UDC: 611.36+616-092.9+616.379-008.64

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MORPHOLOGICAL AND FUNCTIONAL CHANGES AND CLUSTER CHARACTERISTICS OF HEPATOCYTES IN IMMATURE RATS WITH STREPTOZOTOCIN-INDUCED DIABETES

Abstract. *We have studied the cluster distribution of liver hepatocytes in immature rats and their morphological alterations on the 14th and 28th days of development and progress of diabetes mellitus (DM). The study was conducted on 3-month-old male Wistar rats. Diabetes was simulated by a single intraperitoneal administration of streptozotocin made by the firm "Sigma" (USA) at a dose of 7 mg per 100 g dissolved in 0.1 M citrate buffer (pH 4.5). It was established that in the early stages of development (14th and 28th days) of streptozotocin-induced diabetes there is a statistically significant decrease in the area of all cluster hepatocytes due to glycogenolysis, energy supply and inhibition of their protein synthesis function.*
Key words: *morphological alteration, hepatocyte, cluster analysis, streptozotocin-induced diabetes.*

Introduction. The liver is known to play a major role in both detoxification and regulation of metabolism of many substances in the human body. The liver is also involved in the synthesis of protein, vitamin A, cholesterol, and regulates glycogen synthesis from the blood glucose and, conversely, when depositing [2, 11, 12].

Diabetes mellitus (DM) is known to be a metabolic disorder and is characterized by impaired insulin secretion and action, and it results in hyperglycemia [7, 8, 11]. The latter causes disturbances in various organs, including the liver, dysfunction of which is observed in diabetic patients, especially in those patients that are characterized by poor control of blood glucose [9, 10].

The available literature does not use cluster analysis in the morphometric study of the liver.

Objective: to examine morphological and functional changes in hepatocytes of immature rats in the dynamics of a course of experimental diabetes mellitus (EDM) taking into account the results of morphometric and cluster analysis.

Materials and methods. The study was conducted on 20 three-month old male Wistar rats. The animals were divided into 2 groups: the control one (5 animals in each experimental period) and the experimental one (5 animals on the 14th and 28th days of EDM). DM simulating was carried out by a single intraperitoneal administration of streptozotocin made by the firm "Sigma" (USA) at a dose of 7 mg per 100 g of body weight, dissolved in 0.1 M citrate buffer (pH 4.5) [4], and the control group was administered an equivalent dose of 0, 1 M citrate buffer (pH 4.5). The animals were decapitated under thiopental anesthesia and we took their blood and liver for research on the 14th and 28th days of the experiment. To confirm the development of diabetes in rats and to control hyperglycemia throughout the experiment we determined the daily blood glucose in a drop of blood taken from the caudal vein using test strips for blood glucose meter by the company "Accu-Chek" (Germany).

We used histological (hematoxylin-eosin staining) and electron microscope methods. Determination of inclusions of glycogen in hepatocytes was performed on semifine sections stained with polychrome dye Humphrey Ch.D. as modified by Eroshenko H.A. [5] and on histological sections stained by Shabadash.

Morphometry was performed on the photomaterial using the software NIH USA "Image J" in manual mode, considering the magnification. We determined the performance of the profile field area of hepatocytes and their nuclei, nuclear-cytoplasmic ratio (N/C) – the ratio of the area of the profile field of the nucleus to

the area of the cytoplasm.

Computerized data processing was performed using statistical analysis package STATISTICA (StatSoft, Inc. (2011), STATISTICA (data analysis software system), version 10. www.statsoft.com.). We applied Student test (T-tests) for testing hypotheses about the equality of averages, whenever p <0,05, and claim the difference between averages.

