NANOCHROMIUM CITRATE: ANTIHYPERGLYCEMIC AND PANCREATOPROTECTIVE ACTION AGAINST UNDERLYING DEXAMETHASONE-INDUCED DIABETES MELLITUS

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Abstract:

Introduction: The Ukrainian State Scientific-Research Institute of Nanobiotechnology and Resources Saving has received nanochromium citrate (NCC), a new chrome compound, by means of the electric pulse aquanotechnology method.

Objectives: to determine nanochromium citrate efficacy with experimental dexamethasone-induced diabetes through the results of antihyperglycemic activity and its effect on the pancreatic histological structure.

Methods: diabetes mellitus was simulated by dexamethasone administration on 18-month male rats. The morphological structure of the pancreas was examined in comparison with metformin. Examination of the pancreas morphological structure considered the amount, distribution and size of the pancreatic islets and their cellular shape.

Results: on the 14th day of simulated diabetes mellitus the preventive -therapeutic indication of nanochromium citrate promoted a decrease of glycemia level twice. The administration of nanochromium citrate with underlying diabetes mellitus is not inferior to metformin and its protective effect on the pancreas morphological structure exceeds the effect of metformin.

Conclusion: nanochromium citrate of experimental dexamethasone-induced diabetes mellitus in rats decreases the glycemia level twice which does not differ reliably from the effect of metformin antihyperglycemic. The cytoprotective effect of nanochromium citrate produced on the histological structure of the pancreas is found to exceed metformin action.

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Keywords: nanochromium citrate, hypoglycemic activity, experimental diabetes mellitus, metformin

Introduction

The issue of diabetes mellitus (DM) considering its occurrence, severity and complications, is one of the most urgent in the world (Crawford, 2017; Pan'kiv, 2015). The number of DM patients is hypothesized to increase to 642 million by 2040 (Ogurtsova, 2015). In Ukraine, the DM sickness rate increases annually by 5-7%. Nowadays there are over 2 million people with DM in Ukraine (Tkachenko, 2014; Tsytovs'kyi, 2017). In spite of the introduction of new diagnostic and therapeutic technologies into medical practice, the search for effective and safe medicinal means for DM treatment remains a high priority.

Due to nanotechnology, the contemporary state of scientific development has been supplied by new achievements in the fields of nanobiology, nanomedicine, and nanopharmacology (Chekman, 2017). Nanoparticles and nanostructured materials are already used as new remedies, biosensors and devices for visualization and diagnostics (Holovenko, 2008; Yigit, 2012; Malekzad, 2017). Dynamic research of toxicological and pharmacological properties of nanoparticles of biometals such as silica, silver, iron, copper and their composites, is being conducted in Ukraine (Savchenko, 2014; Doroshenko, 2014; Pryskoka, 2016; Simonov, 2016).

Chromium nanoparticles are considered to be promising structures used in nanomedicine. Scientific literature contains sporadic information concerning the biological activity of chromium nanoparticles (Zha, 2007; Chandra, 2016). Since chromium is an essential trace element entering the body from outside, it participates in the regulation of insulin production and metabolism, provides pancreatic functioning, intensifies insulin action when contained in glucose tolerance factor (GTF) (Jeejeebhoy, 1999), and being a low molecular organic complex, chromium compounds can supplement the current treatment of DM (Suslyk, 2014; Dashkevich, 2013). Dietary supplements containing chromium are

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recommended for type 2 DM (“Chromium active”, “Chromium Chelat”, “Solgar Chromium Picolinate”, “Insuvit”) in order to normalize the metabolism of proteins, fats and carbohydrates (Shcherbak, 2004; Lewicki, 2014).

The Ukrainian State Scientific-Research Institute of Nanobiotechnology and Resources Saving (Ltd “Nanomaterials and Nanotechnology”, Kyiv) has received nanochromium citrate (NCC), a new chrome compound, by means of the electric pulse aquanotechnology method (Kosinov, 2008). Our previous screening studies determined hypoglycemic action available in NCC (Sadohurska, 2016). Its pathogenic mechanism requires further study.

