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Стаття надійшла 14.07.2021 р.

DOI 10.26724/2079-8334-2022-3-81-214-218
UDC 616.24+616.092.9+616.379-008.64

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FEATURES OF LIPOPEROXIDATION AND MORPHOLOGICAL CHANGES OF THE LUNGS IN EXPERIMENTAL DIABETES MELLITUS

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In recent decades, diabetes has become one of the most significant medical and social problems. The prevalence and incidence of diabetes have increased dramatically in the last few years. The experiments were performed on white male rats with streptozotocin-induced diabetes. Biochemical analysis has shown that in the conditions of experimental diabetes there is an intensification of lipid peroxidation processes throughout the study period, as evidenced by a significant increase in the content of active products of thiobarbituric acid ($p<0.001$). The probable increase in the level of secondary products of lipid peroxidation leads to a violation of the ultrastructural organization of the components of the respiratory part of the lungs, as indicated by the results of our electron microscopic study. We found that the most expressed changes of dystrophic-destructive nature are observed in the hemocapillaries of the alveolar wall. Red blood cells sludges, adhesion, and aggregation of white blood cells and platelets are observed in the lumen of microvessels that promote the capillary-trophic insufficiency.

Key words: streptozotocin-induced diabetes, lipid peroxidation, respiratory part of the lungs.

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ОСОБЛИВОСТІ ЛІПОПЕРОКСИДАЦІЇ ТА МОРФОЛОГІЧНІ ЗМІНИ ЛЕГЕНЬ ПРИ ЕКСПЕРИМЕНТАЛЬНОМУ ЦУКРОВОМУ ДІАБЕТІ

За останні десятиліття цукровий діабет став однією з найактуальніших як медичних так і соціальних проблем. Поширеність та захворюваність цукровим діабетом різко зросла протягом останніх років. Експерименти проводено на білих щурах-самцях з стрептозотоцин-індукованим діабетом. Біохімічні дослідження сироватки крові показали, що в умовах змодельованого цукрового діабету спостерігається інтенсифікація процесів перекисного окиснення ліпідів протягом всього періоду дослідження про що свідчить достовірне збільшення вмісту активних продуктів тіобарбітурової кислоти ($p<0.001$). Вірогідне підвищення рівня вторинних продуктів перекисного окиснення ліпідів призводить до порушення ультраструктурної організації компонентів респіраторного відділу легень на що вказують результати проведеного нами електронномікроскопіного дослідження. Встановлено, що найбільш виражені зміни дистрофічно-деструктивного характеру спостерігаються в гемокапілярах альвеолярної стінки. У просвіті мікросудин відмічаються еритроцитарні сладжі, адгезія та агрегація лейкоцитів і тромбоцитів, що сприяє розвитку капіляротрофічної недостатності.

Ключові слова: стрептозотоцин-індукований діабет, перекисне окиснення ліпідів, респіраторний відділ легень.

The study is a fragment of the research project "Pathogenetic mechanisms of changes development in the organs of the respiratory, endocrine, and nervous systems in modeled pathological conditions and their correction", state registration No. 0117U001758.

In recent decades, diabetes mellitus (DM) has become one of the most significant medical and social problems [7, 9, 12]. Despite the use of modern treatment strategies, the prevalence and incidence of DM have risen sharply in recent years, indicating a global epidemic. Scientists predict that in the 21st century diabetes will be a disease that reduces life on a global scale [6, 11]. According to the World Health Organization and the International Diabetes Federation (IDF), the number of people with DM worldwide will

increase to 629 million by 2045. According to the IDF, there is a trend toward the highest prevalence of diabetes among the urban working population aged 40–59 in developing countries [9, 11, 12]. Many experimental and clinical studies have shown that the activation of lipoperoxidation processes in blood serum is one of the common pathogenetic links in many diseases, including DM [2–5]. It is known that the reaction of free radical oxidation of lipids is a universal metabolic process, which is present in all organs and tissues. Lipid peroxidation (LPO) reactions in the body are a physiological process and a necessary component of oxidative homeostasis. They can modify the structure and function of cell membranes and can determine the nature of intercellular and interorganic relationships within a particular functional system [3, 5].

