few experimental studies indicate, in particular, the following degenerative changes in AT2 in diabetic animals: presence of abnormal inclusions (deposits) of intracellular proteins, lipid droplets, and violations of the structure of lamellar bodies (Hu et al., 2014; Ruze et al., 2020). Among the probable pathogenetic mechanisms underlying structural changes of the lungs in diabetes, oxidative stress and non-enzymatic glycosylation of proteins deserve special attention, while the known factors leading to the development of pulmonary symptoms are: hyperglycemia, insulin resistance, autonomic neuropathy, pulmonary microangiopathy, fibrosis, disruption of surfactant production and respiratory muscle function (Zheng et al., 2017; Kolahian et al., 2019).

The growing prevalence of DM and the high risks of developing dangerous complications, particularly from the lungs, outline the prospects for further morphological studies. This will illuminate the structural basis of the disease in more detail. The purpose of this study is to identify and analyze ultrastructural changes in AT2 of young rats under conditions of experimental chronic hyperglycemia.

2. Materials and methods

2.1. Animals and treatment

The study was conducted on 24 male Wistar laboratory rats, aged 30 days with average weight of 101.5 ± 0.87 g at the beginning of the experiment. The animals were kept in a vivarium on a diet prepared according to the state standard diet of Ukraine (SSU 7965:2015), with free access to water at 22 ± 2 °C and a 12-hour light/dark cycle, in compliance with the rules approved by the Helsinki Declaration of the General Assembly of the World Medical Association (Helsinki, 2000), the Provisions of the "European Convention on the Protection of Vertebrates animals used for experiments and other scientific purposes" (Strasbourg, 1985), and "General ethical principles of experiments on animals", adopted by the First National Congress on Bioethics (Kyiv, 2001).

The laboratory rats were randomly divided into two groups: experimental (n = 12) and control (n = 12). The experimental group was further divided into two subgroups (6 animals each) depending on the duration of the study: the first - with a duration of hyperglycemia of 30 days, the second - 60 days. For the experimental modeling of hyperglycemia, the animals were injected once, subcutaneously with a solution of alloxan monohydrate at the rate of 20 mg per 100 g of body weight after a 24-hour fasting against the background of normal blood glucose level and glycated hemoglobin concentration. Animals of the control group after 24 h of fasting were injected once subcutaneously with a similar volume of physiological solution (0.9 % NaCl). The animals were withdrawn from the experiment on the 30th and 60th day for dissection under intraperitoneal sodium thiopental anesthesia. After dissection of the chest cavity, ligation of the trachea was performed to prevent atelectasis of the lungs. Later, the lung organ complex was removed together with the trachea, then, the diaphragmatic lobe of the right lung was selected for the study of the ultrastructure changes.

2.2. Evaluation of blood parameters and lung ultrastructure

Before removing the animals from the experiment, blood was taken from the tail vein for biochemical studies. The level of glucose and glycosylated hemoglobin (HbA1c) was determined on a ChemWell-T analyzer (Awareness Technology, USA). The average level of blood glucose in animals of the experimental group on the 30th day of the experiment was 19.3 ± 0.2 mmol/l, on the 60th day of the experiment – 13.8 ± 0.3 mmol/l, the average HbA1c concentration was 7.1 ± 0.1 % and 7.2 ± 0.2 %, respectively, which was considered as a state of persistent hyperglycemia. In animals of the control group, the average blood glucose level on the 30th day of the experiment was 6.3 ± 0.2 mmol/l, on the 60th day of the experiment – 4.9 ± 0.9 mmol/l, the average HbA1c concentration was 4.0 ± 0.1 % and 4.9 ± 0.1 %, respectively (Table 1).

