April 30, 2021

PharmacologyOnLine

Archives • 2021 • vol.1 • 283-287

EFFECT OF CHROMIUM NANOPARTICLES ON ERYTHROCYTE MEMBRANE PERMEABILITY UNDER CONDITIONS OF EXPERIMENTAL DIABETES MELLITUS IN RATS

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Abstract

Diabetes mellitus (DM) is a serious medical-social issue, since it is characterized by considerable occurrence in the world and a stable tendency to the increase of a number of patients. Nowadays the interest to nanobiology, nanomedicine, and nanopharmacolgy increases. Appearance of a new compound of chromium nanoparticles has provoked interest to its activity and perspectives of its use in medicine, and with DM in particular. At the same time, scientific literature contains only isolated facts concerning biological activity of chromium nanoparticles. Effect of chromium nanoparticles on the state of erythrocyte plasmatic biological membranes is examined according to the changes of erythrocyte membrane osmotic resistance (EMOS) in rats under conditions of experimental DM. In experimental DM, when glucose concentration in the blood of rats increased in comparison with the intact control, EMOS decreased. The use of NCC in animals with DM reduced glucose concentration in the blood to the levels close to the physiological ones, and percentage of laky erythrocytes with low urea concentration decreased twice as compared to the appropriate index in animals with experimental DM. While increasing EMOS with underlying experimental DM nanochromium citrate manifests membrane stabilizing properties specified by reduced glucose toxicity and increased antioxidant activity of NCC.

Keywords: *nanochromium citrate, erythrocyte membrane osmotic resistance, experimental diabetes mellitus*

Introduction

Diabetes mellitus (DM) is a serious medical-social issue, since it is characterized by considerable occurrence in the world and a stable tendency to the increase of a number of patients. The WHO estimates occurrence of DM ranging from 1.5 to 4%, with a considerable increase in the developed countries of the world. In Ukraine the number of DM patients doubles every 12-15 years [1]. In spite of a substantial amount of scientific studies, the search of new forms and methods of DM prevention and treatment requires further detailed investigation of the mechanisms promoting development of the disease [2, 3].

Since chromium as a trace element participates in the regulation of insulin production and metabolism, plays an important role in carbohydrate, lipid, and protein metabolism [4], scientists are interested in an organic chromium nanocompound – nanochromium citrate (NCC) obtained at the Scientific-Research Institute of Nanobiotechnologies and Resource-Saving ("Nanomaterials and Nanotechnology", Kyiv) [5]. Literary data contain information that the compound in the animal body increases the content of reduced glutathione and activity of the enzymes of the glutathione system antioxidant protection (glutathione peroxidase, glutathione reductase), which is indicative of its antioxidant effect [6]. The results of the studies can be indirectly indicative of NCC membrane protective action.

The amount of free radicals is known to increase, oxidative and metabolic stresses are known to occur with DM and underlying chronic hyperglycemia associated with increased glucose auto-oxidation, which produces a negative effect on the morphofunctional state of the cellular membranes [2, 7]. Changes in the structure and functions of biological membranes are considered as one of the main universal link in pathogenesis of various diseases. Erythrocytes were used as a cellular model for the studies on the cellular level, since their membrane organization is similar to that of other cells. Erythrocyte membrane osmotic resistance is an integral index of the membrane processes [8]. The data concerning the changes of erythrocyte permeability can be reliably considered as an indicator of general cellular permeability and the state of body health on the whole [9]. The objective of the study was to investigate NCC effect produced on erythrocyte membrane osmotic resistance of the peripheral blood in animals with experimental DM.

Methods

The studies were carried out on albino outbred 18-month male rats with the body mass of 220 –250 g. The animals were provided with free access to food and water. They stayed under standard vivarium conditions. The animals were divided into 4 groups 6 animal each. I control group included intact rats, II group included animals with NCC every day introduced into their stomachs in the dose of 0.01 mg/kg during 14 days [10]. DM was simulated in animals from III and IV groups by means of subcutaneous introduction of dexamethasone (KRKA, Slovenia) in the dose of 0.125 mg/kg of the body weight during 14 days [11], and glucose level in the blood was controlled by means of glucometer (Accu-ChekActiveNew, Germany). In addition to dexamethasone the rats from IV group received NCC in the dose of 0.01 mg/kg during 14 days. The blood for examination was taken with 1-2 drops of heparin added. After centrifugation erythrocytes were removed, and washed with cooled isotonic sodium chloride solution three times.