In recent years numerous studies have shown that DM is a metabolic disorder with deleterious effects on many organs, such as kidneys, liver, eyes, and heart. However, currently, there is also emerging evidence that the lungs are one of the target organs in diabetes because they have a huge, well-developed microvascular network [7, 8, 10, 14]. According to the literature data, the microcirculatory vessels are the first being influenced by pathogenic agents and the first to provide a vascular response to an organ or tissue. Electron microscopic studies allow the detection of changes at the microcirculatory level in the early stages of diabetic angiopathy [8, 12, 14].

It is established that the activation of lipid peroxidation, and in particular thiobarbituric acid reactive substances (TBARS) leads to the damage of cell membranes and cell structures and along with metabolic acidosis contributes to lung tissue damage [3, 4, 5, 14].

The purpose of the study was to investigate the dynamics of the oxidative system of blood and ultrastructural changes in the components of the respiratory part of the lungs in animals with experimental diabetes mellitus.

Materials and methods. The experiments were performed on 88 white male Wistar rats weighing 170–210 g, which were kept on a standard diet with free access to water. Animals were divided into three groups: 1 – intact (n=10); 2 – control (n=40); 3 – experimental (n=38) with a model of diabetes mellitus, which was reproduced by intraperitoneal injection of streptozotocin by “Sigma" company (USA), diluted in 0.1 M citrate buffer with a pH of 4.5, at a rate of 60 mg/kg body weight. The control group of animals received an intraperitoneal injection with an equivalent dose of 0.1 M citrate buffer solution with a pH of 4.5.

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 National Congress on Bioethics (Kyiv, 2013).

The Commission on Bioethics of Ivano-Frankivsk National Medical University believes that the research does not contradict the basic bioethical standards (Minutes № 106/19 of 07.02.2019).

All studies were performed under thiopental-sodium anesthesia at a rate of 60 mg/kg body weight. Blood samples for biochemical testing were performed 14, 28, 42 and 70 days after streptozotocin injection. The serum content of TBARS was determined by the following method [1].

Pulmonary tissue collection for electron microscopy was performed over a similar period. When collecting the material, the generally accepted rules of cutting speed and atraumatic were kept. Samples of lung tissue were fixed in a 2.5 % solution of glutaraldehyde, followed by fixation in a 1 % solution of osmium tetroxide. After dehydration, the material was poured into epon-araldite. Sections obtained on an ultramicrotome “Tesla BS-490” were studied in an electron microscope “PEM-125K”.

The STATISTICA 10 program was used for statistical processing of the obtained results. Using the possibilities of descriptive statistics, all the quantitative data obtained in the study were first checked for the type of their distribution by the Shapiro-Wilk test. Since the vast majority of these data were consistent with Gauss's normal law, the arithmetic mean ± standard error of mean (M±m) was chosen to describe the central trend, and a parametric t-test (Student's test) was chosen to assess the reliability of differences in the results obtained in the comparison groups (experimental and control) and to test the null hypothesis. To assess the reliability of data changes in the dynamics (14, 28, 42, 70 days) within each of the comparison groups we used a non-parametric method for three or more comparison groups – Friedman's test and Kendall's coefficient of concordance (Friedman ANOVA and Kenall Coef. of Concordance).

Results of the study and their discussion. As can be seen in table 1 and fig. 1 below in the conditions of modeled diabetes there is an increase in the content of TBARS in the serum relative to the control group of animals at all stages of the experiment. The value of TBARS in serum was found to be higher by 36.2 % ($p<0.001$) relative to the control group of animals 14 days after modeling diabetes mellitus. 28 days after the experiment, the level of TBARS LPO in the serum was increased by 55.5 % ($p<0.001$) compared with the same control group of animals.

