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Insulin-positive cells in liver and exocrine part of pancreas in animals with experimental diabetes mellitus

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ABSTRACT

Aim. To compare the number of insulin⁺ cells in the liver and exocrine part of the pancreas with the type of experimental diabetes, blood glucose and glycated hemoglobin (HbA_{1c}) level and with the number of Pdx1⁺ cells.

Materials and methods. The experiment was carried out on 25 male Wistar rats (weighting (303.0 ± 25.3) g) that were divided into 3 groups: the first group consisted of intact animals, the second had animals with experimental diabetes type 1, and the third with animals with experimental diabetes type 2. Biochemical, immunohistochemical, ELISA methods and statistical analysis were used.

Results. Insulin⁺ and Pdx1⁺ cells of rats with experimental diabetes were found in the liver and exocrine part of pancreas. The highest number of insulin⁺ cells in the liver was detected in type 2 diabetes (T2D). A strong positive correlation between the number of insulin⁺ cells in the liver and level of glycosylated hemoglobin in the blood was revealed in both type 1 and type 2 diabetes.

Conclusion. Insulin⁺ cells are detected in the liver and acinar part of pancreas of both intact rats and rats with experimental diabetes. Group with T2D is characterized by the highest number of insulin⁺ cells in the liver compared with type 1 diabetes (T1D). The localization of insulin⁺ cells in the liver changes depending on the type of diabetes. In T2D insulin⁺ cells are located in all parts of liver acini, meanwhile in animals with T1D such cells are mainly detected in the periportal area. The expression of Pdx1⁺ in acinar cells of pancreas and liver cells is likely a mechanism for their reprogramming into insulin⁺ cells in experimental diabetes mellitus.

Key words: diabetes mellitus, pancreas, liver, insulin⁺ cells, Pdx1⁺ cells.

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Инсулин-позитивные клетки печени и экзокринной части поджелудочной железы у животных с экспериментальным сахарным диабетом

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РЕЗЮМЕ

Цель исследования: сопоставить количество инсулин-положительных (инсулин⁺) клеток печени и экзокринной части поджелудочной железы с концентрацией глюкозы и гликированного гемоглобина (HbA_{1c}) в крови, а также с количеством Pdx1-положительных (Pdx1⁺) клеток в этих органах при различных типах сахарного диабета в эксперименте.

Материалы и методы. Эксперимент проводился на 25 самцах крыс (линия Wistar, масса (303,0 ± 25,3) г), которые были разделены на три группы: 1-я – интактные животные, 2-я – животные с экспериментальным сахарным диабетом 1-го типа, 3-я – животные с экспериментальным сахарным диабетом 2-го типа. В работе осуществляли биохимический, иммуноферментный, иммуногистохимический и статистический анализы.

Результаты. В печени и экзокринной части поджелудочной железы крыс с экспериментальным сахарным диабетом 1-го и 2-го типов обнаружены инсулин⁺ и Pdx1⁺-клетки. Наибольшее количество инсулин⁺-клеток в печени отмечается при сахарном диабете 2-го типа. Установлена корреляция между количеством инсулин⁺-клеток в печени и концентрацией HbA_{1c} в крови при сахарном диабете 1-го и 2-го типов.

Заключение. Инсулин⁺-клетки определяются в печени и экзокринной части поджелудочной железы интактных животных и крыс, у которых воспроизведена модель сахарного диабета 1-го и 2-го типов. Животные с экспериментальным сахарным диабетом 2-го типа характеризуются большим количеством инсулин⁺-клеток печени по сравнению с крысами с экспериментальным сахарным диабетом 1-го типа. В зависимости от типа сахарного диабета в печени меняется локализация инсулин⁺-клеток. При экспериментальном сахарном диабете 2-го типа инсулин⁺-клетки печени расположены во всех частях печеночной доли, тогда как у животных с экспериментальным сахарным диабетом 1-го типа эти клетки обнаруживаются преимущественно перипортально. Вероятно, экспрессия Pdx1⁺ в ацинарных клетках поджелудочной железы и клетках печени представляет собой механизм их перепрограммирования в инсулин⁺-клетки при экспериментальном сахарном диабете.

Ключевые слова: сахарный диабет, поджелудочная железа, печень, инсулин⁺-клетки, Pdx1⁺-клетки.