Those biochemical methods of examination of membrane function that were associated with detection of erythrocyte hemolysis under the influence of various lytic factors were prioritized. They included various methods of erythrocyte membrane osmotic resistance (EMOS) examination: in the medium with low concentrations of sodium chloride; osmotic erythrocyte hemolysis under effect of various urea concentrations with stable osmolarity of the solution; acidic, spontaneous hemolysis; the method erythrocyte sorption ability [9]. EMOS was studied by means of ureal erythrocyte hemolysis method [8]. The method is based on finding differences of erythrocyte osmotic stability depending on different volumetric content of urea isotonic solutions (0.3 mol/L) and sodium chloride (0.15 mol/L) in the mixture. A series of dilution was prepared in the order of increasing urea concentration (Table). 5 ml of urea solutions with various concentration were filled in 7th centrifuge

tubes, 0.1 ml of erythrocytes was added, mixed, and centrifuged during 10 minutes with 1500 RPM in the centrifuge OPN-3. Centrifugate optic density in relation to distilled water was measured by means of photocolorimetry method with a green light filter (540 nm). The results were calculated in percent taking absorption of the 7th tube content (initial urea solution) as 100% hemolysis. The degree of hemolysis (%) in every tube was calculated in relation to the standard optic density (7th tube content).

All studies were carried out in accordance with the criteria outlined in the European Union Directive 2010/63/EU "On the protection of animals used for scientific purposes".

Statistical processing of the obtained data was performed using the SPSS Statistics 17.0 software. All data are represented as a mean ± standard error of the mean (M±m). Estimation of the differences between the samples was conducted using a parametric Student's t-test and a nonparametric Mann-Whitney U test. The minimum significance level was $p<0.05$.

Results

The dynamics of changes of erythrocyte osmotic resistance in the control and experimental animals depending on the concentration of hemolysis factor (urea) is presented in Table 1. EMOS in the control (intact) animals decreases in proportion to increasing of urea concentration in the solution. First, erythrocytes with lower resistance (worn-out) are hemolyzed, followed by hemolyzation of young erythrocytes with an increased resistance (tubes 1-6 in the Table). When urea concentration increases in the medium (tubes 2, 3) the difference in EMOS as compared to the control decreases gradually, and beginning with the 4th tube, where urea concentration is still higher, the percentage of hemolyzed erythrocytes does not differ from that of the intact animals (Table).

In animals with experimental DM with an increased glucose level of 14.2±0.4 mmol/L, EMOS appeared to be considerably lower than in the previous groups of animals. The percentage of hemoyzed erythrocytes increased the indices of the control animals respectively, especially in the tubes with low urea content. Introduction of NCC to the animals with experimental DM resulted in reduced glucose level in the blood to the values close to those of physiological ones. At the same time, the percentage of hemolyzed erythrocytes with low urea concentrations (tubes 1-3) decreased reliably twice on an average in comparison with the appropriate values of untreated animals. With further increase of urea concentration in the medium EMOS did not differ substantially from the results of NCC effect and from the experimental DM. NCC with underlying experimental DM is considered to increase EMOS and manifests membrane stabilizing properties.

Discussion

Osmotic erythrocyte hemolysis occurs due to the fact that urea molecules by the gradient of concentration penetrate through the membrane pores into the cell, attract water molecules with them, and create an increased osmotic pressure in the cell resulting in erythrocyte hemolysis. Since the pores of erythrocyte membranes are more penetrative for small water molecules than for urea molecules, the test of osmotic resistance is rather sensitive. This method allows evaluation not only minimal and maximal osmotic resistance of cells, but hemolysis dynamics as well depending on the concentration of urea in the solution.

The comparative analysis of the results obtained is indicative of the fact that in comparison with the control the percentage of hemolyzed erythrocytes in animals after NCC introduction appeared to be higher in the medium with low urea content (tube 1). An accelerated erythrocyte worn-out with increase of membrane-destructive processes in them might occur under NCC effect. An increase permeability of erythrocyte membranes leads to reduced osmotic erythrocyte stability and their hemolysis. It is indicative of activation of free radical lipid oxidation and reduced activity of antioxidant protection systems in the animal bodies [8]. Moreover, with experimental DM decrease erythrocyte stability to osmotic hemolysis can occur at the expense of increase of less stable old erythrocytes with increasing membrane-destructive processes [2]. On the other hand, hyperglycemia, found with experimental DM, under conditions of polyol way of glucose breaking down, is known [7]

to result in accumulation of osmotically active metabolites in the cells – sorbitol and fructose, which also promotes swelling and decreased osmotic stability of erythrocytes increasing hemolysis indices. On the one hand, improvement of the erythrocyte functional state in animals with experimental DM in response to NCC introduction can be suggested to result from decreased glucose content in the blood and reduced negative effect of hyperglycemia (glucose toxicity) on the erythrocyte membranes, as well as NCC effect on the state of oxidative-antioxidative balance in the animal bodies (decreased products of lipid peroxide oxidation and increased activity of the antioxidant protection enzymes) [6].

