Journal of Endocrinology and Diabetics Research

Research Article

A SCITECHNOL JOURNAL

# Liver Insulin-Positive Cells in Experimental Diabetes

**Baikenova M[1,2]*, Sokolova K[1,2] and Danilova I[1,2]**

## Abstract

The global diabetes epidemic over the past few decades has been one of the leading causes of death worldwide. The search for alternative sources of insulin producing cells in the body may be one of the possible promising approaches to the treatment of diabetes and its complications. The aim of this work was to study changes in the number and localization of insulin+ and Pdx1+ liver cells on the 30th and 60th days at rats with experimental diabetes type 1 and type 2. Experiment was carried out in accordance with the recommendations of European Parliament and of the Council (Directive 2010/63/EU). 35 male Wistar rats weighting 332.6 ± 12.15 g were used. Type 1 diabetes (T1D) was modeled by IP injections of alloxan (170 mg/kg), Type 2 Diabetes (T2D) IP injections of nicotinamide (110 mg/kg) and streptozotocin (65 mg/kg). Animals were divided into 5 groups:1:intact, 2:experimental T1D 30 days, 3:experimental T1D 60 days, 4:experimental T2D 30 days, 5:experimental T2D 60 days. Biochemical, immunohistochemical and statistics analyses were performed. Increase of the number of insulin ± cells were detected in diabetic rats *vs*.intact. The number of insulin+ and Pdx1 ± cells depends on the type of diabetes. The largest number of insulin ± cells, located in all areas of the hepatic lobule, is observed in rats with T2D. Animals with T1D have less insulin ± cells. On the 30th day of diabetes they are localized mainly in the peripheral zone, while on the 60th day of diabetes they are observed in all areas of the hepatic lobule. Rats with T1D have significantly higher number of Pdx1 ± cells, then rats with T2D.

**Keywords**

Diabetes mellitus; Liver; Insulin+ cells; Pdx1+ cells

## Introduction

Last decades the significant growth spread of diabetes has been demonstrated practically all over the world. About 415 millions of people with diabetes are currently registered [1]. Diabetes mellitus is a metabolic disorder [2], characterized by hyperglycemia due to the absolute (type 1 diabetes:T1D) or relative (type 2 diabetes:T2D) lack of insulin [3], produced by pancreatic β-cells. Complications caused by diabetes, such as cardiovascular disease, retinopathy, nephropathy and peripheral circulations disease often leads to disability or death with ineffective therapy [4]. Currently, replenishment of β-cells mass is a therapeutic alternative of the treatment of this disease [5]. The new sources of β-cells may be transplanting cadaveric islets [6] or differentiation of pluripotent stem cells [7]. Liver cells are a major target for generating insulin-producing cells because of its close developmental origin, accessibility and regenerative ability [8]. Liver and pancreas have many common characteristics: they sensitive to glucose and their tissue express a large group of specific transcription factors [9]. Transcription factors play the most important role in regulation of gene expression. Pdx1, as well as Ngn3 and MafA, is a transcription factor, critically important for developing and maturation of β-cells [10]. Pdx1 is necessary for developing pancreatic exocrine and endocrine cells, including β-cells. Pdx1 also binds with regulatory elements and increases insulin gene transcription [11]. Ectopic expression of Pdx1, Ngn3 and MafA may vary the fate of somatic cells [10]. The aim of the work was studying the change of number and localization of insulin+ and Pdx1+hepatic cells at the 30th and 60th days of T1D and T2D in rats.