To study the ultrastructure, a portion of the lung was removed, while the area of tissue selection was irrigated with a fixative - 3.125 % glutaraldehyde, after which it was cut into fragments of 1 × 1 × 1 mm^3. Prefixation was carried out in a 3.125 % glutaraldehyde solution, then postfixation in a 2 % osmium tetroxide solution in bidistilled water. Tissue fragments were dehydrated using a series of alcohols of increasing concentration. A water-insoluble two-component resin was used for pouring the material in a ratio of 7:3. Ultrathin (40–60 nm) sections were made on an UMTP-6 m ultramicrotome (Selmi, Ukraine). Ultramicrosections mounted on copper grids were first contrasted in a 2.5 % uranyl acetate solution (15 min), then washed with distilled water, and then immersed in a Reynolds solution of lead citrate (15 min). The research was carried out on a PEM-125 K electron microscope (Selmi, Ukraine) at an accelerating voltage of 75–100 kV. Photographing of the obtained preparations at a magnification of 4000 times was carried out using a Baumer/optronic Typ: CX 05c digital video camera.

Ultramicromorphometry was performed in dynamic mode with the measurement of the following parameters: alveolar-capillary membrane thickness (AKM, nm), endotheliocyte thickness (TE, nm); thickness of alveolocytes (TA, nm), thickness of interstitial space (TI, nm) using the certified image analysis program "SEO Scan Lab 2.0" and "SEO Image Lab 2.0" (Selmi, Ukraine). In the lungs of young rats.

2.3. Statistics

The Shapiro-Wilk test was used to check the distribution of data for normality. A descriptive analysis of each sample was performed with the calculation of the mean value (M) and standard deviation (SD). The non-parametric Mann-Whitney *U* test was used to assess differences between independent samples. A difference with a value of $p<0.05$ was considered significant.

Table 1
Ultramicroscopic indicators of lung tissue and blood indicators of young rats with hyperglycemia for 30 and 60 days.

Indicator	30 days, M ± SD		60 days, M ± SD	
	Control	Hyperglycemia	Control	Hyperglycemia
AKM, nm	414,4 ± 0,8	414,8 ± 0,3	414,3 ± 1,1	420,1[a] ± 0,4
TE, nm	167,83 ± 0,1	167,81 ± 0,04	167,83 ± 0,1	170,02[a] ± 0,02
TA, nm	166,9 ± 0,01	166,8 ± 0,6	167,0 ± 0,7	168,1[a] ± 0,4
TI, nm	79,7 ± 0,08	80,2[a] ± 0,1	79,5 ± 0,3	81,9[a] ± 0,8
Blood glucose, mmol/l	6,3 ± 0,2	19,3[a] ± 0,2	4,9 ± 0,9	13,8[a] ± 0,3
HbA1C, %	4,0 ± 0,1	7,1[a] ± 0,1	4,9 ± 0,1	7,18[a] ± 0,2

[a] The difference is significant relative to the control group $p < 0.05$.

3. Results

3.1. Ultrastructural features of type 2 alveolocytes of control group animals

In young rats of the control group, AT2 was located in the corners of the alveoli on the basal membrane that separates the cell from the interstitium of the interalveolar membrane. The cells had a cubic or prismatic shape (without cytoplasmic outgrowths) with a high degree of differentiation, which was evidenced by the presence of a large number of mitochondria, a developed endoplasmic reticulum, ribosomes, Golgi complex cisternae and vesicles in the cytoplasm. The nucleus occupied approximately 30–40 % of the cell, located in the center. A distinctive feature of AT2 was the presence of osmiophilic lamellar bodies, which were ovoid or globular in shape, bounded by a membrane, and contained lamellae of high electron density, which had the ability to accumulate osmium.

3.2. Ultrastructural changes of type 2 alveolocytes in rats with a term of hyperglycemia of 30 days

On the 30th day of the experiment, animals with hyperglycemia showed signs of vacuolization in the cytoplasm of AT2, a massive accumulation of both primary and secondary lysosomes was clearly visible, typical lamellar bodies were grouped in the form of conglomerates, and the granular endoplasmic reticulum (GER) was slightly expanded (Fig. 1). AT2 of different degrees of maturity were present in different fields of view within the same alveolus. The number of mitochondria in the cells was lower compared to the cells of control animals.

The thickness of the AKM remained almost unchanged (414.8 ± 0.3 nm), compared to this indicator in animals of the control group (414.4 ± 0.8 nm, $p = 0.32$). It is worth noting the slight hyperplasia of collagen fibers in the interstitium of the lungs of animals of the experimental group (80.2 ± 0.1 nm) compared to that of animals in the control group (79.7 ± 0.08 nm), which leads to a reliable increase in TI by 0. 6 % ($p = 0.0002$) (Fig. 2).

