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THE ROLE OF ENDOGENOUS INTOXICATION IN THE LUNG INJURY DEVELOPMENT IN EXPERIMENTAL DIABETES MELLITUS

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Key words: diabetes mellitus; Streptozotocin; lung injury

Today diabetes mellitus (DM) takes one of the major places among such common human diseases as cancer, cardiovascular and mental diseases. However, not all aspects of the pathogenesis of the disease are sufficiently studied, and the proof of this is the steady increase in morbidity, high mortality and complication rate. The object of the study was the blood of 48 albino Wistar male rats divided into 2 groups: the 1st group is intact (8 animals) and the 2nd group is experimental animals with streptozotocin-induced diabetes (n = 40). The markers of endogenous intoxication (EI) degree were determined in the blood with the help of the level of the medium molecular weight peptides (MMWP), leukocyte intoxication index (LII) by Kalf-Kalif, lactate content (LC) and the coefficient of leukocyte lung regulation (CLLR). As a result of the study conducted a significant increase in the indices of EI was found due to progression of $MMWP_1$ (254 nm) and $MMWP_2$ (280 nm) by 14.89% and 17.08%, respectively, on the 14-th day of the experiment. After 28 days of the study LC increased almost 4 times, LII – twice and CLLR – by 3.4 times. The research results obtained have shown increase in EI markers of diabetes, among them CLLR appeared to be the most sensitive. CLLR proposed by us indicates a pronounced delay of leukocytes in the microcirculatory bloodstream of the lungs under conditions of streptozotocin-induced diabetes mellitus; in its turn, it leads to the lung injury.

Diabetes mellitus (DM) is a disease of metabolism; its basis is persistent hyperglycemia, the lack of insulin secretion and/or insulin resistance. Chronic hyperglycemia is the basis of numerous complications of the cardiovascular system, nervous system, eyes, kidneys and other organs [7, 11].

According to the WHO data in highly developed countries up to 4-6% of the population suffers from diabetes. With age the incidence of DM increases and after 65 years reaches to 10-15%. Moreover, almost 80% are chronic complications of diabetes. Mortality in diabetes increases by 2-3 times, and the life expectancy is reduced by 10-30%. The WHO Director-General Margaret Chan (2008) referred DM to four common human diseases such as cancer, cardiovascular and mental diseases being the major burden for health care. It is known that every 13-15 years the number of people with diabetes is doubled. Mostly it refers to the number of patients with type 2 DM [10].

Recent decades have been marked by significant advances in understanding the nature of type 1 and type 2 DM, its diagnosis and treatment. However, not all aspects of the pathogenesis of the disease are sufficiently studied, and the proof of this is the steady increase in morbidity, high mortality and complication rate. Diabetic microangiopathy and neuropathy are in the basis of them. In diabetic patients there is a significant risk of development of atherosclerosis and coronary heart disease. More than 40% of amputations of lower limbs are the consequence of diabetic foot syndrome. Diabetes is also the most common cause of blindness in human. All of the abovesaid leads to considerable investments aimed at treating diabetes and its complications. According to the data of the International Diabetes Federation the treatment cost per one patient with diabetes with at least one chronic complication is 6-18 thousand dollars a year [9].

It is known that diabetes causes disorder of carbohydrate metabolism, first of all, but all links of metabolic processes in the body are affected [3, 7]. Therefore, the study of the endocrine system, as well as the lungs as one of the main organs of disintoxication in diabetes, is an urgent task.

The aim of the research was to study the role of the main markers of endogenous intoxication in experimental diabetes mellitus.

Materials and Methods

The object of the study was the blood of 48 albino Wistar male rats divided into 2 groups: the 1st group is intact (8 animals) and the 2nd group is experimental animals with streptozotocin-induced diabetes (n = 40). To model diabetes we selected animals aged 2 months (with the weight of 90-100 g). Streptozotocin ("Sigma", USA) was introduced as a single intraperitoneal injection in the dose of 60 mg/kg, immediately before the injection it was dissolved in 0.5 ml of 0.1 M citrate buffer (pH = 4.5) [4]. The solution was injected quickly as within 1 minute its activity reduced twice. Our study and manipulations complied with the requirements of the Law of Ukraine "About protection of animals against cruel treatment" (No. 1759-VI from 15.12.2009) and the international principles of the European Convention for the Protection of Ver-

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tebrate Animals Used for Experimental and Other Scientific Purposes.