## Methods

35 male Wistar rats, weighting 332.6 ± 12.15 g, were randomly divided into 5 groups:1:intact animals, 2:T1D 30 days, 3:T1D 60 days, 4:T2D 30 days, 5:T2D 60 days. T1D was modeled by Intraperitoneal (IP) injections of alloxan, dilluted in 0.85% NaCl, in a total doze 170 mg/kg body weight, according to the modified author technique [12]. T2D was modeled by IP injections of streptozotocin, diluted in citrate buffer, in a dose 65 mg/kg, with preventive (15 minutes before) IP injections of nicotinamide, diluted in water (110 mg/kg) [13]. Rats were euthanized an overdose of Zoletil in the 30th and 60th days after beginning of experiment. Before euthanasia blood from tail vein was taken for analysis. Liver was removed during mediallaparotomy. Tissues were fixed with 10% neutral formalin for 24 hours, washing for 8 hours, embedded in paraffin, using LeicaEG 1160 (Leica Microsystems, Germany), and then manually sectioned with a microtome LeicaSM 2000 R (LeicaMicrosystems, Germany) to obtain 3-4 μm thick paraffin sections. To verify diabetes blood glucose and glycosylated hemoglobin (HbA1c) were determined, using standard kits for determination (VectorBest, Russia and GLIKOGEMTEST, Russia). Immunohistochemical investigation of liver were made, using antibody for proinsulin and insulin (clone INS04+INS05, Invitrogen, CIIIA) and for Pdx1 (Abcam, CIIIA) according to standard protocols. Incubation with the primary antibodies was done for 16 hours at 4°C a 1:200 dilution. For detection of insulin avidin-biotin peroxidase complex is used, goat anti-rabbit Igs, conjugated with Texas Red (Thermo Fisher, USA) were used for visualization of Pdx1. To check the protocol and exclude non-specific binding positive and negative controls were made. Positive and negative control for immunohistochemical detection of insulin was pancreas from intact rats [14,15]. Visualization and counting the number of insulin+ cells were performed, using a light microscope Leica (Leica DM 2500, Germany) and Video Test Morphology 5.0 software (VIDEOTEST, Russia), Pdx1+ cells confocal laser scanning microscope LSM 710 (Carl Zeiss, Германия) and ZEN 2.0 software (Carl Zeiss, Германия). The number of insulin+ and Pdx1+ cells in hepatic plates in all areas of

***Corresponding author:** Baikenova M, Ural Federal University, Yekaterinburg, Russia, Tel: +79122889470; E-mail: m.b.baikenova@urfu.ru

**Received Date:** December 22, 2020; **Accepted Date:** January 06 , 2021 ; **Published Date:** January 13, 2021

hepatic lobule per 1 mm² of liver parenchyma was counted. Statistical analysis performed, using Statistica 6.0 (DELL, USA), OriginPro 9.0 (Origin Lab, USA) and Microsoft Excel 2003 (Microsoft, USA). To check the hypothesis of homogeneity of two independent samples the non-parametrical Mann-Whitney U criteria also Kruskal-Wallis test were used with p<0,05. Data are presented as mean ± errors of the mean.

## Results

Concentration of blood glucose and HbA1c at T1D and T2D significantly higher *vs.* intact animals. In T1D there were no differences between these parameters at the 30th and the 60th days of experiment, in T2D at the 60th days contrary to the 30th day glucose was significantly higher and HbA1c was lower (Table 1). Immunohistochemical investigation of liver reveals the presence of insulin+ and Pdx1+ cells in liver in all experimental groups. The number of insulin+ cells in the liver of diabetic animals is higher *vs.* intact animals; the highest amount is in T2D groups. The number of insulin+ cells between the 30th and the 60th day of experiment at T1D and T2D is not significantly different. It was found, that on the 30th day of T1D insulin+ cells are localized mainly in the peripheral area of hepatic lobules, meanwhile on the 60th day of T1D and at T2D these cells are detected in all parts of the hepatic lobule. The number of Pdx1+cells increased in animals with diabetes. The number of Pdx1+cells on the 30th and 60th days of diabetes does not differ between each other within the same type of diabetes, however, their number is significantly higher at T1D contrary to T2D (Table 2).

