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Use of glucagon-like peptide-1 agonists in treatment of morphologicalmanifestations of diabetic gastroparesis

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Abstract:-The research included 18 mature male Wistar rats. They were divided into 3 groups: Group I - 6 animals (the control group); Group II - 6 animals with streptozotocin-induced DM (DM was induced by a single intraperitoneal administration of streptozotocin at a dose of 6 mg/100 g body weight); Group III - 6 animals with streptozotocin-induced diabetes mellitus receiving a subcutaneous injection of exenatide at a dose of 0.04 mcg/100 g body weight once a day since the 14th day of DM development. Diabetic gastroparesis was observed on the 56th day of the development of streptozotocin-induced DM being morphologically manifested as destructive changes in neurons of the myenteric plexus (vacuolar degeneration, apoptosis), axonal degeneration of unmyelinated nerve fibers, apoptosis of the interstitial cells of Cajal, vacuolar degeneration of myocytes and the development of diabetic microangiopathy. The use of exenatide in the early stages of DM development (up to 28th day) led to the normalization of glycemic profile and the restoration of structural elements of the myenteric plexus and smooth myocytes of the muscular membrane of the stomach indicating the adequacy of the proposed therapy. However, the use of exenatide as a monotherapy for 1.5 month resulted in elevated blood glucose and HbA_{1c} levels as well as destructive changes in neurons and the interstitial cells of Cajal of the myenteric plexus and smooth myocytes of the muscular membrane of the stomach. Considering the conducted research, we can conclude that the use ofexenatide as a monotherapy in diabetic gastroparesis is advisable in the early stages of disease development only, in the late stages – only combinations with other hypoglycemic agents should be used.

Keywords:- *Diabetic gastroparesis, exenatide, interstitial cells of Cajal, stomach,streptozotocin-induced diabetes mellitus*

I. INTRODUCTION

Diabetes mellitus (DM) is considered as a relevant problem due to its significant prevalence as well as the fact that it is the basis for the development of serious chronic complications, early incapacitation and high mortality rates among patients. Nowadays the progression of diabetic polyneuropathy is found in most patients with DM; however, the manifestations of autonomic neuropathy of the gastrointestinal tract are diagnosed too late as they are often considered as the manifestations of other diseases.The increase in the duration of DM, poor glycemic control and the number of other factors may cause numerous gastrointestinal disorders, the severest manifestation of which is gastroparesis. Diabetic gastroparesis (DG) involves a variety of neuromuscular dysfunctions of the stomach including abnormal gastric contractility as well as myoelectrical activity in patients with DM which impair their life quality [5, 10, 16].A new direction in treatment of type 2 DM and its complications is based on the effects of incretins glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) which contribute to regeneration and improvement ofislet cell function.Hormones – incretinmimetics are a modern class of antihyperglycemic agents used in treatment of type 2 DM – GLP-1 agonists.GLP-1 consists of 30 amino acid residues; it is synthesized by alpha cells in the small and large intestines and acts on pancreatic alpha and beta cells, the gastrointestinal tract, the central nervous system, the heart and the thyroid gland through specific receptors.GLP is synthesized by K cells of the duodenum affecting pancreatic alpha, beta and delta cells, osteoblasts, adipocytes, nerve endings. The secretion of incretins is due to food stimulation of the proximal intestine, postprandial hyperglycemia and dyslipidemia.In patients with type 2 DM, the concentration of GLP-1 is significantly lower than in healthy individuals contributing to the development of postprandial hyperglycemia which aggravates the clinical course of DM. GLP-1 stimulates neogenesis of insulin-producing cells from epithelial progenitor cells (beta cells) and promotes the correction of progressive beta-cell dysfunction as well as other mechanisms of glucose homeostasis regulation [1, 4, 6, 11].Synthesized GLP-1 agonist medications (exenatide, liraglutide) allow regulating the digestive process through the slowing down of gastric motility and prolongation of time which is necessary for food to enter the intestine.The aforementioned mechanisms facilitate glycemic control as well as

contribute to a feeling of satiation; they eliminate peripheral insulin sensitivity in patients with type 2 DM and prevent the development of osteoporosis and osteopenia. The results of a number of clinical trials indicated that therapy with GLP-1 agonist medications provides a significant improvement of glycemic control, normalizes glucagon secretion, reduces body weight and improves lipid metabolism [7, 12, 15].Experimental studies have proven that the aforementioned class of antidiabetic drugs stops the process of damage to the black substance in the midbrain due to the interaction with receptors on their surface, hence possessing neuroprotective as well as cardioprotectiveproperties.However, in literature, there are insufficient data on the effect on the nervous apparatus of the stomach [3, 13, 17].Therefore, the objective of our research was to determine morphological changes in the nervous apparatus of the stomach and its smooth myocytes in streptozotocin-induced diabetes and its correction.

