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Реферати

МОРФОМЕТРИЧНА ХАРАКТЕРИСТИКА ЄМНІСНОЇ ЛАНКИ ГЕМОМІКРОЦИРКУЛЯТОРНОГО РУСЛА СЛИННИХ ЗАЛОЗ ЩУРІВ В НОРМІ ТА ПРИ ХРОНІЧНІЙ ІНТОКСИКАЦІЇ ЕТАНОЛОМ

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В роботі представлені дані морфометричного дослідження при хронічній інтоксикації етанолом. Встановлено, що хронічна інтоксикація етанолом впливає на ємнісну ланку гемомікроциркуляторного русла часточок піднижньощелепної слинної залози. На ранніх термінах спостереження визначається розширення венул, що підтверджується достовірним збільшенням зовнішнього діаметру та діаметру просвіту із зменшенням товщини судинної стінки. З дванадцятої доби спостерігається тенденція до відновлення метричних показників. Нормалізація показників до тридцятої доби не визначається.

Ключові слова: хронічна інтоксикація етанолом, щури, слинні залози, венули.

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МОРФОМЕТРИЧЕСКАЯ ХАРАКТЕРИСТИКА ЕМКОСТНОГО ЗВЕНА ГЕМОМИКРОЦИРКУЛЯТОРНОГО РУСЛА СЛЮННЫХ ЖЕЛЕЗ КРЫС В НОРМЕ И ПРИ ХРОНИЧЕСКОЙ ИНТОКСИКАЦИИ ЭТАНОЛОМ

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В работе представлены данные морфометрического исследования при хронической интоксикации этанолом. Установлено, что хроническая интоксикация этанолом влияет на емкостное звено гемомикроциркуляторного русла частиц поднижнечелюстной слюнной железы. На ранних сроках наблюдения определяется расширение венул, что подтверждается достоверным увеличением наружного диаметра и диаметра просвета с уменьшением толщины сосудистой стенки. С двенадцатого дня наблюдается тенденция к восстановлению метрических показателей. Нормализация показателей до тридцатого дня не определяется.

Ключевые слова: хроническая интоксикация этанолом, крысы, слюнные железы, венулы.

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MORPHOFUNCTIONAL CHANGES IN THE LIVER OF 24-MONTH-OLD RATS IN THE EARLY STAGES OF EXPERIMENTAL DIABETES MELLITUS DEVELOPMENT

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The purpose of the research work was to establish the peculiarities of morphofunctional changes in the liver in the early stages of streptozotocin-induced diabetes mellitus (DM). Diabetes mellitus was induced by a single intraperitoneal injection of streptozotocin (5mg per 100g of body weight). Specimens for the study were collected on the 14th and 28th day of the experiment. Histologic, electron-microscopic, and biochemical methods of investigation were used. The study revealed that in streptozotocin-induced diabetes mellitus with the increase of levels of glucose and glycosylated hemoglobin the granular degeneration of hepatocytes with the transition to vacuolar degeneration takes place. Structural changes in the liver lead to its dysfunction, which is confirmed by the increased levels of specific hepatic aminotransferases and the decreased De Ritis ratio starting from the 14th day of the experiment. Such changes are followed by the increased activity of pro-oxidant system which is confirmed by the significant increase of levels of superoxide dismutase in blood serum and liver homogenates.

Key words: liver, liver transaminase, superoxide dismutase, streptozotocin-induced diabetes mellitus.

The article is a fragment of the academic research project "Age peculiarities in pathomorphogenesis of some organs of neuroendocrine, cardiovascular, digestive and respiratory systems in diabetes mellitus" (state registration No. 0116U003598).

Various pathological processes occurring in the liver as a result of its damage occupy one of the most important places in gastroenterology [6, 7]. These changes may be caused by a large range of

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factors, one of which is diabetes mellitus (DM) [2, 6, 10]. The medical-and-social problem of diabetes mellitus is that it is one of the most common endocrine diseases in the world, and its complications (angio-, neuropathies) lead to patients' performance degradation, disability and mortality [2, 7]. The number of patients suffering from diabetes mellitus grows at an exponential rate annually, motivating scientists to improve already existing and find new methods of diagnosis and treatment of this disorder. Quite often, diabetes leads to the development of diabetic hepatopathies, which are accompanied by liver dysfunction [10]. Therefore, a comprehensive study of morphofunctional liver changes in diabetes mellitus is important and prospective for theoretical and practical medicine.

The purpose of the study was to establish the peculiarities of morphofunctional changes in the liver of 24-month-old rats in the early stages of experimental streptozotocin-induced diabetes mellitus development.