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INTRODUCTION

The relevance of the study of diabetes mellitus (DM) is due to its prevalence, high level of disability in patients and their high mortality rate. Currently, more than 422 million people worldwide have diabetes [1]. The exponential increase in the number of patients with diabetes requires new therapeutic strategies to reduce the socio-economic burden of this disease [2]. Diabetes is a chronic disease characterized by hyperglycemia, which is the result of absolute or relative insulin deficiency. Both classic forms of diabetes are characterized by the inability of pancreatic beta (β) cells to satisfy the body's need for insulin secretion due to either an almost complete loss in type 1 diabetes (T1D) or a deficit of β -cells as a result of insulin resistance in type 2 diabetes (T2D). The deficit in β -cell mass, which is up to 90% in long-standing T1D and approximately 65% in long-standing T2D is considered to be a consequence of β -cell destruction [3].

Insulin synthesis is specific for pancreatic islets' β -cells and is tightly controlled at the transcription level. The transcription factor Pdx1 determines the transcription rate and stability of insulin m-RNA. Pdx1 is both a factor of β -cell differentiation during embryogenesis and a regulator of the insulin-producing function of islet cells in mature islets of Langerhans [4]. In the differentiation process of pancreatic cells, the expression of the Pdx1 gene is enhanced in β -cells, while in exocrine and duct cells, the expression of this gene, on the contrary, is gradually reduced. It is believed that the activity of the Pdx1 gene is preserved in a differentiated state only in β -cells [5]. However, currently insulin⁺ cells have been detected in exocrine part of the pancreas, in the brain, bone marrow, spleen, liver and adipose tissue of animals [6, 7].

Since the liver and pancreas are of endodermal origin in ontogenesis, there is an assumption that both tissues include the same precursor cells that can differentiate both into hepatocytes and pancreatic β -cells. Moreover, hepatocytes and pancreatic β -cells express a large group of specific transcription factors and also use the same type of glucose transporter into the cell (GLUT2) including it in the metabolism through phosphorylation with glucokinase [8].

Based on the preceding, the aim of our research was to compare the number of insulin⁺ cells in the liver and exocrine part of pancreas with the blood glucose and glycated hemoglobin (HbA_{1c}) levels and

with the number of Pdx1⁺ cells in these organs in different types of diabetes mellitus in the experiment.

MATERIALS AND METHODS

Experiment was carried out on 25 male Wistar rats weighting 303.0 ± 25.3 g that were divided into 3 groups: the first of intact animals ($n = 10$), the second of experimental diabetes type 1 (T1D) ($n = 8$), the third of experimental diabetes type 2 (T2D) ($n = 7$). T1D was modeled by intraperitoneal (i.p.) injection of alloxan, diluted in 0.85% sodium chloride solution in a total dose 170 mg/kg of animal's body mass, according to the modified author's method [9]. T2D was induced by i.p. injection of streptozotocin diluted in citrate buffer in a dose 65 mg/kg with preventive (15 minutes before) i.p. injection of nicotinamide dissolved in water in a dose 110 mg/kg [10]. The animals were sacrificed by ether overdose on the 30th day from the beginning of the experiment after taking blood from the tail vein. After median laparotomy pancreases and livers were removed and fixed in 10% neutral formalin for 24 hours. After being washed for 8 hours, tissue was subjected to standard histological protocol using Automatic Tissue Processor Leica TP 1020 (Leica Microsystems, Germany) and Tissue Embedding Station Leica EG1160 (Leica Microsystems, Germany). Tissue slides 3–4 μ m thick were made using Leica SM2000 K Sliding microtome (Leica Microsystems, Germany).

To confirm experimental diabetes, plasma glucose, glycated hemoglobin (HbA_{1c}) and insulin concentrations were determined in the blood. To detect glucose and glycated hemoglobin (HbA_{1c}) concentration, standard kits for biochemical analysis were used (VectorBest, Russia; GLIKOGEMTEST, Russia). To measure blood insulin, Rat/Mouse Insulin ELISA kit (Millipore, USA) and Automatic immunofermental analyzer LAZURITE (Dynex Technologies, USA) were used.

Immunohistochemical examination of the pancreas and liver was performed using mouse anti-proinsulin and insulin (clone INS04+INS05, Invitrogen, USA) and anti-Pdx1 antibodies (Abcam, USA) according to standard protocols. Tissue sections were incubated with primary antibodies in 1: 200 dilution for 16 hours at 4 °C. Detection of insulin was performed, using the avidin–biotin–peroxidase complex. To detect Pdx, Goat anti-Mouse IgG (H + L) conjugated with fluorescent dye Texas

Red (ThermoFisher, USA) was used. Negative and positive tissue controls were used to check the protocol and exclude non-specific binding. As positive control for immunohistochemical determination of insulin and Pdx1, pancreatic tissue sections of intact rats were taken [11, 12]. Negative control was carried out on the similar tissue sections and using the same protocols, excluding primary antibodies [12, 13].