According to the indicators of TE and TA, no significant difference between the animals of the experimental and control groups was established.

3.3. Ultrastructural changes of type 2 alveolocytes in rats with a term of hyperglycemia of 60 days

On the 60th day of the experiment, nuclei with uneven outlines, karyoplasmic outgrowths and intussusceptions were often found in AT2 animals with hyperglycemia, and heterochromatin was distributed along the periphery of the nucleus. GER was extended. The number of cytophospholiposomes in osmiophilic bodies was reduced. The osmiophilic material was loosely located in electron transparent vacuoles and was deformed. Empty autophagic vacuoles were often found, some of them contained single osmiophilic plates (Fig. 3). This indicates a violation of the synthesis of osmiophilic material, which is the main component of the surfactant system, against the background of increased destructive changes. In endotheliocytes, there was swelling and lightening of the cytoplasm, which changed the configuration and thickness of the cells (170.02 ± 0.02 nm). Thus, TE in hyperglycemic animals on the 60th day of the experiment was 0.72 % more compared to the corresponding average indicator in hyperglycemic animals on the 30th day of the experiment (167.81 ± 0.04 nm) and significantly more compared to the animals of the control group (167, 83 ± 0.1 nm, $p < 0.001$). Also, the TI index increased by 2.12 % (81.9 ± 0.8 nm) compared to that of animals in the experimental group on the 30th day of the experiment (80.2 ± 0.1 nm). The increase in TI occurred due to hyperplasia of collagen fibers in the interstitium and is reliable in comparison with that of animals in the control group (79.5 ± 0.3 nm, $p = 0.003$).

The indicators of AKM and TA in hyperglycemic animals on the 60th day of the experiment were 420.1 ± 0.4 nm and 168.1 ± 0.4 nm, respectively, which is 1.3 % and 0.8 % higher, respectively, compared to such indicators on the 30th day and significantly more compared to the indicators of animals of the control group (414.3 ± 1.1 nm and 167.0 ± 0.7 nm, $p < 0.001$ and $p = 0.012$, respectively).

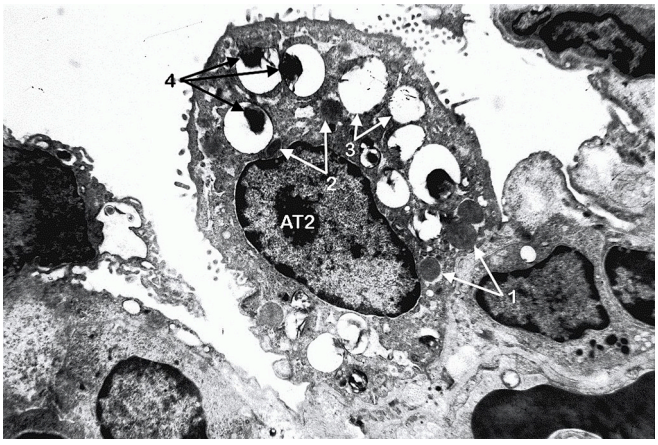

Fig. 1. Ultrastructure of the lung tissue of a young rat with a term of alloxan hyperglycemia of 30 days. Contrast with uranyl acetate and lead citrate; ×4000: AT2 – type 2 alveolocyte; 1 – primary lysosomes; 2 – secondary lysosomes; 3 – vacuoles; 4 – osmiophilic bodies.

4. Discussion

In a model experiment to reproduce chronic hyperglycemia in young male rats, structural changes in AT2 were detected. The AT2 pool was about 15 % of peripheral pulmonary cells. AT2 - differentiated cells are well-described structures with characteristic lamellar bodies. They are the source of the pulmonary surfactant and the precursors of the alveolar epithelium, which ensure its regeneration after damage. The surfactant, in turn, facilitates breathing mechanics and prevents the collapse of the alveoli and pulmonary edema. In addition, surfactant has antimicrobial and anti-inflammatory properties. It consists mainly of phospholipids and also has 4 bound proteins (SP-A, SP-B, SP-C, SP-D). If SP-B and SP-C are hydrophobic proteins that determine the structure and properties of the pulmonary surfactant itself, then SP-A and SP-D are collectins, which are involved in the reactions of innate immunity, which protects the lungs from inflammatory processes under the influence of many exogenous factors which they are constantly exposed to. The secretory activity of AT2, which creates peripheral protection of the lung, is also associated with the production of lysozyme, beta-defensins 2, cathelicidin, lipocaine 2, restored glutathione, various chemokines, and components of the complement system (C3). Another important feature of AT2 is the ability to facilitate transepithelial transport of sodium ions (from the apical surface to the interstitium), ensuring adequate clearance of the alveolar fluid (Mason, 2006; Da Silva et al., 2021).