Materials and methods. The investigation involved 16 24-month-old male Wistar rats. The animals were divided into 2 groups: experimental group (10 animals), and control group (6 animals). In experimental group of animals, diabetes mellitus was induced by a single intraperitoneal injection of streptozotocin ("Sigma", USA) at a dose of 5mg per 100g of body weight, diluted in 0.1 M solution of citrate buffer (pH 4.5) [8]. Control group animals were injected 0.1 M solution of citrate buffer (pH 4.5) in the equivalent dose intraperitoneally.

Animals were decapitated under thiopental anesthesia and their blood and liver specimens were taken for study. In order to control the level of hyperglycemia and confirm the development of diabetes, blood glucose levels (blood samples from caudal vein) were determined after an overnight fast every day during the whole study period using test strips for Accu-Chek glucometer (Germany).

The levels of liver transaminases were studied in order to evaluate the changes in the liver, namely, the levels of ALT (Alanine aminotransferase) and AST (Aspartate aminotransferase) in blood serum and liver homogenates were determined with the help of standard diagnostic kits «Felicit-Diagnostics» (Ukraine). Diagnostic set «Accent-200 HbA_{1c} Direct» (Poland) was used to determine the levels of glycosylated hemoglobin. The activity of superoxide dismutase was determined on the basis of quantitative content of nitroformazane, the product of nitrotestrazole recovery, and expressed in IU per 1mg of hemoglobin (IU/ml).

Computer data processing was performed with the help of statistical analysis software – STATISTICA (StatSoft, Inc. (2010).

Results of the study and their discussion. It should be noted that the level of glucose and glycosylated hemoglobin in the blood of the experimental group of animals statistically significantly increases on the 14th and 28th day of streptozotocin-induced diabetes mellitus (DM) development (table 1).

Table 1

Indices of glycemic profile in streptozotocin-induced diabetes mellitus

Indices	Groups of animals	14th day	28th day
Glucose (mmol/L)	Experimental group	14.06±0.41*	15.74±0.49*$^{\#}$
	Control group	5.62±0.66	5.43±0.6
Glycosylated hemoglobin (%)	Experimental group	7.21± 0.19*	8.18±0.41*$^{\#}$
	Control group	2.41± 0.37	2.34±0.33

Notes: 1) * – relevant difference as compared to the control indices, $p<0.05$; 2) $^{\#}$ – relevant difference as compared to the previous period of experiment, $p<0.05$.

The study made it possible to distinguish the following zones in the liver lobule of 24-month-old rats of the experimental group: peripheral (periportal), central and intermediate. Small amount of connective tissue was present around the portal triad. Hepatocytes are of typical structure on the ultrastructural level. The nuclei are located centrally and have somewhat elongated or rounded shape with condensed heterochromatin along the inner nuclear membrane and diffusely scattered fine-grained euchromatin in their nucleoplasm. Nuclear membrane is clearly visualized with minor invaginations. Perinuclear spaces are filled with granules of glycogen. The cytoplasm of hepatocytes contains mitochondria of various forms and densities. Some of them are increased in size with slightly dilated mitochondrial cristae; others have loosened membrane and low electron-optical density matrix, still others have matrix of high density and are elongated in shape. The Golgi complex contains a small number of vesicles and tubules. Granular endoplasmic reticulum is represented by cisternae with ribosomes concentrated on their surface. There are some lipid droplets, lysosomes, autophagosomes and myelin-like bodies in the cytoplasm (fig. 1a).

Dysplasia of hepatic plate and leukocytic infiltration of portal triads of hepatic lobules were observed in 24-month-old rats on the14th day of streptozotocin-induced diabetes mellitus. Great veins,

sinusoid hemocapillaries, as well as near-sinusoidal spaces were significantly enlarged. Significant number of binuclear hepatocytes is also observed. The number of lipid droplets of varying size increases in the cytoplasm of hepatocytes. The quantitative content of glycogen granules decreases leading to the decrease of optical density of cytoplasm with the formation of larger and smaller bare fields. Glycogen granules are quite often observed in the nuclei of liver cells. At the same time the number of Kupffer's cells is also increased (fig. 1b).