Table 1

The content of TBARS (nmol/ml) in the serum of white rats in experimental diabetes mellitus

Group	14 days		28 days		42 days		70 days		p_2
	M	±m	M	±m	M	±m	M	±m	
Experiment	5.64*	0.11	6.39*	0.09	6.94*	0.08	7.71*	0.14	<0.001
Control	4.14	0.05	4.11	0.02	4.12	0.03	4.10	0.04	>0.05
p_1	<0.001		<0.001		<0.001		<0.001		x
Intact	4.11±0.02								

Notes: p_1 – the reliability of the difference between the data of the experimental and control groups; p_2 – reliability of data within the group in the dynamics; * – reliability of the data difference compared to the intact group.

Determination of serum TBARS content after 42 days showed a further increase in this indicator. In particular, it was found that the level of TBARS in the serum at the time of the study exceeded the value of the same indicator of the control group of animals by 68.4 % ($p<0.001$). With increasing duration of the experiment (70 days), the level of TBARS LPO was higher compared to the control group of animals by 88.0 % ($p<0.001$).

The next stage of our study was the analysis of ultrastructural changes in the components of the respiratory part of the lungs in modeled diabetes mellitus. Conducted submicroscopic analysis showed that 14 days after the start of the experiment, the nucleus of type I alveolocytes (A-I) and type II alveolocytes (A-II) mainly were with a matrix of medium electron-optical density and equitable distribution of chromatin granules. Mitochondria were of various sizes and shapes. Components of the Golgi apparatus (GA) and granular endoplasmic reticulum (ER) were without significant structural changes. The basement membrane throughout retained its characteristic structure. The lamellar bodies

(LBs) of various degrees of maturity, size and form were noted in the cytoplasm of A-II. The individual mitochondria were found enlarged in volume with single reduced crystals in the endotheliocytes of hemocapillaries. The constituent components of GA and ER were moderately expanded. A large number of micropinocytic vesicles were observed in the peripheral parts of endotheliocytes. An increased number of neutrophils was detected in the lumen of some hemocapillaries.

The swollen mitochondria with single disoriented cristae were observed in some A-I with increasing study duration (28 days). Cisterns and tubules of GA and ER were expanded. In the peripheral part of the cells, there was a large number of micropinocytic vesicles. In A-II, some mitochondria were enlarged with an enlightened matrix and shortened cristae. The constituent components of GA and ER were expanded. The deformed LBs, partially filled with phospholipid matrix, were determined in the cytoplasm of some A-II. Nuclei of endothelial cells were with fine-grained matrix and marginal localization of chromatin granules. Mitochondria were with a matrix of low electron-optical density. Fragmentation of ER membranes was sometimes observed along with expanded AG cisterns. The basement membrane was locally thickened. Adhesion and aggregation of leukocytes and erythrocyte aggregates were determined in the lumen of some hemocapillaries.

At the electron microscopic level, it was found that the most significant changes in the respiratory part of the lungs were observed 42-70 days after the start of the experiment. Thus, after 42 days, nuclei of A-I were enlarged in size with a nucleoplasm of low electron-optical density and marginally placed chromatin. The nuclear membrane formed shallow intussusception. Mitochondria were swollen with single reduced crystae. The GA was presented by vesicular dilated cisternae and a small number of vesicles. The tubules of the ER were deformed and filled with fine-grained content of weak electron density. The number of ribosomes on their membranes was reduced. Fragmentation of ER membranes was observed in some cells. A-I with local ruptures of the apical plasmalemma, especially its peripheral parts, were sometimes observed. The basement membrane of A-I was significantly thickened. The phenomena of hyperhydration were determined in A-II too. Mitochondria of cells were of different sizes with an enlightened matrix and disoriented cristae. Signs of destruction were observed in GA and ER. Numerous lamellar cells were at different stages of vacuolation. The number of microvilli on the apical surface was reduced. The basement membrane was thickened in many areas with blurred contours. Severe edema was also observed in the endothelial cells of hemocapillaries. The nuclei of endothelial cells were with an enlightened matrix. The perinuclear space was expanded. Mitochondria were with a matrix of low electron-optical density and disorganization of the cristae. Cisternae and tubules of the GA and the ER were expanded. The number of ribosomes on the membranes of the latter was reduced. Along with this, fragmentation of ER membranes was noted. The basement membrane was sharply thickened. In some hemocapillaries, a violation of the integrity of the lumenal membrane of endothelial cells was detected, which was accompanied by the release of intracellular contents into the lumen of the microvessel. The characteristic feature of this period of research was the presence of erythrocyte aggregates in the lumen of hemocapillaries and leukocyte adhesion (fig. 2). In addition, adhesion and aggregation of platelets were observed in some hemocapillaries.