The structural disorders we studied, which arised and developed in the lungs of diabetic animals during the 60 days of the experiment, are a reflection of certain features of the compensatory-adaptive mechanism at the cellular and tissue levels.

On the 30th day of the experiment, destructive changes in AT2 (vacuolization of the cytoplasm, disruption of the structure of lamellar bodies, slight expansion of the hEPR, destruction of mitochondria) are probably compensated to some extent. This is in good agreement with the indicators of AKM, TI, TE and TA at this stage of the experiment. Only a significant increase in interstitium thickness (by 0.6 %) due to hyperplasia of collagen fibers (beginning of fibrosis formation) was observed compared to the control.

On the 60th day of the experiment, the pathological structural

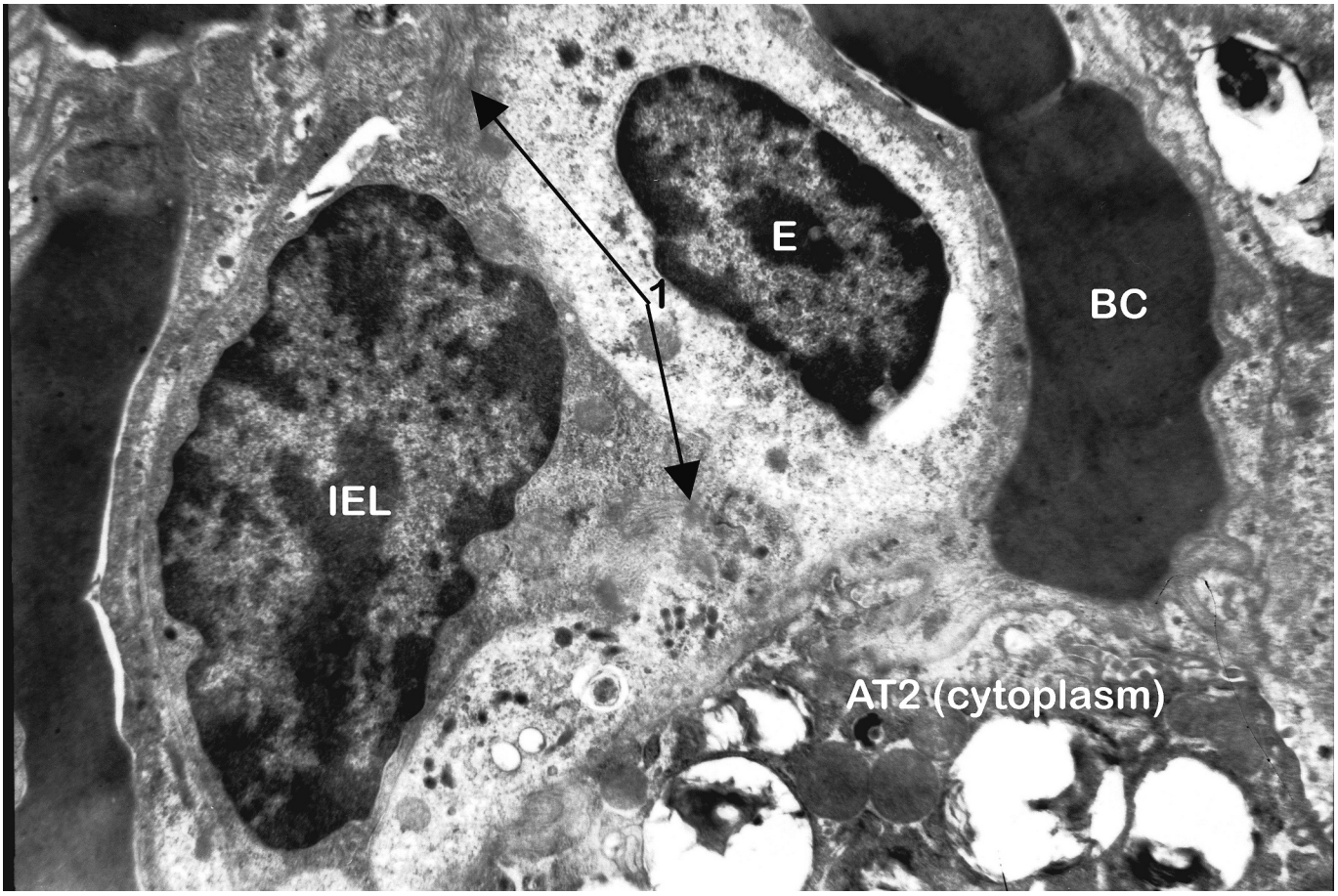

Fig. 2. Ultrastructure of the lung tissue of a young rat with a term of alloxan hyperglycemia of 30 days. Contrast with uranyl acetate and lead citrate; ×4000: AT2 (cytoplasm) – cytoplasm of alveolocyte type 2; E – endotheliocyte; IEL - interepithelial lymphocyte; BC – blood capillary; 1 – hyperplasia of collagen fibers in the lung interstitium.

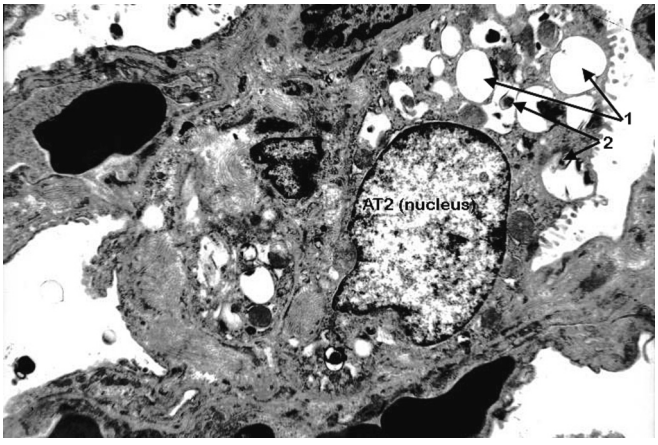

Fig. 3. Ultrastructure of the lung tissue of a young rat with a term of alloxan hyperglycemia of 60 days. Contrast with uranyl acetate and lead citrate; ×4000: AT2 (nucleus) – the nucleus of a type 2 alveolocyte with a karyoplasmic outgrowth and intussusceptions; 1 – emptied autophagic vacuoles; 2 – loosely packed deformed osmiophilic material.

changes in AT2 increased, affecting the cell nuclei and reached a greater expression in the cytoplasm and organelles. Accordingly, at this stage of the experiment, signs of microangiopathy were revealed (swelling and lightening of the cytoplasm of endotheliocytes, significant thickening of the endothelium compared to the 30th day of the experiment). The hyperplasia