The Pharma Innovation Journal 2016; 5(4): 80-83

www.ThePharmaJournal.com

The Pharma Innovation

ISSN: 2277- 7695
TPI 2016; 5(4): 80-83

www.thepharmajournal.com
Received: 07-02-2016
Accepted: 11-03-2016

Olga Ivantsiv
Postgraduate of human anatomy, gross anatomy and operative surgery of Ivano-Frankivsk National Medical University (supervisor - prof. Yu. Popovych), Ivano-Frankivsk, Ukraine.

Correspondence
Olga Ivantsiv
Postgraduate of human anatomy, gross anatomy and operative surgery of Ivano-Frankivsk National Medical University (supervisor - prof. Yu. Popovych), Ivano-Frankivsk, Ukraine.

Morphometric characteristic of the pancreatic islets in the treatment of experimental diabetes with insulin

Olga Ivantsiv

Abstract
Morphometric parameters of pancreatic islets of Wistar male rats were studied during treatment of experimental diabetes mellitus (EDM) with insulin. For this diabetes was simulated by intraperitoneal Streptozotocin administration. Starting with the 14-th day of diabetes development, every day animals were injected subcutaneously Insulin glargine("Lantus"). Material sampling was performed during the 42-nd, the 56-th and the 70-ieth day of the experiment. Correction associated with EDM with insulin in animals a mild reduction of lymphocytic infiltration, a slight decrease in intracellular edema of beta and alpha cells were observed. The decrease of glycemia level in fasting and the improvement of the overall health of animals were noted.

Keywords: pancreatic islet, experimental diabetes mellitus, Insulin glargine treatment.

1. Introduction
Diabetes mellitus Type I is a disease which is based on insufficient insulin production caused by the decreased number of beta cells of the pancreatic islets with the following increase of blood glucose level and the development of numerous complications [4, 14]. The main treatment for Type I diabetes is the replacement therapy with the use of different types of insulin [5-7]. However, conventional insulin preparations of short and medium spectrum, administered in injections for various dosing regimen were unable to normalize fully a daily insulin administration and require several everyday injections [8-12]. Today, the necessary insulin ‘’without spikes’’ is glargine (Lantus) of long 24-hour acting [15-17]. The drug is a biosynthetic human insulin analogue. It is synthesized by three ways of DNA of human insulin: a) joining the C-terminus of B-chain of insulin two positively charged arginine molecules (with a shift of isoelectric point of pH 4.5 to 6.7 – so, its solubility in physiological pH of the subcutaneous fat decreases); b) substitution of asparagine with glycin in the 21st position of A-chain (molecule gets a neutral charge, thereby increasing the bioavailability of insulin); c) addition of $Zn2^{+}$ to stabilize the contact between hexamers (long drug acting) [20-24]. Such modifications of Insulin glargine determine its prolonged absorption from the injection site, action ‘’without spikes’’ and also consistently reproduce the effect for at least 24 hours, reducing the frequency of night hypoglycemia [13, 18, 19]. As stated above, the aim of our study was to determine the features of morphometric changes of rats’ pancreas in the experimental diabetes treatment with insulin.

2. Material and Methods
Experimental studies were carried on 30 matured Wistar male rats, stimulated with experimental diabetes mellitus by intraperitoneal Streptozotocin administration. The animals were divided into two groups: 1) control group (animals with Streptozotocin-induced diabetes); 2) study group (animals, with subcutaneously injected prolonged Insulin glargine 1 time per day during the 14-th day). Recalculating of an average therapeutic dose, recommended for a human per 1 kg of body weight, in rats’ weight is carried out by constant biological activity. The animals were kept in standard vivarium conditions, care for animals and all the other manipulations are not contradicted «the European Convention for the Protection of vertebrate animals used for experimental and scientific purposes" [3]. The development of EDM was controlled by blood glucose level, determined by blood glucose meter «Accu Chec» (Germany). A drop of blood was obtained from the tail vein of the animal by making superficial cuts in the area of the tail. At the end of the experiment during the 42-nd, the 56-th and the 70-ieth day (5 rats for each sampling period) the animals in anesthesia were decapitated and portions of pancreas sampling was performed. Fixation of material and manufacturing of blocks were performed according to the standardized methods [3]. To study

the structural components of pancreas the histological sections were stained with hematoxylin and eosin. Histological preparations were examined under a light microscope MS 300, photographing of micropreparations of pancreas was made by Digital camera for microscope DCM 900. Electronomicroscopical study was carried out with PYEM 125 K (with a voltage of 75 kV) microscope. Identification of the structural components of pancreas was performed in accordance with International histological terminology. The average number of islets per 1 mm^2, the average area of the islands (mkm^2) and the average number of beta and alpha cells and their relationship were estimated [1].