Conclusion. Experimental DM with underlying hyperglycemia in rats promotes EMOS decrease, activates destructive processes in the erythrocytes, and increases their destruction. NCC increases EMOS, decreases glucose level in the blood of animals with experimental DM.

References

1. Tkachenko, V.I. (2014). Analysis of the prevalence and incidence of diabetes among the population of the world and Ukraine for 2003-2013. Medicines of Ukraine, 4(21), 55-59.

2. Ferents, I.V., Brodyak, I.V., Luta, M.Ya.,Kulachkovsky, O.R., Sibirna, N.O. (2014). The effect of the decarboxylation product of L-arginine on the morphofunctional parameters of erythron under conditions of experimental diabetes mellitus in rats. Physiological Journal, 4(60), 70-79.

3. Pankiv V.I. (2015). Modern approaches to drug prevention of type 2 diabetes.Clinical endocrinology and endocrine surgery.Clinical endocrinology and endocrine surgery, 2(50), 58-63.

4. Pechova, A., Pavlata, L. (2007). Chromium as an essential nutrient: a review. VeterinarniMedicina, 52(1), 1–18.

5. Kosinov, M.V., Kaplunenko, V.H. (2008). Sposibotrymannyaakvakhelativnanometaliv «Eroziyno-vybukhovananotekhnolohiyaotrymannyaakvakhelativnanometaliv» [Method of obtaining nanometals aqua chelates "Erosion-explosive nanotechnology of obtaining nanometals aqua chelates"] (Ukraine Patent identifier № 29856), 2008.

6. Iskra, R.Ya.,Svarchevska, O.Z., Maksymovych, I.Ya. (2012). Glutathione antiperoxid system in blood and tissues of rats for the action of nanocryane citrate. Bulletin of problems of biology and medicine; 2(93): 32 – 35.

7. Bondar, T.P., Kozinets, H.I. (2002). Morphofunctional conditions of peripheral blood erythrocytes in late vascular complications of type 2 diabetes mellitus. Clinical laboratory diagnostics, (12): 22 – 34.

8. Kamyshnikov, V.S. (2000). Spravochnikpokliniko-biokhimicheskoilaboratornoidiahnostike [Reference book on clinical and biochemical laboratory diagnostics]. Minsk: Belarus.

9. Moiseenko, V.A., Antonenko, L.I., Arshynikova, L.L., Arutiunova, K.Sh.,Pasko, I.V. (2007). The permeability index of erythrocyte membranes in assessing the functional state of the organism.Crimean therapeutic journal, 2(2), 103 – 106.

10. Sadohurska, K.V. (2016). On the safety of nanochrome citrate under repeated administration.Pharmacology and drug toxicology, 4-5(50), 95–100.

11. Stefanov, O.V. (2001). Doklinichnidoslidzhennyalikars'kykhzasobiv [Preclinical studies of medicines]. Kyiv: Avitsena.

Table 1. Nanochromium citrate effect on the osmotic resistance of erythrocyte membranes in intact rats and with experimental DM

Tubes / Conditions of the experiment	1	2	3	4	5	6
	Urea /sodium chloride (ml)					
	40/60	45/55	50/50	55/45	60/40	65/35
Hemolysis degree (%)						
Intact animals	2.35±0.10	3.55±0.11	13.11±0.37	36.44±0.39	66.25±1.08	87.64±1.11
Nanochromium citrate	4.24±0.36*	5.84±0.47*	16.48±0.36*	37.06±0.16	69.47±0.31	88.19±0.29
Experimental DM (dexamethasone)	19.20±0.31	24.08±0.89	29.87±0.42	42.23±0.93	76.15±0.35	93.66±0.43
Dexamethasone + Nanochromium citrate	8.31±0.32	11.50±0.25	18.80±0.12	39.46±0.34	73.61±0.53	90.02±1.08

* - difference of the indices is probable in comparison with the intact control ($p<0.05$)