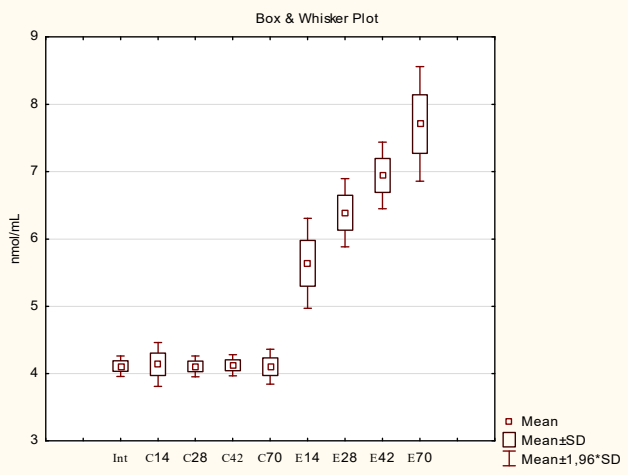

Fig. 1. Dynamics of TBARS content (nmol/ml) in the serum of white rats in experimental diabetes mellitus. Notes: groups of animals: Int – intact; C – control; E – experimental. 14, 28, 42, 70 – days of the experiment

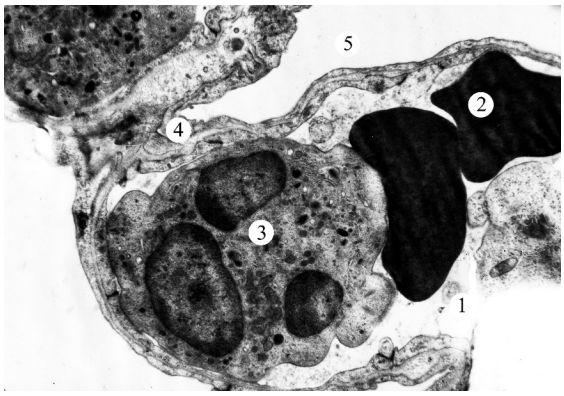

Fig. 2. Ultrastructural organization of the alveolar wall 42 days after the start of the study. Electronic microphotography. x6400. 1 – hemocapillary lumen; 2 – erythrocyte; 3 – leukocyte; 4 – peripheral part of the alveolocyte type I; 5 – lumen of the alveoli.

With increasing study time (70 days), pronounced violations of the structural organization of the components of the respiratory part of the lungs led to the release of blood cells into the interstitial tissue and alveoli with the development of interstitial and intraalveolar edema.

Our biochemical studies of blood serum showed that in the conditions of modeled DM there is an intensification of free radical oxidation of lipids during the whole period of the experiment, as evidenced by the increase in the content of thiobarbituric acid reactive substances of LPO.

It is noteworthy that in each subsequent period of the study, the content of thiobarbituric acid reactive substances of LPO in serum was significantly higher than in the previous one ($p<0.001$). The maximum increase in the level of secondary products of LPO was observed 70 days after modelling of DM in comparison with the same indicator of the control group of animals ($p<0.001$). The results of our research confirm the data of other authors, which indicate a significant activation of LPO processes in the long-term DM [3, 7, 13]. Numerous studies have shown that under the action of various exogenous and endogenous factors there is an overproduction of secondary products of lipid peroxidation, which leads to cell damage and plays a key role in disorganizing the ultrastructure of membranes [7, 13].