Results and discussion. It should be noted that many papers deal with structural and functional changes in the liver in diabetic mature rats [6, 9, 10], and the papers that describe changes in immature animals are rare. It should be noted that some authors point out different areas of hepatocytes in different zones of the hepatic lobule in the study of pathological conditions [2, 3], which shows their morphological heterogeneity. Other researchers take into account the zonal distribution of hepatocytes in the classic liver lobule, but do not note any morphological difference [8, 10]. The majority of the findings in scientific sources are based on comparing average values of the size of hepatocytes in the hepatic lobule, without specifying the differences of morphological structure in different areas of the liver lobule [3, 6]. It should be also noted that we did not find any description of the changes of the hepatic lobule in immature rats using cluster distribution (analysis) of hepatocytes into different groups based on their morphological characteristics.

Based on the above, we decided to perform the morphometry of mononuclear hepatocytes of the hepatic plate and conduct morphometric, histological and ultrastructural analysis of the liver hepatocytes in different zones of the hepatic lobule in immature rats. While studying the histological preparations of the liver of 3 month-old rats in the control group it was established that a polygonal shape lobule is the structural and functional unit, where we distinguish between the following zones: central (zone III) - hepatocytes of which are around the central vein, peripheral (zone I) - near the portal triad and intermediate (II zone) which is located between the peripheral and central areas. Two rows of hepatocytes form the hepatic plates that go radially from the central vein to the portal triad. It should be noted that the ultrastructural and histological features of the liver structure in the control group of immature animals are not different from the norm. [1] After analyzing the morphometric findings, all the cells were divided into 3 clusters (C). For instance, the hepatocytes C1 have the largest area of both the cells and the nuclei and small N/C (Table). Hepatocytes C2 have a medium area of cells, a large area of the nucleus and large N/C, while C3 is characterized by the smallest cell area, small nucleus and small N/C (see. Table).

In order to compare the average values of standardized characteristics between the

Table

Cluster characteristic of hepatocytes of 3 month-old rats with EDM

Cluster	Experiment	Hepatocyte area	Hepatocyte nuclear	N/C
C1	Control 14 d	236,48±27,46	50,39±5,45	0,28±0,04
	DM 14 d	227,58±33,10*	51,33±7,42	0,30±0,06*
	Control 28 d	254,17±34,56	51,11±7,18	0,25±0,05
	DM 28 d	233,91±36,55*	44,69±6,72*#	0,24±0,03*#
C2	Control 14 d	189,05±25,48	51,68±6,75	0,38±0,07
	DM 14 d	159,88±27,52*	47,23±8,75*	0,42±0,06*
	Control 28 d	191,19±33,73	49,60±5,99	0,35±0,08
	DM 28 d	173,03±23,81*	41,03±4,91*	0,32±0,05
C3	Control 14 d	167,29±28,80	35,42±4,93	0,27±0,04
	DM 14 d	151,03±25,59	33,25±5,46	0,29±0,05
	Control 28 d	186,86±25,68	36,60±5,99	0,24±0,05
	DM 28 d	153,53±27,03*	33,45±3,98	0,28±0,03*

Notes: *1) *compared to the control, p<0,05; 2) # compared to the previous duration of the experiment, p<0,05.*

clusters in each period of the experiment and in different areas of the hepatic lobule, we used t-Student test for independent samples. Pairwise correlation showed that clusters reliably differ ($p < 0.05$) in the area of their hepatocytes and their nuclei. Considering the zones in which measurements of the cells were carried out, we analyzed the content of their clusters, checking the hypothesis of the links by using chi-square test of independence. Whenever p-value <0,05, we argue about the connection. Pearson's Chi-squared test ($p < 0,05$) shows that there is a connection between the cells belonging to the certain cluster and its localization in the hepatic lobule. For instance, the hepatocytes of the control group of animals C1 are mainly localized in the peripheral zone, those with C3 - in the center, with C2 - in the intermediate zone.