II. MATERIALS AND METHODS

18 sexually mature male Wistar rats weighing 180 to 220 g were examined.They were divided into 3 groups: Group I included 6 animals (the control group); Group II comprised 6 animals with streptozotocin-induced DM; Group III included 6 animals with streptozotocin-induced DM receiving exenatide. DM was induced by a single intraperitoneal administration of streptozotocin (6 mg/100 g body weight); animals of the control group were injected with an equivalent amount of 0.1 M citrate buffer pH 4.5.Animals of Group III received a subcutaneous injection of exenatide at a dose of 0.04 mcg/100 g body weight once a day since the 14^{th} day of the development of DM.The samples were collected on the 28^{th} and 56^{th} days of the experiment.Euthanasia was performed using decapitation under thiopental anaesthesia; then, the blood samples were collected into the test tubesfor biochemical analysis. The development of DM was evaluated measuring blood glucose level which was determined collecting a drop of blood from tail vein with the help of a blood glucose meter (Accu-Chec, Germany) and test strips.The level ofglycated hemoglobin (HbA_{1c}) was measured using an extremely sensitive ion exchange liquid chromatography method.For electron-microscopic study, small pieces of the stomach wall1x1 in size were fixed in a 2% solution of osmium tetroxide using standard contrasting technique.The material was investigated at a magnification of 1,200 to 12,000 times by means of electron microscope PEM-125K at an acceleration voltage of 75 kV.Semi-thin sections stained with a 2% solution of methylene blue were examined using the light microscope 300 MC (TXP) and photographed using a Digital camera for microscope DCM 900.Morphometry was performed using image processing program NIH USA ImageJ in a manual mode considering the magnifications.Structural changes at a certain stage of the study were analyzed in 50 fields of view. The surface area of the profile of neurons and their nuclei, the nucleocytoplasmic index (NCI), and nuclear shape factor (NSF) were determined.

For statistical processing of the material at all stages of the research, several Microsoft Excel-based computer programs were developed (calculation of relative values, their deviations, t-test).The mean values were calculated by the statistical software package Microsoft Excel. Nonparametric methods of the investigations, descriptive statisticsin particular, were used. The interval scale indicators were presented in the form of the mean values and standard deviations. The value of $p<0.05$ was considered statistically significant.

III. RESULTS AND DISCUSSION

Two weeks after treatment with exenatide, in animals of Group III, blood glucose level decreased by 9,38±0,14mmol/l ($p=0.03$) compared to animals of Group II (blood glucose level was 12.51±0.94mmol/l); however, these indicators were significantly higher compared to the control group (5.03±0.08mmol/l, $p=0.03$ in all cases).In animals of Group III, HbA_{1c} level decreased by 7.29±0.27% ($p=0.02$) comparedtoGroup II (8.22±0.14%) aswell; such HbA_{1c} levels were higher than control values (the control group – 4.31±1.11%, $p=0.03$), however, they were found to bewithin the permissible limits.On the 28^{th} day of the experiment, in animals of Group I, there was observed a significant increase inthearea of neuronal profile as well as their nuclear profile in the myentericplexus compared to control values (Table 1); the area of profile of neuronal nuclei decreased significantly.At the ultrastructural level, neural microtubules and neurofibers, oval mitochondria with reduced cristae, dilated cisternae of therough endoplasmic reticulum (RER), isolated small vacuoleswere identified in the perikarya of most neurons.The nuclei were of moderate electron density with diffusely scattered granules of euchromatin and 1-2 electron dense nucleoli. The nuclear membrane formed significant invaginations. Pycnomorphic neurons were observed in the myentericplexus. On the one hand, such morphometric and ultrastructural neuronal changes indicated their increased functional activity [2, 9, 14] that was obviously associated with polyphagia and constant gastric motility for evacuating intestinal contents; on the other hand, vacuolar degeneration was observed which might be irreversible. In unmyelinated nerve fibers, there was found the axoplasm of low electron optical density containing mitochondria with enlightened matrixand destroyed cristae.The Schwann cell cytoplasm was characterized by an increase in the number and size of breakdown products of myelin and the RER.