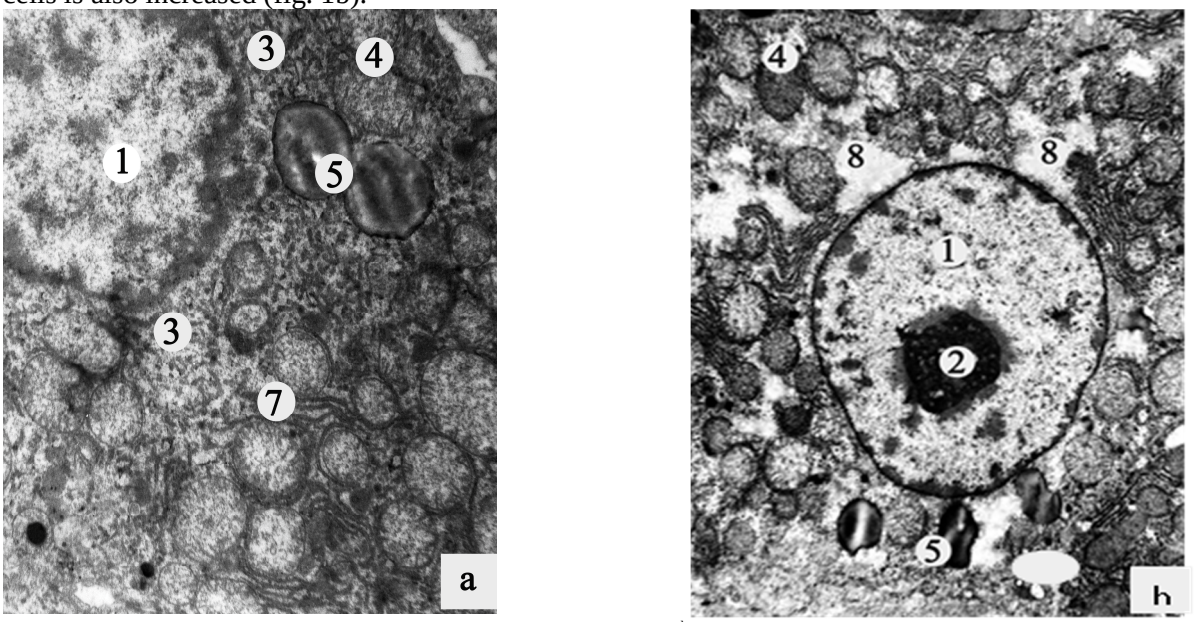

Figure 1. Ultrastructure of control and diabetic group rat hepatocytes on the 14th day of experiment. Magnification: a)x6400, b) x4800. 1– hepatocyte nucleus; 2 – nucleolus; 3 – glycogen granules; 4 – mitochondria; 5 – lipid droplet; 6 – slightly dilated cisternae agranular endoplasmic reticulum; 7 – granular endoplasmic reticulum; 8 – vacuole-like non-glycogen opacities.

The nuclei are round, electron-light, containing one or two nucleoli, with certain regions of defibration of external nuclear membrane. Mitochondria are swollen with reduced cristae. The Golgi complex contains a small number of vesicles and tubules. The number of ribosomes attached to the cisternae of the granular endoplasmic reticulum is visually reduced as compared to the control group of animals. On the 28th day of experiment structural changes are more progressive. Thus, the number of glycogen granules decrease, while there are hepatocytes the perinuclear space of which does not contain any glycogenic inclusions and is "empty". Thereat, the hepatocytes of the control group of animals contain rosette-like glycogen granules throughout the cytoplasm, especially in the central part of the hepatocyte, around the nucleus.

The nuclei of hepatocytes have spherical shapes, chromatin is condensed in the form of lumps along the inner surface of the nuclear membrane. The latter has uneven contours due to invaginations. The nuclear space is extended.

The mitochondrial cristae are contracted, and the matrix space is clear. The cisternae of the granular endoplasmic reticulum are somewhat enlarged. Golgi complex contains tubules, vesicles and saccules (fig. 2a, 2b.). Accumulation of large quantities of neutral fat and overload with Ito cells (perisinusoidal cells) is observed at early stages of streptozotocin-induced diabetes mellitus.

Increased enzyme activity of hepatocyte cytolysis was observed in blood serum of rats at early stages of streptozotocin-induced DM and is two times higher than in the control group animals (Table 2). On the 28th day of experiment the level of hepatic transaminase continues to increase both in blood serum and homogenate. De Ritis ratio (correlation between ALT and AST levels) is lower than the control value on the 14th day of the experiment and increases up to the 28th day and does not significantly differ from the control one (table 2).

These changes indicate abnormalities of the liver function, mainly in hepatocytes. The decrease of De Ritis ratio, consequently a slight difference between the ALT and AST levels on the 14th day of experiment, may point to the release of these enzymes from the cytoplasm of hepatocytes, since ALT is predominantly present in the cytoplasm of hepatocytes and AST has two fractions – cytoplasmic and mitochondrial.