Development of the disease was monitored by increase of the glucose level in the animals' blood, which was 10-15 mmol/L. Blood sampling for the study was carried out under ketamine anesthesia (40 mg/kg); for this purpose 3 ml of blood were taken from the tail vein on the 14 and 28 days of the experiment. Along with traditional laboratory parameters the degree of endotoxemia was analyzed with the help of the level of the medium molecular weight peptides (MMWP) determining the leukocyte intoxication index (LII) by Kalf-Kalif and lactate content (LC) [6].

The assessment of the regulatory activity of the lungs was determined by the coefficient of leukocyte lung regulation (CLLR) proposed (the patent of Ukraine No.UA 71009) [1]. The methodological basis for its implementation was the comparative analysis of the white blood cells of the venous and arterial blood, the sampling was performed simultaneously. To calculate the WBC count in rats 0.4 ml of blood from the right ventricle (V) and 0.4 ml of blood from left ventricle (A) were collected. CLLR was calculated using the formula: (V-A) / A x 100% where A is the number of leukocytes in the arterial blood, V is the number of leukocytes in the venous blood.

The data obtained were processed using the nonparametric criteria on a personal computer with the help of "Statistica 6" programme ("Statsoft, Inc." – USA). The reliability was assessed by Wilcoxon test. In correlation analysis the Spearman's rank correlation coefficients (*R*) were determined. Differences were considered to be reliable if the value *R* was 95% and more ($p<0.05$).

Results and Discussion

As a result of the study conducted it has been found that the concentration of the major markers expressing the intoxication syndrome – MMWP significantly increased after 14 days from the start of simulation of DM, exceeding the reference levels of $MMWP_1$ (254 nm) and $MMWP_2$ (280 nm), respectively, by 14.89% and 17.08%. On the 28-th day of the study MMWP continued to grow significantly over the parameters of intact animals. Thus, the main components of MMWP fraction are medium molecular weight peptides. Decomposition of the protein molecules resulting in the formation of medium molecular weight peptides occurs with the action of proteinases. Since the level of MMWP depends, on the one hand, on intensity of biopolymer decomposition, and, on the other hand, on the rate of excretion through detoxication organs, we can think of a violation of both components of this process [2, 5, 6].

Another indicator that shows the development of endogenous intoxication (EI) is the level of the lactate content (LC). More than two-fold increase in the concentration of LC already at the first stage of the study should be noted, and it, in turn, may indicate hypoxic conditions as a result of the inadequate oxygen supply to tissues. With the extension of the experiment duration the level of LC increases exceeding significantly the control results more than 4 times on the 28-th day. The LC elevation from the early stages of diabetes may also indicate the involvement and increased use of leukocytes as a reaction of the nonspecific cellular links of immune protection in response to development of EI, as well as their retention in the lung tissue due to excessive activation and sequestration and subsequent destruction of the structural components of the respiratory system with general progression of destructive phenomena [9].

To confirm assumptions about active participation of leukocytes in the processes of EI development, taking into account the results obtained, it was decided to evaluate the response of white blood cells to development of DM and lung injury in rats. With this purpose the LII was determined by Kalf-Kalif. In 14 days from the beginning of the experiment a significant increase of the LII by 48.23% was recorded, it continued to increase and on the 28-th day exceeded the indices of the first group almost twice. These results may indicate the excessive involvement of leukocytes, particularly neutrophils in the inflammatory process and their significant death [2].

To assess the role of leukocytes in lung injury we analyzed the quantitative difference of white blood cells in the venous and arterial blood, and CLLR was calculated. Thus, it was found that at the first stage of the experiment CLLR was significantly higher than in control animals and was 4.38± ±1.32% in relation to 1.52±0.99% in intact animals. In 28 days from the beginning of the study the increase of CLLR was determined; it prevailed by 3–4 times over those data observed in the first group of rats. These results indicate a significant delay of leukocytes activated by endotoxins in the microcirculatory bloodstream of the lungs, and subsequently it leads to pathological changes in the respiratory organs as we have confirmed in the previous morphological studies [3].

Thus, hyperglycemia causes development of the oxidative stress and alters the activity of antioxidant enzymes. These facts were published earlier [2, 6, 11]. That is why the processes of lipid peroxidation are significantly activated by prolonged duration of diabetes and play a certain role in development of its late complications, including lung injury [5, 8]. So, we believe that one of the key and perhaps a turning point in progression of respiratory pathology in DM is a significant delay of leukocytes activated by endotoxins in the respiratory system with their further aggression as for a "target

organ", which is the microcirculatory bloodstream of the lungs [3, 10].