Table 1: Biochemical characteristics of blood of experimental animals. *p<0.05 *vs.* intact; #: p<0.05 *vs.* T1D 30 days; ^: p<0.05 *vs.* T2D 30 days.

| Parameter/ Experimental group | Intact | T1D 30 days | T1D 60 days | T2D 30 days | T2D 60 days |
|---|---|---|---|---|---|
| Glucose mmol/L | 5 ± 0.3 | 10.88 ± 0.46* | 10.46 ± 0.49 | 10.9 ± 0.50* | 12.3 ± 0.23*, ^ |
| HbA1c % | 4.4 ± 0.3 | 6.73 ± 0.78* | 6.53 ± 0.20* | 6.58 ± 0.97* | 5.6 ± 0.55*, ^ |

Table 2: Number of insulin+ and Pdx1+ cells in the liver of experimental animals (cells per mm2 of the pancreatic tissue), M ± m. *: p<0.05 *vs.* intact; #: p<0.05 *vs.* T1D 30 days; ^: p<0.05 *vs.* T2D 30 days.

| Parameter | Intact | T1D 30 days | T1D 60 days | T2D 30 days | T2D 60 days |
|---|---|---|---|---|---|
| Number of insulin+ cells in hepatic plates N/mm² | 14.26 ± 0.8 | 24.86 ± 2.36* | 22.78 ± 1.93* | 151.5 ± 7.34* | 151.81 ± 4.04* |
| Number of insulin+ cells in hepatic plates in peripheral area N/mm² % | 0 | 13.58 ± 3.08* (54.62%)* | 6.87 ± 2.37*-30.15% | 41.1 ± 4.93* (27.12%)* | 51.82 ± 2.61*,^ (34.13%)*, ^ |
| Number of Pdx1+ hepatocytes N/mm² | 32.11 ± 2.14 | 42.72 ± 1.59* | 43.54 ± 2.48* | 34.09 ± 2.46*, # | 36.24 ± 2.16 *,^ |

## Discussion

Inadequate amount and dysfunction of β-cells in islets of Langerhans at T1D and T2D are the main reason of hyperglycemia and connected to it complications [16]. Methods, aimed at compensation of lack of insulin and search for alternative sources of it are a promising strategy for the treatment of T1D and T2D. About 20 years ago the plasticity of mature cells was considered rather amazing, since the leading dogma of that time was that finally differentiate mammalian cells cannot be transformed into other types of cells. However, a study, inwhichPdx1 induced the insulin gene expression in liver and ameliorated hyperglycemia in diabetic mice treated with Streptozotocin [17], demonstrate the approach for generating mature cells from other mature cells. Advantages of using the liver as the source for generating of insulin-producing cells are based on their close developmental familiarity with the pancreas (the both organs derived from the tissue of foregut endoderm) and the regenerative ability of hepatic cells [18]. It was founded, that the number of insulin+ and Pdx1+cells at T1D and at T2D differs; meanwhile duration of diabetes does not affect the number of such cells. Number of insulin+and Pdx1+cells at diabetic animals are higher vs.intact animals, the highest number is in T2D groups. It was found, that on the 30th day of T1D insulin+ cells are localized mainly in the peripheral area of hepatic lobules. In groups with T2D and on the 60th day of T1D these cells are detected in all parts of the hepatic lobule. The number of Pdx1+cells in liver of animals with experimental diabetes increased compared to intact animals. It was revealed, that the number of Pdx1+ hepatic cells is affected by the type of diabetes (at T1D the number is higher, that at T1D), but not it duration.

## Conclusion

Results of the investigation indicate that little amount of insulin+ and Pdx1+ hepatic cells are founded both in intact and diabetic animals. Number and localization of these cells depends from the type of diabetes, effect of the duration of diabetes was not fixed. In any case, reprogramming of hepatic cells into insulin-producing may be one of the possible promising approaches to the treatment of diabetes and its complications.

The study was supported by a grant from the Russian Science Foundation No: 16-15-00039-П.