Table 1. Morphometric parameters of neurons of the myentericplexus of the stomach in streptozotocin-induced

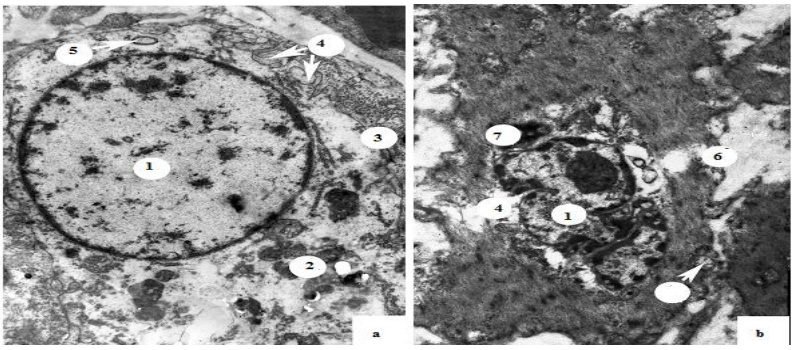
diabetes and its correction (M±m)

Groups	Surface area of cell(μm^2)	Surface area of the nucleus(μm^2)	NSF	NCI
the 28thday				
I	90.83±5.38	29.59±1.01	0.82±0.02	0.47±0.05
II	106.30±2.80*	34.49±1.18***	0.69±0.05*	0.76±0.02
III	94.02±3.42	30.47±1.57	0.98±0.02	0.44±0.02
the 56th day				
I	92.51±5.11	29.20±1.04	0.83±0.02	0.45±0.05
II	146.92±3.74$^{***, ###, §}$	46.32±4.73$^{**, ###, §}$	0.94±0.02$^{**, ##, §}$	0.45±0.04
III	106.22±2.62$^{*,###, §}$	36.66±1.95$^{**, # §}$	0.86±0.02$^{##, §}$	0.49±0.03

N o t e s :

1. * - significant difference compared to Group I, * -p<0.05, ** -p<0.01, ***-p<0.001;
2. $^{#}$ - significant difference between Group II and Group III, $^{#}$ - p<0.05, $^{##}$ - p<0.01, $^{###}$ - p<0.001;
3. § - significant difference between animals of the same group in different time periods, p<0.05.

The interstitial cells of Cajal contained irregular nuclei with predominant heterochromatin; lysosomes, autophagosomes, isolated small vacuoles were identified. In myocytes, reversible dystrophic changes in mitochondria were detected.In the microcirculatory bloodstream, erythrocyte sludges, erythrocyte and thrombocyte adhesion, early manifestations of diabetic microangiopathy were observed.On the 28th day of the experiment, in animals of Group III, the surface area of neurons as well as their nuclei in the myentericplexus reduced significantly compared to animals of Group II being not statistically different from control values (Table 1).Neurons, gliocytes, unmyelinated nerve fibers as well as the interstitial cells of Cajal of the myenteric plexus restored their ultrastructural organization; however, in some light neurons, vacuolar degeneration was observed. In light myocytes, some modified mitochondria were detected.Incapillaries, nummular erythrocyte sludge was often observed. Thus, the use of exenatide in streptozotocin-induced DM led to the restoration of structural elements of the myenteric plexus and smooth myocytes of the muscular membrane of the stomach as well as normalized the motor-evacuation function of the stomach indicating the adequacy of the proposed therapy.On the 56th day of the experiment, in animals of Group II, blood glucose level continued to increase being 19.27±2.88mmol/l (the control group – 4.86±0.62 mmol/l, p=0.04); HbA_{1c} level increased by 9.82±0.83% (the control group – 3.88±0.18%, p=0.03).On the background of pronounced uncompensated DM, pathological changes in the nervous apparatus and the muscular membrane of the stomach developed. According to the results of morphometry, the surface area of neurons and their nuclei increased significantly as compared to the previous observation period.NSF increased as well indicating edematous changes in the nucleus being confirmed at the light-optical and submicroscopic levels.On semi-thin sections, central and peripheral chromatolysis, vacuolization of the neuroplasm were seen in neurons. At the ultrastructural level, light neurons were destroyed due to colliquative necrosis. Enlarged light nuclei were seen; membranous organelles were destroyed (Fig. 1a).The neuroplasm contained myelin-like inclusions, lipofuscin granules. Alongside with destructively changed neurons dark pycnomorphic neurons with apoptotic bodies as well as neurons with preserved ultrastructure were detected. However, the latter were rare.Such morphological changes in neurons were associated with hypoxia which occurred due to diabetic microangiopathy as well as impaired metabolic processes as low insulin levels led to the impairment in protein synthesis in neurons of the brain and damage to neurofilamentsresulting in diabetic encephalopathy [8, 9].In the Schwann cell cytoplasm,lysosomes and autophagosomes were identified. The axoplasm of unmyelinated nerve fibers contained isolated mitochondria with destroyed cristae and many neurofilaments which indicated the delay of axonal transport in DM [3, 13, 16].