CONCLUSIONS

1. Under the conditions of the experimental streptozotocin-induced diabetes mellitus increase in markers of endogenous intoxication, such as the medium molecular weight peptides, lactate, leukocyte intoxication index and the coefficient of leukocyte lung regulation has been observed.

2. The research results obtained have shown that CLLR proposed by us indicates a pronounced delay of leukocytes in the microcirculatory bloodstream of the lungs under conditions of streptozotocin-induced diabetes mellitus; in its turn, it leads to the lung injury.

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РОЛЬ ЕНДОГЕННОЇ ІНТОКСИКАЦІЇ У РОЗВИТКУ ЛЕГЕНЕВОГО УШКОДЖЕННЯ ПРИ ЕКСПЕРИМЕНТАЛЬНОМУ ЦУКРОВОМУ ДІАБЕТІ

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Ключові слова: цукровий діабет; стрептозотоцин; легеневе ушкодження

Сьогодні цукровий діабет (ЦД) посідає одне з провідних місць серед основних поширених захворювань людини: серцево-судинних, раку, психічних хвороб. Однак не всі особливості патогенезу цього захворювання достатньо вивчені, і доказом цього є неухильний ріст захворюваності, висока смертність і частота ускладнень. Об'єктом дослідження була кров 48 білих щурів-самців лінії Вістар, яких було розділено на 2 групи: 1 – інтактна (8 тварин) і 2 – тварини з експериментальним стрептозотоцин-індукованим діабетом (n=40). У крові визначали маркери ступеня ендогенної інтоксикації (ЕІ) за допомогою рівня молекул середньої маси (МСМ), лейкоцитарного індексу інтоксикації (ЛІІ) за Кальф-Каліфом, за вмістом лактату (ЛТ) та коефіцієнтом легеневої регуляції за лейкоцитами (КРЛ). У результаті дослідження встановлено достовірне зростання показників ЕІ за рахунок прогресування $МСМ_1$ (254 нм) та $МСМ_2$ (280 нм) відповідно на 14,89% та 17,08% уже на 14 добу експерименту. ЛТ зріс до кінця дослідження майже у 4 рази, ЛІІ – удвічі, а КРЛ – у 3,4 рази. Отримані результати свідчать про наростання маркерів ЕІ при ЦД, серед яких найбільш чутливим виявився КРЛ. Запропонований нами КРЛ вказує на виражену затримку лейкоцитів у гемоциркуляторному руслі легень при стрептозотоциновому діабеті, що, в свою чергу, спричиняє легеневе ушкодження.

РОЛЬ ЭНДОГЕННОЙ ИНТОКСИКАЦИИ В РАЗВИТИИ ЛЕГОЧНОГО ПОВРЕЖДЕНИЯ ПРИ ЭКСПЕРИМЕНТАЛЬНОМ САХАРНОМ ДИАБЕТЕ

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Ключевые слова: сахарный диабет; стрептозотоцин; легочное повреждение

Сегодня сахарный диабет (СД) занимает одно из ведущих мест среди основных распространенных заболеваний человека: сердечно-сосудистых, рака, психических болезней. Однако не все стороны патогенеза этого заболе-

вания достаточно изучены, и доказательством этого является неуклонный рост заболеваемости, высокая смертность и частота осложнений. Объектом исследования была кровь 48 белых крыс-самцов линии Вистар, которые были разделены на 2 группы: 1 – интактная (8 животных) и 2 – животные с экспериментальным стрептозотоцин-индуцированным диабетом (n = 40). В крови определяли маркеры степени эндогенной интоксикации (ЭИ) с помощью уровня молекул средней массы (МСМ), лейкоцитарного индекса интоксикации (ЛИИ) по Кальф-Калифу, содержания лактата (ЛТ) и коэффициента легочной регуляции по лейкоцитам (КРЛ). В результате исследования установлено достоверное увеличение показателей ЭИ за счет прогрессирования МСМ1 (254 нм) и МСМ2 (280 нм) соответственно на 14,89% и 17,08% уже на 14 сутки эксперимента. ЛТ вырос к концу исследования почти в 4 раза, ЛИИ – вдвое, а КРЛ – в 3,4 раза. Полученные результаты свидетельствуют о нарастании маркеров ЭИ при СД, среди которых наиболее чувствительным оказался КРЛ. Предложенный нами КРЛ указывает на выраженную задержку лейкоцитов в гемоциркуляторном русле легких при стрептозотоциновом диабете, что, в свою очередь, способствует легочному повреждению.