of collagen fibers was progressing, which caused a significant increase in TI by 2.12 % compared to the previous observation period. Also, the thickness of AKM and TA increased significantly. Therefore, fibrotic changes in the lung tissue of diabetic animals on the 60th day of the experiment became more pronounced and significant.

The violation of the structure of the plate bodies and the organization of osmiophilic material in the AT2 experimental animals, especially for 60 days of hyperglycemia, indicates impaired function of the production of pulmonary surfactant, which is associated with an increase in the risk of collapse of the alveoli, development of edema and insufficiency of respiratory function. Our observations confirm similar results obtained by J.F. Hu et al. in an experiment on modeling diabetes in rats (Hu et al., 2014). Additionally, R. Ruze et al., using the streptozocin model of diabetes, showed an increase in the number of lamellar bodies in AT2 diabetic animals, which can be considered as a compensatory reaction in response to glucotoxicity (Ruze et al., 2020). In this context, it should also be noted that fewer mitochondria found in AT2 were hyperglycemic animals compared to the animals of the control group. This is evidence of existing energy deficiency, which negatively affects the functioning of energy-dependent transepithelial sodium transport, and therefore prevents normal clearance of alveolar fluid and promotes edema (Mason, 2006; Vadász et al., 2007; Gonzales et al., 2015). The accumulation of pathological structures (inclusions) in AT2 under conditions of chronic inflammation under the influence of hyperglycemia (glucotoxicity) modulates the function of cells and indicates a violation of the regulation of a number of cellular processes, such as the synthesis of proteins, lipids, etc. (Ruze et al., 2020) In the study of S. Yu et al., on the streptozocin model of diabetes, a significantly higher number of glycogen