3. Results and Discussion

Macroscopically, pancreas of both experimental groups did not differ (located intraperitoneally, head, body and tail were distinguished). The structure of the cross-section pancreas cut, microscopically, has lobed structure typical for rats' pancreas. Acinuses were formed by exocrinocytes in the cytoplasm. There zymogenic granules were visualized (Figure 1). The endocrine part is represented by pancreatic islets. There were distinguished among endocrinocytes' islets such cells as: beta and alpha cells, somatostatin cells, D1 cells, enterochromaffin cells, pancreatic gastrin cells and others.

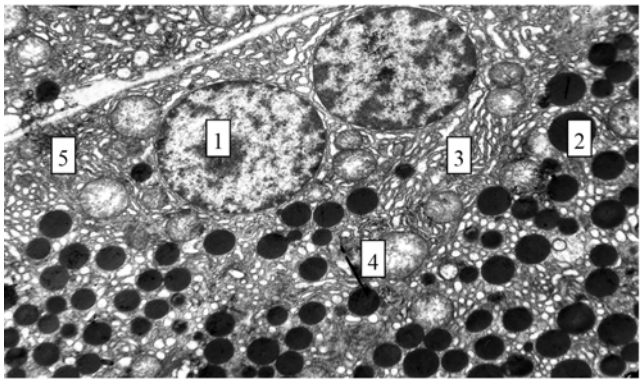

Fig 1: Zymogenic granules in the apical cytoplasm of dual exocrinocyte of rat's pancreas in the study group during the 42-nd day of EDM.

1 – two nuclei in the basal area of exocrinocyte, 2 – zymogenic granules in the apical zone of exocrinocyte, 3 – endoplasmic reticulum, 4 – mitochondria, 5 – cytoplasm. Electronography: x4200.

The introduction of insulin in EDM showed that during the 42-nd day of the experiment blood glucose level of animals was (9.02±0.34) mmol/L (which 1.69 times less than in control group), the average number of islets of 1 mm^2 – 1.51±0.38, and the average size of islets - (5031.48±361.41) mkm^2, the ratio beta/alpha cells – 2.52±0.04. Histologically, in the structural arrangement of pancreatic islets in insulin treatment the substantial positive changes (compared to the control group) in this study period were not observed. Among endocrinocytes the cells with dystrophic altered cytoplasm were visualized. Some beta cells were with the morphological features of the cytoplasm vacuolization. The nuclei were irregular in shape and the space around the nuclei was narrow. Heterochromatin indefinitely turned into euchromatin, compared to untreated animals, its number was slightly higher. Sometimes a violation of the integrity of the outer and inner nuclear membranes and the increased number of nuclear pores were noted. Secretory beta cell granules were arranged evenly throughout the cytoplasm, and they did not form clusters (Fig. 2), unlike the control group. During the 42-nd day of the experiment the intracellular destruction of the cytoplasm area with the formation of small vacuoles was observed in insulin treatment. Thus, in the control group the signs of vacuole dystrophy progression was seen without treatment. Such progression was shown, at the ultrastructural level with the significant expansion of the space around the nucleus and tanks of the granular endoplasmic reticulum. The formation of large vacuoles was also noted.

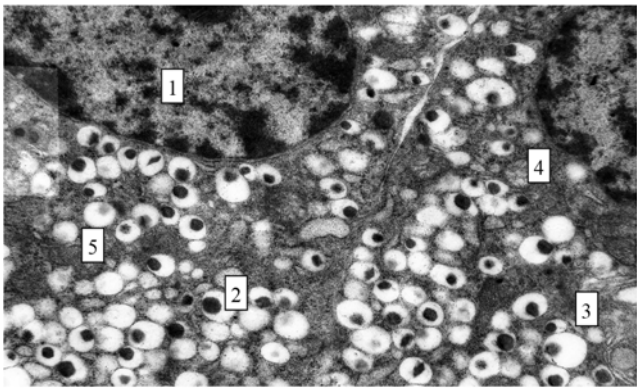

Fig 2: Ultrastructural organization of beta cell of the pancreatic islets during the 42-nd day of EDM in insulin administration.

1– nucleous of beta cell, 2– secretory granules of B-type, 3– endoplasmic reticulum, 4 – mitochondria, 5– cytoplasm. Electronography: x 8000.

In morphometric evaluation of micropreparations during the 56th day of the experiment there were found, that in animals of the control group without treatment the average number of islets per 1 mm^2 was 1.50±0.18; the average size of islets - (5314.31±393.54) mkm^2; and the ratio beta/ alpha cells was 2.70±0.14. In animals treated with insulin, these indexes were not significantly different from the control group and were lower than 0.7%; 0.4% and 3%. It was believed, that such indexes were due to a slight decrease of endocrinocytes' hypertrophy of islets in the study group. In animals treated with insulin, blood glucose during the 56-nd day of the experiment was 1.73 times lower than in the control group without treatment. It indicates the improvement of carbohydrate metabolism in the insulin replacement therapy. In this period of the experiment, electronomicroscopic examination showed, that in some endocrinocytes of the control group there were the signs of ballooning degeneration (small vacuoles merge to form a large and giant ones). While in drug therapy with insulin the slowing of the degenerative processes, reducing of vacuolization of cytoplasm, noted in the previous period of the experiment without treatment were shown. There was an ultrastructural manifestation of the decrease in intracellular edema of endocrinocytes and the increase the electron density of cytoplasm of alpha cells and the increased number of ribosomes of granular endoplasmic reticulum (Fig. 3).