Analysis of tissue slides and calculation of insulin⁺ cells were performed using light microscope Leica DM 2500 (Leica, Germany) and Video TesT-Morphology 5.0 software (Video TesT Ltd., Russia), calculation of Pdx1⁺ cells was carried out with the help of a confocal laser scanning microscope LSM 710 (CarlZeiss, Germany) and ZEN 2.0 (CarlZeiss, Germany) software.

The number of insulin⁺ cells and Pdx1⁺ cells in exocrine part of pancreas per 1 mm² of a pancreatic slide was calculated. In the liver, the number of insulin⁺ cells and Pdx1⁺ cells in hepatic plates in all zones of hepatic lobule was determined. In sinusoidal capillaries, the following parameters were calculated: the total number of sinusoidal cells throughout the entire hepatic lobule and the total number of insulin⁺ sinusoidal cells, located in all parts of hepatic lobule per 1 mm² of a hepatic tissue slide. Functional activity of insulin⁺ cells was evaluated based on the optical density (OD) of their cytoplasm.

Statistical analysis was performed using Statistica 6.0 (DELL, USA) and Microsoft Excel 2003 (Microsoft, USA). To test the hypothesis of homogeneity of two independent samples, the nonparametric Mann – Whitney *U*-test and Kruskal – Wallis test were used. The results were considered significant at $p < 0.05$. The data were presented as the mean and the standard error of the mean ($M \pm m$). To reveal the relationship between the number of insulin⁺ cells, Pdx1⁺ cells and the concentration of glucose and glycated hemoglobin (HbA_{1c}) in blood, the Pearson pairwise linear correlation coefficient (r) was calculated.

RESULTS

Blood glucose and glycated hemoglobin (HbA_{1c}) levels in experimental T1D and T2D rats are significantly higher in comparison with intact rats, while blood insulin concentration decreases only in T1D. In T2D rats the level of blood insulin is significantly higher than in the T1D group, which is typical of T2D (Table 1).

Table 1

Concentration of glucose, HbA _{1c} and insulin in the blood of rats in all experimental groups, $M \pm m$			
Parameter	Group 1 (intact animals)	Group 2 (animals with T1D)	Group 3 (animals with T2D)
Glucose, mmol/L	5.00 ± 0.30	10.88 ± 0.46*	10.90 ± 0.50*
HbA _{1c} , %	4.40 ± 0.30	6.73 ± 0.78*	6.58 ± 0.97*
Insulin, mkg/L	1.28 ± 0.19	0.50 ± 0.09*	1.00 ± 0.13 #

*difference as compared with intact animals ($p < 0.05$); #difference as compared with T1D group ($p < 0.05$) (here and in Tables 2–4).

Immunohistochemical analysis showed the presence of insulin⁺ and Pdx1⁺ cells in the exocrine part of pancreas and in all zones of the hepatic lobule of experimental animals (Figure).

The number of insulin⁺ and Pdx1⁺ cells in the exocrine pancreas in T1DM and T2DM does not differ from their number in intact animals. The number of insulin⁺ cells in the liver of animals with T1DM and T2DM is higher than in intact animals. In T1D insulin⁺ cells in the liver are primarily localized in the periphery of the hepatic lobule, while in T2D such cells are in all zones of the hepatic lobule. The total amount of insulin⁺ and Pdx1⁺ cells was counted throughout the hepatic lobule. Additionally, the data on the number of insulin⁺ cells in the peripheral zone of the hepatic lobule are shown separately. The number of Pdx1⁺ cells in the liver of rats with experimental diabetes is bigger than in intact rats. This parameter in T1D rats is higher than the similar parameter in intact animals (Table 2).

Optical density (OD) of cytoplasm in insulin⁺ cells of the liver and pancreas in T1D is less than in the intact group (Table 3). In animals with experimental T1D, contrary to rats with experimental T2D, insulin production in insulin⁺ sinusoidal cells increases, since the optical density of cytoplasm in these cells is higher in comparison with the similar parameter in intact animals and animals with experimental T2D (Table 3).

The number of insulin⁺ sinusoidal cells increases in different types of diabetes in comparison with the similar parameter in intact animals. This parameter reaches the highest value in rats with experimental T2D, while these cells are localized in all zones of the hepatic lobule (Table 4).