Therefore, the hypoxic factor may be the most obvious starting from the 28th day of streptozotocin-induced diabetes mellitus development, as it indicates the predominate increase of both

ALT and AST levels in blood serum and homogenate and may be considered the marker of diabetes mellitus progression [1, 3].

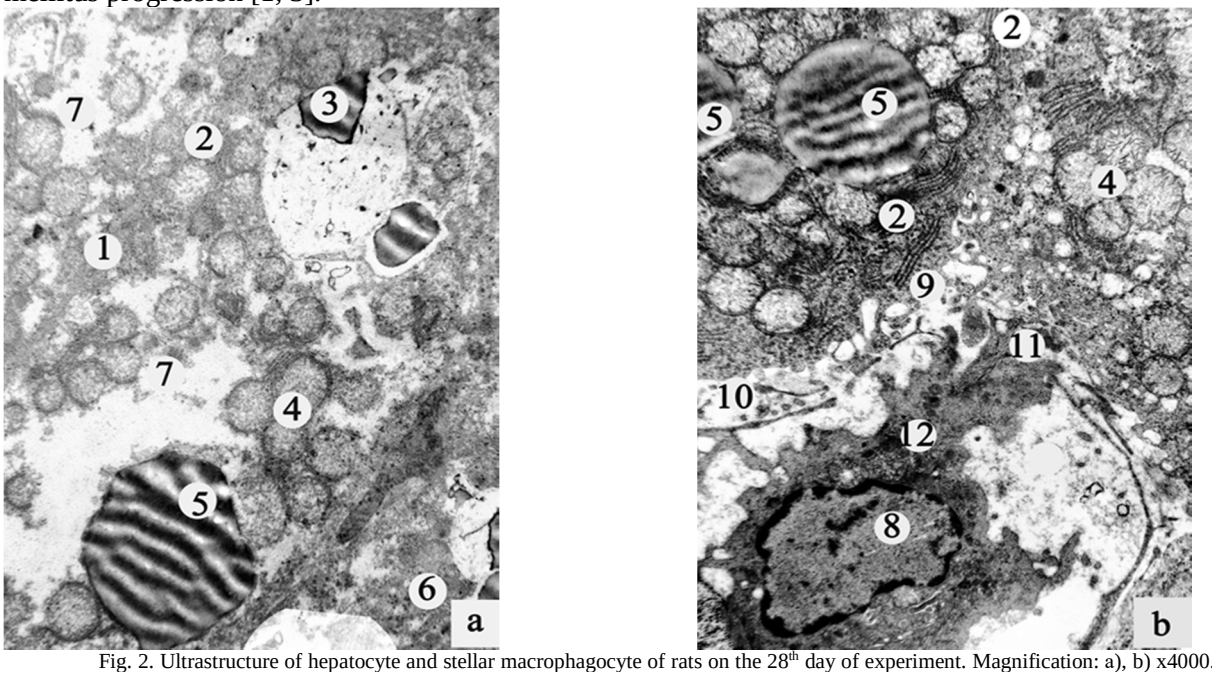

Fig. 2. Ultrastructure of hepatocyte and stellar macrophagocyte of rats on the 28th day of experiment. Magnification: a), b) x4000. 1 – hepatocyte; 2 – enlarged cisternae of the granular endoplasmic reticulum; 3 – lipid droplet in destroyed nucleus of hepatocyte; 4 – mitochondria; 5 – lipid droplet; 6 – agranular endoplasmic reticulum; 7 – vacuole-like non-glycogen opacities; 8 – nucleus of macrophagocyte; 9 – processes of sinusoidal pole of hepatocyte; 10 – Disse's space; 11 – processes of macrophagocyte; 12 – intracellular inclusion of macrophagocyte.

We think that the increase of blood levels of glucose and glycosylated hemoglobin leads to structural liver changes and consequently causing its dysfunction. This is confirmed by the data of other authors, who also observed liver dysfunction due to the increased blood glucose levels [10].

Table 2

Indices of antioxidant defense and hepatic transaminases of blood and homogenates in streptozotocin-induced diabetes mellitus

Timing of experiment		ALT, µmol \h*L		AST, µmol \h*L		De Ritis ratio
		Blood serum	Homogenate	Blood serum	Homogenate	
14th day	Experiment	0.7± 0.13*	1.85±0.2*	0.78±0.14*	1.95±0.22*	1.11±0.04*
	Control	0.28±0.02	1.41±0.11	0.33±0.02	1.5±0.8	1.21±0.03
28th day	Experiment	1.02±0.05*,#	2.22±0.9*,#	1.27±0.11*,#	2.04±0.18*	1.24±0.05#
	Control	0.28±0.02	1.35±0.08	0.33±0.02	1.5±0.7	1.2±0.01

Notes: 1) * – relevant difference as compared to the control indices, $p<0.05$; 2) # – relevant difference as compared to the previous period of experiment, $p<0.05$.