Thus, the aim of this study was to determine nanochromium citrate efficacy within experimental dexamethasone-induced diabetes by the results of antihyperglycemic activity and its effect on the pancreatic histological structure.

Material and Methods

The study was conducted on albino outbred 18-month male rats with a body weight of 220-270 g. Experimental diabetes mellitus (EDM) was simulated by subcutaneous injection of dexamethasone in a dose of 0.125 mg/kg of the body weight for 14 days (Stefanov, 2001). Metformin Sandoz was used as a drug of comparison in the form of a water suspension. It was introduced into the stomach by means of a probe in a dose of 200 mg/kg (Poltorak, 2000). Simultaneously with dexamethasone NCC was introduced in the stomach in a dose of 0.01 mg/kg (Sadohurska, 2016) or metformin. The animals were divided into 4 groups. I – intact rats, II – control pathology, III – dexamethasone + NCC, IV – dexamethasone + metformin. Antihyperglycemic properties of NCC were assessed by basal glycemia (in the dynamics on the 1st, 7th, 14th day) by means of a glucometer (Accu-Chek Active New, Germany). To assess morphological changes histological sections of tissues stained with hematoxylin and eosin (Venerucci, 2016) were studied by means of a light microscopy method (microscope “LUMAM-P8”, digital camera Olympus C 740UZ). All interventions were conducted in accordance with the criteria outlined in the European Union Directive 2010/63/EU “On the protection of animals used for scientific purposes” (2010).

Statistical analysis of the data was performed using SPSS 17.0 software. All data are represented as a mean $\pm$ standard error of the mean ($M \pm m$). Estimation of the differences between the samples was conducted using a parametric Student’s t-test and nonparametric Mann-Whitney U test. The values $p < 0.05$ were considered significant.

Results and Discussion

In the first day glucose concentration in the blood of intact and experimental animals ranged within the norm and did not differ among animals from the other groups (Table 1). On the 7th day of modeling control pathology, the glucose concentration in the blood of animals after dexamethasone administration increased by 1.3 times. Similar glycemia increase was observed among animals from groups III and IV. On the 14th day dexamethasone-induced glycemia increased practically twice as much, and the glucose level in the blood increased 2.6 times in comparison to the intact control, which is a reliable sign of experimental type 2 DM reproduction (Poltorak, 2000). The glycemia level in animals receiving NCC as a preventive-therapeutic measure remained on the initial level on the 14th day (similar to that of the 7th day), though it appeared to be 2.1 times lower than that of the animals with EDM. A similar effect was observed when metformin was introduced (Table 1), which is indicative of hypoglycemic action of NCC similar to that of the reference-drug metformin. Literary data indicate that correction hypoglycemic effect of NCC is likely to occur in case of streptozotocin-induced DM as well (Iskra, 2014), and when chromium citrate is administered for alloxan-induced DM (Li, 2011).

Since the major insulin producer in the body is beta-cells of islets of Langerhans, the effect of NCC on the morphological structure of the pancreas with underlying EDM was examined. The islets of Langerhans in intact animals were found to occur practically in every visual field (99%). Their size was on an average 205.0 ± 12.65 mcm, the cellular profile on one histological section of the islet was 84.0 ± 5.69 (Fig. 1).

In animals with EDM, the general amount of the islets is considerably smaller (under 27% in the visual field). Their size is 4.8 times smaller (up to 43.0 ± 4.27 mcm), and their shape is changed to an

irregular one. The cellular profile of the islets on one section was 3.3 times less (up to 25.5 ± 3.56), and the majority of cells were found to be in the state of necrosis (Fig. 2).