To determine the relationship between the number of hepatic insulin⁺ cells and Pdx1⁺ cells and the level of glucose in rat blood, a pairwise linear correlation coefficient was used (Table 5).

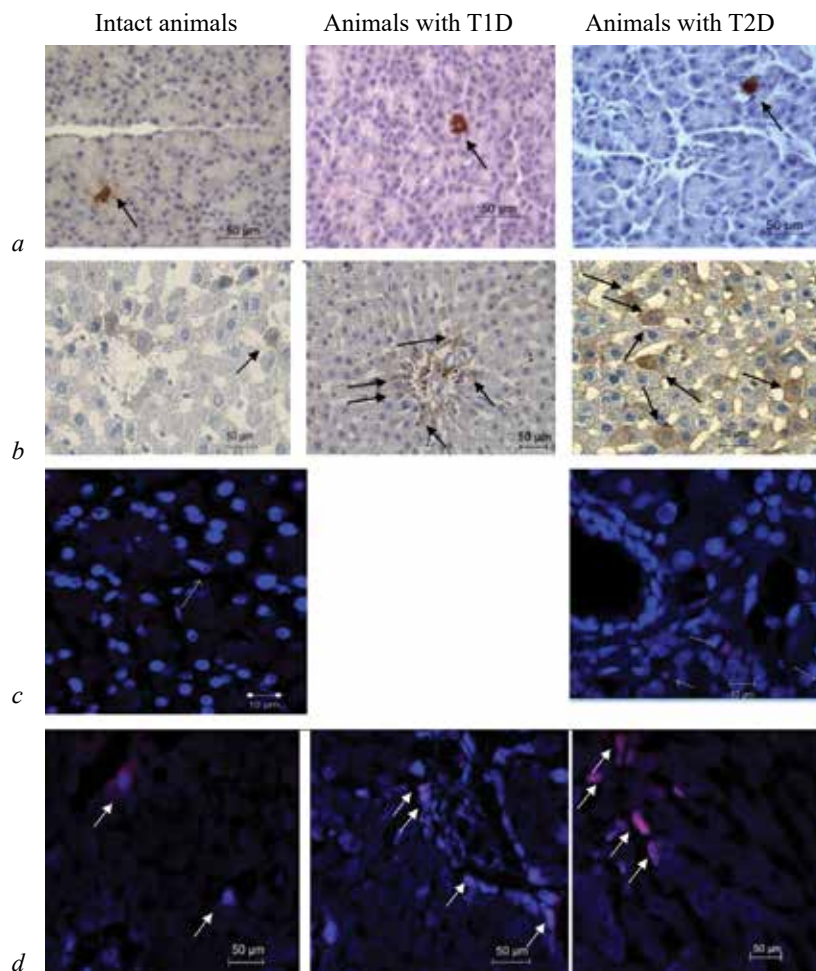


Figure. Pancreas (a, c) and liver (b, d) of intact animals, animals with experimental T1D and animals with experimental T2D; immunohistochemical staining for insulin (a, b) and immunofluorescent staining for Pdx1⁺ (c, d). Insulin⁺ and Pdx1⁺ cells are shown by arrows, $\times 400$

Table 2

Number of insulin ⁺ and Pdx1 ⁺ cells in the liver and exocrine part of pancreas (N/mm ² of the organ tissue), $M \pm m$			
Parameter	Group 1 (intact animals)	Group 2 (animals with T1D)	Group 3 (animals with T2D)
Number of insulin ⁺ cells in exocrine pancreas, N/mm ² of pancreas	3.50 ± 0.54	3.67 ± 0.73	3.15 ± 0.34
Number of Pdx1 ⁺ cells in exocrine pancreas, N/mm ² of pancreas	27.34 ± 4.92	—	21.54 ± 3.22
Number of insulin ⁺ cells in liver, N/mm ² of liver	14.26 ± 0.84	$24.86 \pm 2.36^*$	$151.50 \pm 7.34^{* \#}$
Number of insulin ⁺ cells in hepatic plates in the peripheral zone, N/mm ²	0	$13.58 \pm 3.08^*$	$41.10 \pm 4.93^* \#$
Number of Pdx1 ⁺ cells in liver, N/mm ² of liver	32.11 ± 2.14	$42.72 \pm 1.59^*$	34.09 ± 2.46