granules was found in AT2 diabetic rats than in control animals (Songyan et al., 2019).

The structural changes associated with the appearance and increase in the number of vacuoles, primary and secondary lysosomes, which occupy a large part of the intracellular space, found by us on the 30th day of the experiment, only increased with increasing the period of hyperglycemia, indicating increased imbalance and irregular metabolic processes. Extended GER in AT2 hyperglycemic animals, noted both for 30 days and for 60 days of the experiment is probably evidence of hyperplasia of its membranes and imbalance of the processes of protein synthesis and their intracellular transport. This condition can be defined as the endoplasmic reticulum (ER) Stress, in response to which a cascade of reactions called unfolded Protein Response (UPR) unfolds in the cells. The initial compensatory nature of UPR under chronic ER stress begins to play a critical pathogenic role in particular in the development of lung fibrosis (Burman et al., 2018). Signs, although volatile, ER stress were installed by K. Kida et al. in the study of morphological changes and elasticity of the lungs in rats on the streptosocin model of diabetes (Kida et al., 1983). Quite clear changes were acquired by the nuclei of AT2 in experimental animals. The loss of the correct form with pronounced outgrowths and invaginations of karyolema was accompanied by marginal condensation of heterochromatin in the form of lumps of different density, especially at the 60th day of the experiment, which indicates an increase in destructive phenomena and a decrease in the functional activity of AT2 nuclei. The development of chronic inflammation due to glucotoxicity causes the growth of connective tissue elements with the subsequent formation of fibrosis, as evidenced by our noted increase in the number of interstitial collagen fibers in hyperglycemic animals. Hyperglycemia induces epithelial-mesenchymal transition in the lungs and the development of fibrosis (Talakatta et al., 2018; Chen et al., 2018; Kida et al., 1983; Yang et al., 2011). In accord with this, Ch.M. Chen et al., found in AT2 of hyperglycemic animals, high expression of Alpha-Smooth Muscle Actin (α-SMA) - actin isoforms, which plays an important role in fibrogenesis (Chen et al., 2018). Morphological changes that reflect pulmonary fibrosis were reproduced by X. Zou et al. in an experimental model of streptozocin diabetes in rats. The authors describe increased deposition of collagen in the interstitium of the lungs against the background of decreased expression of E-cadherin and increased expression of Vimentin, as an important marker of fibroblasts (Zou et al., 2017). We noted the progressive thickening of the AKM, of which the structural component is made of endothelial cells in hyperglycemic animals within the dynamics of the experiment leads, and violation in diffusion of gases. The thickening was also associated with the loss of integrity of the AKM during respiratory movements of the alveoli, which may also be accompanied by the release of blood components into the alveolar spaces and the filling of the alveoli with fluid, thus increasing the insufficiency of respiratory function (McCloud et al., 2004; Forgiarini et al., 2009; Da Silva et al., 2021). Thickening of the alveolar walls and basal membranes, as quite pronounced and important structural changes in the lungs, were noted in experimental studies using both the alloxan model and the streptozocin model of diabetes (Kida et al., 1983; Zou et al., 2017; Li et al., 2018; Al-Qudah et al., 2018; Park et al., 2022). The potential mechanisms that underlie the morphological changes in the lungs in diabetes, including those described by us, form a pathogenetic complex, each link of which requires a thorough explanation. In a review study by Zheng et al., the following mechanisms are highlighted to be activated under the influence of hyperglycemia: 1) non-enzymatic glycosylation of proteins, potentiated by oxidative stress (accumulation of glycation end products disrupts the structure and function of proteins, stimulates the formation of collagen, laminin and fibronectin in the extracellular matrix through the transformation of growth factor -β); 2) activation of Protein kinase C (in turn, activates NADPH oxidase with subsequent generation of reactive oxygen species and increased oxidative stress); 3) activation of the nuclear factor (NF)-κB signaling pathway (increasing oxidative stress); 4) activation of the polyol pathway (one of the main mechanisms of formation of reactive oxygen species in diabetes); 5) oxidative stress (disruption of pro-antioxidant balance due to hyperproduction of reactive oxygen species and depletion of antioxidant protection) (Zheng et al., 2017). Pathomorphological changes in the lungs as a result of the described interconnected mechanisms determine the development of pulmonary microangiopathy, fibrosis, and surfactant deficiency, which, in combination with a decrease in the function of the respiratory muscles, manifests itself in lung function disorders in diabetes with corresponding clinical symptoms (a decrease in the parameters of lung ventilation, an increased tendency to obstructive and infectious diseases). In view of this, the state of the system of pro-antioxidant balance of the body in conditions of diabetes deserves special attention from the point of view of a possible therapeutic target for the prevention of the development of comorbid conditions, related to the lungs in particular.