Table 1: Glycemia dynamics in rats in the process of diabetes mellitus simulation and the effect of nanochromium citrate ($M \pm m$)

Conditions of the experiment	Glucose content in the blood, mmol/L		
	1 day	7 day	14 day
Intact control	5.30 ± 0.20	5.40 ± 0.07	5.47 ± 0.21
Dexamethasone (0,125 mg/kg) (control pathology)	5.37 ± 0.26	7.20 ± 0.20 $p < 0.01$	14.22 ± 0.36 $p < 0.01$
Dexamethasone + Nanochromium citrate (0,01 mg/kg)	5.52 ± 0.19	6.47 ± 0.30 $p < 0.01$	6.63 ± 0.34 $p < 0.05, p_1 < 0.01$
Dexamethasone + Metformin (200 mg/kg)	5.65 ± 0.16	7.05 ± 0.35 $p < 0.01$	6.98 ± 0.41 $p < 0.01, p_1 < 0.01$
p – reliable difference compared with the intact control; p_1 – reliable difference compared with the control pathology			

Source: Authors

Figure 1: Slide mount of the pancreatic islet of an intact rat. 1 - islet of Langerhans. H&E. x100.

Source: Authors

Figure 2: Slide mount of the pancreatic islet with experimental diabetes mellitus. 1 - islet of Langerhans. H&E. x100.

Source: Authors

It should be noted that in comparison with EDM animals, the administration of NCC promoted considerably decreased alternative signs in the endocrine part of the gland (Fig. 3). Though the size of the islets did not differ considerably from that of the intact animals (217.5 ± 19.36 mcm), their cellular profile appeared to be 5 times larger than with EDM, and it even increased the profile of the intact rats (133.0 ± 12.4 against 84 ± 5.69 in the control). Reduced degenerative changes in the morphological structure of the gland in the process of EDM simulation and NCC effect are evidenced by the fact that only single cells of the islets were in the state of necrosis. Thus, in the process of EDM simulation NCC decreases the toxic effect of dexamethasone on the morphological structure of the gland.

The size of the islets of Langerhans in animals receiving metformin (Fig. 4) ranged within 140.0 ± 11.1 mcm and they were 1.5 times smaller than when NCC was administered under similar conditions. Though the cellular profile on one histological section of the islet (67.5 ± 4.98) was close to the level of intact animals, it appeared to be two times smaller than that of the cellular profile under conditions of NCC action. Necrotic signs were found in 3-12% of cells.

Therefore, pathomorphologic studies of the pancreas of rats with EDM are indicative of a more marked protective effect of NCC by morphometric parameters in comparison with metformin action (Table 2).

Figure 3: Slide mount of the pancreatic islet under nanochromium citrate action with underlying experimental diabetes mellitus. 1 - islet of Langerhans, 2 – group of necrotized cells. H&E. x100.	Figure 4: Slide mount of the pancreatic islet under metformin action with underlying experimental diabetes mellitus. 1 - islet of Langerhans, 2 - group of necrotized cells. H&E. x100.

Source: Authors	Source: Authors

Table 2: Morphometric parameters of the pancreatic islets under conditions of nanochromium citrate and metformin action with underlying experimental DM (M±m)			
Conditions of the experiment	Size of the islets of Langerhans, mcm	Cellular profile	Terms of the experiment
Intact control	205.00±12.65	84.0±5.69	lacking
Control pathology (EDM)	43.00±4.27*	25.5±3.56*	majority of cells
EDM+NCC	217.5±19.36#	133.0±12.4#	single cells
EDM+metformin	140.00±11.1#	67.5±4.98#	single cells
* – reliable difference compared with the intact control (p<0.05), # – reliable difference compared with the control pathology (p<0.05)			
Source: Authors			

Conclusion

Thus, NCC administration with underlying EDM decreases glucose concentration in the blood twice as much, which does not differ reliably from the antihyperglycemic effect of metformin. The cytoprotective effect of NCC produced on the histological structure of the pancreas is found to exceed the metformin action (1.5 times larger size and twice as much cellular profile of the pancreatic islets of Langerhans).

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