Table 3

Optical density (OD) of cytoplasm in insulin ⁺ cells of the liver and exocrine part of the pancreas (in conventional units), $M \pm m$			
Parameter	Group 1 (intact animals)	Group 2 (animals with T1D)	Group 3 (animals with T2D)
OD of cytoplasm in insulin ⁺ cells in the exocrine pancreas	0.44 ± 0.02	$0.37 \pm 0.02^*$	0.42 ± 0.02
OD of cytoplasm in insulin ⁺ cells in hepatic plates	0.20 ± 0.01	$0.17 \pm 0.012^*$	0.19 ± 0.01
OD of cytoplasm in insulin ⁺ sinusoidal cells	0.29 ± 0.24	$0.35 \pm 0.01^*$	$0.30 \pm 0.01^{\#}$

Table 4

Number of sinusoidal and insulin ⁺ sinusoidal cells in the liver of experimental animals, $M \pm m$			
Parameter	Group 1 (intact animals)	Group 2 (animals with T1D)	Group 3 (animals with T2D)
Total number of sinusoidal cells, N/mm ² of liver	387.11 ± 14.19	645.22 ± 33.95*	713.15 ± 33.47*#
Total number of insulin ⁺ sinusoidal cells, N/mm ²	13.82 ± 0.63	19.19 ± 0.89*	37.80 ± 3.39*#
Number of insulin ⁺ sinusoidal cells in the peripheral zone of the hepatic lobule, N/mm ² of liver parenchyma, N/mm ² / (%)	0.63 ± 0.03 (4.6 ± 0.02%)	5.64 ± 0.32* (29.7 ± 2.80%)*	17.91 ± 2.71*# (48.1 ± 7.70%)*#

Table 5

Coefficients of pairwise linear correlation between the number of insulin ⁺ cells and Pdx1 ⁺ cells and concentration of glucose and HbA _{1c} in the rat blood. Coefficients of pairwise linear correlation between the number of insulin ⁺ cells and Pdx1 ⁺ cells in the rat liver			
Type of cell	Glucose	HbA _{1c}	Pdx1 ⁺ cells
Insulin ⁺ cells in liver in T1D	0.13	0.84	–0.49
Insulin ⁺ cells in liver in T2D	0.58	0.98	–0.25
Pdx1 ⁺ cells in liver T1D	0.84	0.47	–
Pdx1 ⁺ cells in liver in T1D	0.26	0.41	–

The obtained data indicate a strong positive correlation between the number of insulin⁺ liver cells and the level of glycated hemoglobin in blood, which is evidence of strong hyperglycemia during a month, both in T1D and T2D. At the same time, positive correlation between the number of hepatic insulin⁺ cells and concentration of glucose, measured in rat blood on the 30th day of the experimental diabetes is weak in T1D and of moderate strength in T2D. It was established that there is a weak inverse correlation between the number of insulin⁺ cells and Pdx1⁺ cells in liver (Table 5). Thus, the number of insulin⁺ cells in T1D is less than in T2D, however, the number of Pdx1⁺ cells increases as compared with T2D. Interrelation between the HbA_{1c} concentration and the number of Pdx1⁺ cells in T1D and T2D is weak. The coefficient of pairwise linear correlation between concentration of glucose and Pdx1⁺ cells in T1D is relatively high, while T2D is characterized by a lower interrelation between these parameters.

DISCUSSION

Insufficient quantity and dysfunction of insulin-producing β -cells in pancreatic islets are the main causes of hyperglycemia and the associated complications arising in T1DM and T2DM [14]. The search for methods, aimed at increasing the number and functional activity of preserved β -cells in diabetes, is a promising strategy for treatment of both diabetes types. Insulin⁺ cells, found in various organs,

are attracting more and more researchers' attention because they can partially compensate for the damage of β -cells of pancreatic islets in diabetes mellitus [15]. The quantity and localization of insulin⁺ cells in the liver and exocrine pancreas, depending on the type of the experimental diabetes mellitus, were studied in this work.

Insulin⁺ cells are found in the parenchyma of the liver and pancreas (outside the pancreatic islets) in small quantity in intact animals. In the rat pancreas, the number of these cells remains constant in T1D and T2D. The optical density of the cytoplasm of these cells also does not differ under the conditions of T1D and T2D models.

Depending on the type of diabetes, there are differences not only in the number of insulin⁺ cells (in T2D their number is almost 5 times greater than in the liver of animals with T1D), but also in the localization of these cells. In T2D, insulin⁺ cells are located in all parts of the hepatic lobule, while in animals of the T1D group these cells are found mainly in the peripheral zone. The structure, size and location of insulin⁺ cells in the liver correspond to hepatocytes.