Finally, the data obtained by us can be considered as experimental confirmation of ultrastructural changes in the lungs in diabetes, described in a number of examinations and clinical studies (Rajasurya et al., 2020).

Limitations of our research include, longer intervals between “control points” (30, 60 days) and impossibility of the applied methods to establish in animals of the experimental group, the degree of violation of the synthesis of osmiophilic material in type 2 alveolocytes which is the main component of the surfactant system. Given the peculiarities of compensatory and adaptive reactions in animals during the development of diabetes in the experiment, studies with shorter intervals (5, 10, 15, etc. days) may be promising (Ighodaro et al., 2017). Studies of the relationship between morphological changes and the immunocompetent properties of AT2 at successive stages of the development of alloxan diabetes seem promising, because it is known that collectins SP-A and SP-D (surfactant components) participate in the reactions of innate immunity (Mason, 2006; Da Silva et al., 2021). It is also necessary to resolve the issue of determining the specifics and strength of the specific influence of factors such as: hyperglycemia, insulin resistance, microangiopathy and neuropathy on the formation of the diabetic pathomorphological complex in the lungs.

5. Conclusion

The results of our study demonstrate complex ultrastructural changes in type 2 alveolocytes and lung tissue in general animals with chronic hyperglycemia. These changes are primarily the disorganization of the plate bodies, the reduction of the number of mitochondria, the structural features of the ER stress, the destruction of the cytoplasm and nucleus, the development of fibrosis, and the thickening of the alveolar-capillary membrane. All of the above is the morphological basis of the formation of the pulmonary symptom complex against the background of diabetes.

Ethical statement

This scientific study was reviewed and approved by the bioethics committee of Medical Institute of Sumy State University and was carried out under the guidelines of the European Convention for the Protection of Vertebrate Animals (Strasbourg, March 18, 1986), directives of the European Parliament and the Council of the EU dated 22.09.2010 and “General ethical principles of animal experiments” adopted by the First National Congress on Bioethics (Kyiv, 2001, Law of Ukraine “On Medicinal Products”, 1996), Articles 7, 8, 12, ICH GCP (2008), GLP (2002), in accordance with the requirements and standard provisions on ethics of the Ministry of Health of Ukraine N№690 dated 09/23/2009 (excerpt from protocol N№ 5/5 dated 05/15/2019).

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CRediT authorship contribution statement

Toufik Abdul-Rahman: Conceptualization, Methodology, Data curation, Writing – original draft, Validation, Software, Writing – review & editing. **Andrew Awuah Wireko:** Conceptualization, Methodology, Data curation, Writing – original draft, Validation, Writing – review & editing. **T.P. Teslyk:** Conceptualization, Methodology, Data curation, Writing – original draft, Validation, Software. **Serhii Dmytruk:** Writing – original draft, Writing – review & editing. **Iryna Shkolna:** Writing – original draft, Writing – review & editing.

Declaration of competing interest

Authors wish to declare no conflict of interest. Authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability

Data will be made available on request.

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