Our electron microscopic studies of the respiratory part of the lungs are consistent with data from other researchers and indicate the important role of free radical oxidation of lipids in the mechanisms of lung tissue damage in diabetes and other diseases [4, 13].

Conclusions

1. Experimental diabetes mellitus is accompanied by an intensification of lipoperoxidation processes throughout the study period, which indicates a significant ($p<0.001$) increase in serum thiobarbituric acid reactive substances.

2. Disturbances of ultrastructural organization of components of respiratory part of lungs in the conditions of the modelled diabetes mellitus are most expressed in hemocapillaries of an alveolar wall as evidenced by the existence of erythrocyte aggregates, adhesion and aggregation of neutrophils and platelets.

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

The prospect of further research is the correction of lipid peroxidation processes and structural changes in the components of the respiratory part of the lungs in experimental diabetes mellitus.

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Стаття надійшла 25.07.2021 р.

DOI 10.26724/2079-8334-2022-3-81-218-222
UDC 612.127.2:616.314–002.4+616.314.17–008.1[612.015.6]

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INFLUENCE OF COMPLEX OF PREPARATIONS ON THE STATE OF THE ORAL CAVITY TISSUES IN RATS UNDER THE CONDITIONS OF EXPERIMENTAL HYPOTHYROIDISM

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The research is dedicated to studying the state of the dentoalveolar system and tissues of the oral cavity of rats under the influence of the complex under the action of experimental hypothyroidism. Experimental hypothyroidism was simulated in 8 1-month-old rats (group 2). The intact group (group 1) consisted of 8 rats. 7 rats received a complex under conditions of experimental hypothyroidism (group 3). The duration of the study was 30 days. The complex of preparations in conditions of insufficiency showed a significant caries-preventive and periodontal-protective effect. Under the action of the complex, an increase in the activity of alkaline phosphatase in the periodontal bone tissue and dental pulp was revealed, as well as a decrease in the activity of acid phosphatase in the bone of the alveolar process.

Key words: plant polyphenols, rats, biochemical markers, hypothyroidism modeling, stinging nettle preparation.

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ВПЛИВ КОМПЛЕКСУ ПРЕПАРАТІВ НА СТАН ТКАНИН РОТОВОЇ ПОРОЖНИНИ ЩУРІВ ЗА УМОВ ЕКСПЕРИМЕНТАЛЬНОГО ГІПОТИРЕОЗУ

Дослідження присвячено вивченню стану зубощелепної системи та тканин ротової порожнини щурів під впливом комплексу при дії експериментального гіпотиреозу. У 8 щурів 1-місного віку проводили моделювання експериментального гіпотиреозу (2-а група). Інтактну групу (1 група) склали 8 щурів. У 3-й групі 7 щурів отримували комплекс на тлі експериментального гіпотиреозу. Тривалість дослідження становила 30 днів. Комплекс препаратів в умовах недостатності виявив значну карієс-профілактичну та пародонтопротекторну дію. Під дією комплексу виявлено збільшення активності лужної фосфатази в кістковій тканині пародонту та пульпі зубів, а також зниження активності кислої фосфатази в кістці альвеолярного відростка.

Ключові слова: рослинні поліфенолі, щури, біохімічні маркери, моделювання гіпотиреозу, препарат кропиви дводомної.

The work is a fragment of the research project "Influence of hypoxia on the processes of collagen formation and mineralization on models of dental pathology and correction of the obtained disorders", state registration No. 0118U006963.

Hypothyroidism is a syndrome, a condition of the body associated with a reaction to a low level of concentration of thyroid hormones (TH). Hypothyroidism is associated with functional insufficiency of TH. Iodine is a trace element involved in the construction of TH. Between 60 % and 80 % of the total thyroid hormone produced by TH enters the blood in the form of thyroxine [1, 3]. Thyroxin affects all body tissues; there are no specific target cells for it. This hormone is able to cross the membrane and bind to