Liver sinusoidal cells (LSC) are located along the hepatic sinusoids and make up about 33% of liver parenchyma cells. Endothelial cells, stellate macrophages (Kupffer cells), perisinusoidal lipocytes (Ito cells), pit cells, and dendritic cells are referred to as LSC. LSC are capable of phagocytosis and pinocytosis and are involved in a wide range of immunological reactions [17].

When analyzing the number of LSC, it was determined that their number increases in both types of DM, but to a greater extent in DM2. According to the results of the study, in T2D the number of insulin⁺ sinusoidal cells is higher than in T1D. Counting the number of insulin⁺ sinusoidal cells in the peripheral zone of the hepatic lobule is necessary to compare this parameter with the number of insulin⁺ cells in hepatic plates (presumably hepatocytes), located in the similar zone. Since there is evidence [6] that

insulin⁺ hepatocytes are localized mainly in the first (peripheral) zone of the hepatic lobule, the number of insulin⁺ sinusoidal cells, located in the intermediate (second) zone, is not indicated. As compared with pancreatic β -cells, insulin⁺ hepatocytes are generally characterized by a lower optical density of cytoplasm, which indicates a relatively low concentration of insulin in these cells and reflects their low functional activity. An increase in the number of this type of cells can be both the result of insulin synthesis in the cells themselves and their ability for endocytosis of extracellular insulin [18].

Pancreatic and duodenal Pdx1 is a key factor in development, proliferation, and functioning of β -cells in pancreatic islets [19]. The protein is able to bind to the insulin gene promoter, GLUT2, glucokinase and other, regulating gene expression of these proteins [20]. It is believed that Pdx1 can reprogram any cells by stimulating insulin synthesis in them [21]. We observed differences in change in the number of Pdx1⁺ liver cells in experimental models of diabetes mellitus: in T1DM their number significantly increases, in T2DM their number does not change. The pairwise linear correlation coefficient indicates a strong relationship between the number of Pdx1⁺ liver cells and the HbA_{1c} concentration in blood, which reflects the severity of hyperglycemia during the experiment. Likely, in the model of T1DM, insulin-producing cells in liver do not compensate for the lack of insulin, therefore, additional production of Pdx1 is required. On the contrary, in animals with T2D, the normalization of blood insulin concentration does not require further reprogramming of hepatocytes into insulin-producing cells. It also cannot be ruled out that the reason of the revealed differences between animals with T1DM and T2DM may be explained by the involvement of other transcription factors, Nkx 6.1 in the differentiation of insulin-producing cells in particular [5]. The obtained data allow us to put forward a hypothesis about the existence of an inverse relationship between the insulin concentration in blood and the production of Pdx1 in hepatocytes as a mechanism for compensating for hypoinsulinemia in diabetes.

Thus, the lack of insulin in experimental DM1 and DM2 can be compensated for by increasing the number of insulin-producing cells in liver and exocrine pancreas. Reprogramming of differentiated cells such as hepatocytes into insulin⁺ cells can be considered a mechanism for obtaining more insulin-producing cells. New approaches in the treatment of diabetes,

aimed at increasing the expression of Pdx1 and the number of insulin⁺ cells in various organs, appear to be extremely promising.

CONCLUSION

It has been shown for the first time that in experimental T1DM and T2DM there is an increase in the number of insulin⁺ and Pdx1⁺ cells in the rat liver. In animals with experimental T1DM, insulin⁺ liver cells are found mainly in the peripheral zone of the hepatic lobule. In experimental T2DM, insulin⁺ cells are located in all parts of the hepatic lobule, and the number of Pdx1⁺ cells does not differ from that in intact animals. A strong positive correlation between the number of insulin⁺ cells in the liver and the HbA_{1c} concentration in blood was found in both T1DM and T2DM. The correlation between the number of insulin⁺ cells in liver and the glucose concentration in rat blood on the 30th day of the experiment is weak in T1DM and moderate in T2DM.

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Baykenova M.B. – work with experimental animals, implementation of the laboratory part of the study, statistical processing of the results. Chereshev V.A. – critical revision of the manuscript for important intellectual content. Sokolova K.V. – work with experimental animals, implementation of the laboratory part of the study, statistical processing of the results. Gette I.F. – conception and design of the study, setting up of the experiment, modeling of DM. Emelianov V.V. – analysis and interpretation of the obtained data, drafting of the text of the article. Danilova I.G. – analysis and interpretation of the obtained data, drafting of the text of the article.

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