## References

1. Ogurtsova K, da Rocha FJD, Huang Y, Linnenkamp U, Guariguata L, et al. (2017) IDF Diabetes atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. Diabetes Res Clin Pract 128: 40-50.
2. Bluestone JA, Herold K, Eisenbarth G (2010) Genetics, pathogenesis and clinical interventions in type 1 diabetes. Nature 464: 1293-1300.
3. Moin ASM, Butler AE (2019) Alterations in beta cell identity in type 1 and type 2 diabetes. Current Diabetes Reports 19.
4. Harding JL, Pavkov ME, Magliano DJ, Shaw JE, Gregg EW (2019) Global trends in diabetes complications: A review of current evidence. Diabetologia 62: 3-16.
5. Aguayo-Mazzucato C, Bonner-Weir S (2018) Pancreatic β cell regeneration as a possible therapy for diabetes. Cell Metab 27: 57-67.

6. Hering BJ, Clarke WR, Bridges ND, Eggerman TL, Alejandro R, et al. (2016) Clinical islet transplantation consortium. Phase 3 trial of transplantation of human islets in type 1 diabetes complicated by severe hypoglycemia. Diabetes Care 39: 1230-1240.

7. Rezania A, Bruin JE, Arora P, Rubin A, Batushansky I, et al. (2014) Reversal of diabetes with insulin-producing cells derived *in-vitro* from human pluripotent stem cells. Nat Biotechnol 32: 1121-1133.

8. Ruzittu S, Willnow D, Spagnoli FM (2019) Direct lineage reprogramming: Harnessing cell plasticity between liver and pancreas. Cold Spring Harb Perspect Biol.

9. Otsuka M, Hoshida Y, Kato N, Moriyama M, Taniguchi H, et al. (2003) Liver chip and gene shaving. J Gastroenterol 38: 89-92.

10. Zhu Y, Liu Q, Zhou Z, Ikeda Y (2017) PDX1, Neurogenin-3, and MAFA: Critical transcription regulators for beta cell development and regeneration. Stem Cell Res Ther 8.

11. Jennings RE, Berry AA, Kirkwood-Wilson R, Roberts NA, Hearn T, et al. (2013) Development of the human pancreas from foregut to endocrine commitment. Diabetes 62: 3514-3522.

12. DanilovaI G, GetteI F (2014) A method for modeling alloxan diabetes. Patent for Invention №2534411.

13. Spasov AA, Voronkova MP, Singur GL, Cheplyaeva NI, Chepurnova MV (2011) Experimental model of diabetes mellitus type 2. Biomedicine 3: 12-18.

14. Hewitt SM, Baskin DG, Frevert CW, Stahl WL, Rosa-Molinar E (2014) Controls for immunohistochemistry: The Histochemical Society's standards of practice for validation of immunohistochemical assays. J Histochem Cytochem 62: 693-697.

15. Seeberger KL, Anderson SJ, Ellis CE, Yeung TY, Korbutt GS (2014) Identification and differentiation of PDX1 β-cell progenitors within the human pancreatic epithelium. World J Diabetes 5:59-68.

16. Geerts A (2001) History, heteogeneity, developmental biology, and functions of quiescent hepatic stellate cells. Semin Liver Dis 21: 311-35.

17. Ferber S, Halkin A, Cohen H, Ber I, Einav Y, et al. (2000) Pancreatic and duodenal homeobox gene 1 induces expression of insulin genes in liver and ameliorates streptozotocin-induced hyperglycemia. Nat Med 6: 568-72.

18. McKimpson WM, Accili D (2019) Reprogramming cells to make insulin. Journal of the Endocrine Society 3: 214-1226.

## Author Affiliations

Top

[1]*Ural Federal University, Yekaterinburg, Russia*

[2]*Institute of Immunology and Physiology, Ural Branch of the Russian Academy of Science, Yekaterinburg, Russia*