After 14 days of experimental diabetes there was a statistically reliable decrease of hepatocytes area in the liver due to the breakdown of glycogen, it is especially true for C1 and C2 (see. Table). The area of hepatocyte nuclei of these clusters does not change and it increases N/C, while the morpho-metric analysis of hepatocytes C3 indicates an unreliable reduction in their area. We noticed a significant decrease in glycogen granules in periportal and intermediate zones of the liver lobule at the light optical level. Therefore, we believe that reducing the area of hepatocytes C1 and C2 is due to the activation of glycogenolysis. Our data are also confirmed by those of some authors, indicating that hepatocytes of the periportal areas perform glycogen synthesizing and glycogen accumulating functions [2, 8], while hepatocytes of the central zone (to our knowledge this is C3) carry out primarily antitoxic function.

We could observe some light and dark hepatocytes at the ultrastructural level on the 14^{th} day of EDM. The first ones are characterized by the cytoplasm with moderate electronic-optical density and by a small number of organelles, which, in turn, have antireflective matrix, in particular, they are mitochondria and the nucleus. Dark hepatocytes have the cytoplasm with increased density, which is filled with organelles. It should be noted, that both dark and light hepatocytes can be found in one field of view of the electronic microscope. Their nuclei are mostly rounded, located in the center of the cell, chromatin granules are evenly dispersed throughout the area of the nucleus. In some places there are some hepatocyte nuclei, where chromatin is redistributed and condenses along the inner surface of the nuclear membrane. Mitochondria are swollen, sometimes vacuolated. The number of free ribosomes and those associated with tanks of granular endoplasmic reticulum (GER) decreases somewhat. The tanks of GER are dilated, with some areas of destruction. The membranes of the Golgi complex lose their parallelism, the number of vesicles decreases. Compared to the control group of animals, biliary and vascular plasmolemma of hepatocytes have fewer microvilli.

After 28 days of EDM course the area of hepatocytes of all clusters continues reducing (see. Table). At the same time the area of the nuclei reduces as well (see Table). Reducing the sizes of the nuclei in many hepatocytes when they were exposed to other toxic compounds was observed by several authors [2], and they associated these changes with reduced functional activity of hepatocytes. There was no glycogen in specimens stained by Shabadash and in semifine sections, stained with polychrome dye, which is confirmed by the findings of electron microscopic studies. At the ultrastructural level, the most distinctive feature of EDM is "desolation" of hepatocytes or so-called "empty hepatocytes". In these hepatocytes the perinuclear cytoplasm is empty, while in the control animals we noted the accumulation of rosette-like glycogen granules in the areas around the nucleus. The light hepatocytes were in the state of granular and vacuole dystrophy here and there, and the dark ones contained apoptotic cells. Different populations of hepatocytes had: shallow invaginations of the nuclear membrane, the reduction of GER and Golgi complex, expanding perinuclear space, antireflection of mitochondria matrix and destruction of their crests.

The appearance of dark and light hepatocytes with the prevalence of the latter is also observed with other effects on the liver and may indicate the depletion of the cells themselves [6]. Some data on such cells in diabetes are also found in the papers of other researchers [7]. Our findings are consistent with the results of other researchers who noted a reduction in the area

of hepatocytes, especially in the peripheral area, due to the impaired depositing of glycogen by hepatocytes and its excess release [2, 7, 11, 12]. Derangements in energy supply with the change in the number and destruction and lysis of mitochondrial membranes in immature animals are also noted by other scientists [10].

Conclusions. In the early stages of streptozotocin-induced diabetes (14th and 28th days) there is a reduction in the area of hepatocytes and their nuclei, which is associated with the expressed glycogenolysis process, and disturbances in detoxification function, which was confirmed by histological and ultrastructural studies. An increase in destructively-modified mitochondria with lysis of their crests indicates a derangement in energy supply of hepatocytes. Electron-transparent nuclei, reduced number of ribosomes, the expansion of GER and perinuclear space is one of the signs of inhibition of protein synthesis hepatocyte function.

Prospects for further research. The findings of our research could be used to further study the impact of various factors and pathological action of a drug substance in the liver hepatocytes, in both the treatment of diabetes and other diseases.

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