Concerning the biochemical indices of antioxidant defense, the level of superoxide dismutase increased by 14% in blood serum on the 14th day of experimental diabetes mellitus, and by 16% by the 28th day, and in the liver homogenate its level increased by 12% and 13% respectively, as compared with the control group of animals.

Other researchers have also observed the increased activity of lipid peroxidation in hepatocytes in streptozotocin-induced type I DM [5], that pointed to the development of oxidative stress; and the findings of our researches indicated that this stress may lead to increased activity of pro-oxidant system[4, 8].

Conclusions

The study revealed granular degeneration of hepatocytes with the transition to vacuolar degeneration caused by the increase of levels of glucose and glycosylated hemoglobin at early stages of streptozotocin-induced diabetes mellitus (14-28 days). Structural changes in the liver lead to its dysfunction, which is confirmed by the increased levels of specific hepatic aminotransferases and the decreased De Ritis ratio starting from the 28th day of the experiment. Such changes are followed by the increased activity of pro-oxidant system which is confirmed by the significant increase of levels of superoxide dismutase in blood serum and liver homogenates.

Prospects for further research: the findings of our study may be used in further research of various antidiabetic agents effects on liver hepatocytes in diabetes mellitus treatment.

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Реферати

МОРФОФУНКЦІОНАЛЬНІ ЗМІНИ В ПЕЧІНЦІ 24-МІСЯЧНИХ ЩУРІВ НА РАННІХ СТАДІЯХ РОЗВИТКУ ЕКСПЕРИМЕНТАЛЬНОГО ЦУКРОВОГО ДІАБЕТУ

О.Я. Жураківська, Ю.В. Боднарчук, В.М. Перцович, Х.Б. Кулинич, В.А. Мєсоєдова

Метою роботи було встановлення особливостей морфофункціональної перебудови печінки у ранні терміни перебігу стрептозотоцинового цукрового діабету (ЦД). ЦД моделювали одноразовим внутрішньоочеревинним введенням стрептозотоцину (5 мг на 100г маси тіла). Матеріал для дослідження забирали на 14 та 28 доби експерименту. Використали гістологічний, електронно-мікроскопічний, біохімічні методи дослідження. При стрептозотоциновому ЦД на тлі зростання глюкози і глікозильованого гемоглобіну виявляється зерниста дистрофія гепатоцитів з переходом у вакуольну. Структурні зміни печінки призводять до порушення її функції, що підтверджується підвищеним рівнем специфічних печінкових амінотрансфераз та зменшенням коефіцієнта Рітіса з 14 доби експерименту. Такі зміни супроводжуються підвищенням активності прооксидантної системи, на що вказує достовірне зростання супероксиддисмутази в сироватці крові та гомогенатах печінки.

Ключові слова: печінка, печінкові трансамінази, супероксиддисмутаза, стрептозотоциновий цукровий діабет.

МОРФОФУНКЦИОНАЛЬНЫЕ ИЗМЕНЕНИЯ В ПЕЧЕНИ 24-МЕСЯЧНЫХ КРЫС НА РАННИХ СТАДИЯХ РАЗВИТИЯ ЭКСПЕРИМЕНТАЛЬНОГО САХАРНОГО ДИАБЕТА

О.Я. Жураковская, Ю.В. Бондарчук, В.М. Перцович, Х.Б. Кулинич, В.А. Месоедова

Целью работы было установление особенностей морфофункциональной перестройки печени в ранние сроки развития стрептозотоцинового сахарного диабета (СД). СД моделировали однократным внутрибрюшинным введением стрептозотоцина (5 мг на 100г массы тела). Материал для исследования забирали на 14 и 28 сутки эксперимента. Использовали гистологический, электронно-микроскопический, биохимические методы исследования. При стрептозотоциновом СД на фоне роста глюкозы и гликолизированного гемоглобина наблюдается зернистая дистрофия гепатоцитов с переходом в вакуольную. Структурные изменения печени приводят к нарушению ее функции, что подтверждается повышенным уровнем специфических печеночных аминотрансфераз и уменьшением коэффициента Ритиса с 14 сутки эксперимента. Такие изменения сопровождаются повышением активности прооксидантного системы, на что указывает достоверное увеличение супероксиддисмутазы в сыворотке крови и гомогенатах печени.

Ключевые слова: печень, печеночные трансаминазы, супероксиддисмутаза, стрептозотоциновый сахарный диабет.

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