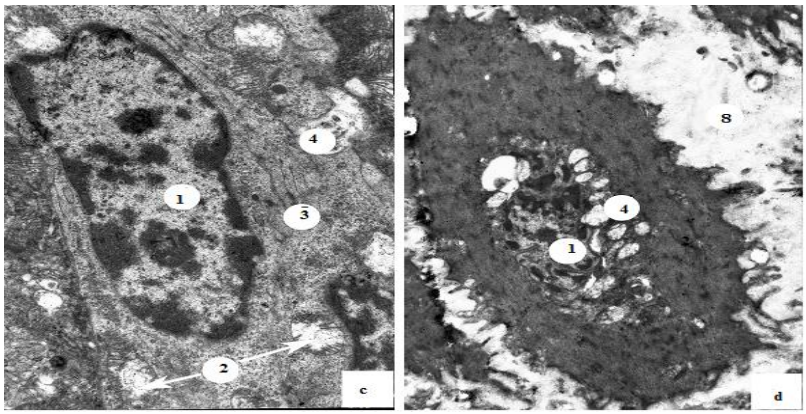


Fig. 1. Ultrastructuralalterations in neuron (a, c) and myocytes (b, d) in animals of Group II (a, b) and Group III (c, d) on the 56th day of the development of experimental DM. Electron diffraction patterns. Mag.: a, d) × 6,400;b, c) × 8,000

1 - nucleus, 2 - mitochondria, 3 - RER; 4 - vacuoles; 5 - myelin-like inclusions, 6 - lysis of microfilaments, 7 - autophagosomes, 8 - interstitial edema.In most interstitial cells of Cajal, there was observedcaryorrhexis with further nuclear fragmentation and the formation of apoptotic bodies. Their cytoplasm was of different electron optical density and contained lysosomes, autophagosomes and vacuoles. Other researchers indicated a reduced number of the interstitial cells of Cajal in DM as well [9, 14] and according to the results of our study, it is caused by their apoptosis and colliquativenecrosis.On the background of circulatory and hemic hypoxia, there were detected destructive changes in myocytes of the stomach wall due to hydropic degeneration and colliquative necrosis (Fig. 1b). Interstitial edema was found; the overgrowth of connective tissue between myocytes was observed (Fig. 1b)In animals of Group III receiving daily injections of exenatide, blood glucose level decreased significantly as compared to animals of Group II - 6.88±0.84 mmol/l ($p=0.03$); however, such indicators were higher than in the control group ($p=0.03$).In animals of Group III, HbA_{1c}level decreased significantly as compared to animals of Group II as well – 5.93±0.12% ($p=0.02$); however, such HbA_{1c}levels were higher than control ones ($p=0.02$).According to the results of morphometric analysis, in animals of Group III, the surface area of neurons and their nuclei was significantly lower as compared to animals of Group II; however, it did not match control values (Table 1).At the ultrastructural level, a moderate vacuolar degeneration was observed in most light neurons (Fig. 1c) which manifested itself as the destruction of the inner mitochondrial membrane, the dilated RER, increased amount of heterochromatin in the nucleus. Swollen and destructed mitochondria (some of which were converted to vacuoles and found around the nucleus) (Fig. 1d), dilated cisternae of the RER and the Golgi complex were identified in myocytes.The spaces between myocytesbecame distended and filled with loose connective tissue. Considering the aforementioned data, we can conclude that the use of exenatide for 1.5 month in case of streptozotocin-induced DM is advisable only in combination with other hypoglycemic agents as it does not result in the normalization of chronic glycemia and, as a result, destructive changes in structural elements of the myenteric plexus and smooth myocytes of the muscular membrane of the stomach are observed.

IV. CONCLUSION

Diabetic gastroparesis in rats was observed on the 56th day of the development of experimental DM being morphologically manifested as destructive changes in neurons of the myenteric plexus (vacuolar degeneration, apoptosis), axonal degeneration of unmyelinated nerve fibers, apoptosis of the interstitial cells of Cajal, vacuolar degeneration of myocytes and the development of diabetic microangiopathy. The use of exenatidein the early stages of the development of experimental DM (up to 28th day) led to the normalization of glycemic profile and the restoration of structural elements of the myenteric plexus and smooth myocytes of the muscular membrane of the stomach indicating the adequacy of the proposed therapy. However, the use of exenatide as a monotherapy for 1.5 month resulted in elevated blood glucose and HbA_{1c} levels as well as destructive changes in structural elements of the myenteric plexus and smooth myocytes of the muscular membrane of the stomach. Considering the conducted research, we can conclude that the use ofexenatide as a monotherapy in case of diabetic gastroparesis is advisable in the early stages of disease development only, in the late stages – only combinations with other hypoglycemic agents should be used. Further study of changes in the nervous apparatus of the stomach in diabetes mellitus which will serve as the theoretical basis for the development and pathogenetic substantiation of actions aimed at the correction and prevention of diabetic gastroparesis is promising.

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