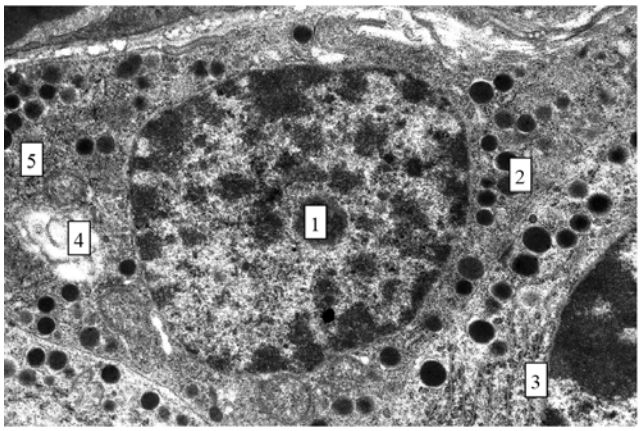

Fig 3: Reduction of dystrophy in the cytoplasm of alpha cell of pancreatic islets during the 56-nd day of EDM development in insulin administration.

1–alpha cell nucleus, 2–secretory granules of A-type, 3–endoplasmic reticulum, 4–dystrophy area, 5–cytoplasm. Electronography: x 9600.

During the 70-ieth day of the experiment of glargine administration as a replacement therapy of diabetes showed that the average number of islets per 1 mm^2 was 1.50±0.24; the average size of islets – (5534.26±643.17) mkm^2; and the ratio of beta /alpha cells – 2.90±0.13, which were not significantly different from that of animals with EDM without treatment. Blood glucose level in the insulin administration was 1.66 times lower than in the control group. Monotherapy with insulin at the time of the experiment reduces the progression of the disease, that histologically manifested by a decrease of endocrinocytes'vacuolization of cytoplasm (Fig. 4). Large vacuoles actually were not found, compared to untreated animals. The secretory granules were with a bright rim without the signs of merging. The cytoplasmic membrane was also without significant changes.

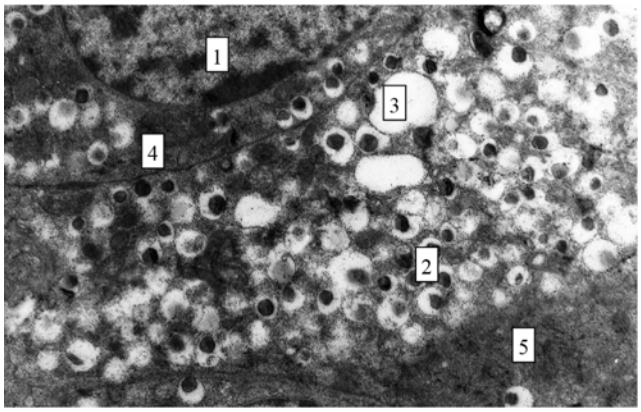

Fig 4: Small vacuoles in beta cell cytoplasm of rat's pancreatic islets during the 70-ieth day of the EDM in the insulin administration.

1–beta cell nucleus, 2–secretory granules of B-type, 3–vacuoles, 4–area around the nucleus, 5–cytoplasm. Electronography: x 8000.

4. Conclusions

1. Treatment of rats with Streptozotocin-induced diabetes by insulin replacement therapy with the use of the long acting Insulin glargine allowed to reduce the increase of glucose level, compared with such levels in the untreated animals (1.69; 1.73; 1.66 times respectively during the 42-nd, the 56t-h and the 70-ieth day of the experiment).
2. We have not found significant differences of the morphometric indexes: the average area of islets (mkm^2), the average number of islets per 1 mm^2, the ratio beta/alpha cells in rats with EDM. The differences were not found in treatment of diabetes mellitus with Insulin glargine in the period of the experiment. However, histologically in the structural organization of the pancreatic islets of the long acting insulin replacement therapy of Streptozotocin-induced diabetes the significant slowing of degenerative processes, compared to the untreated animals was noted. The proof of this process is the reduction of intracellular beta cell edema and slowing the progression of endocrinocytes' vacuole degeneration of the most pancreatic islets. So, monotherapy of diabetes with Insulin glargine provides the delayed progression and stabilization of the disease.

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