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The influence of nanodispersed cerium oxide on the development of oxidative stress and the production of nitric oxide in patients with type 2 diabetes mellitus

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Abstract. ***Background.*** *In the pathogenesis of complications of diabetes mellitus (DM), in addition to glucotoxicity, the development of oxidative stress plays a leading role. Antioxidants have been of great interest for physicians in recent years. Contemporary diabetology have been focused on the search and practical implementation of pathogenetic medications that can affect the main chains of DM and prevent its negative consequences. Purpose of the study is to determine the effect of nanodispersed cerium oxide (NCO) on the production of nitric oxide (NO), the activity of antioxidant enzymes and the intensity of lipid peroxidation in the blood of patients with type 2 diabetes mellitus.* ***Materials and methods.*** *Seventy-two patients aged 36 to 66 years, average age of 55.20 ± 6.82 years, who received treatment at the Municipal Clinical Hospital 2 in Poltava from July to December 2022 have been involved in the study. They have been divided into 2 groups: controls (n = 35), which included people without diabetes; experimental group (n = 37), which consisted of patients diagnosed with type 2 DM.* ***Results.*** *The use of NCO in patients with type 2 DM significantly reduces the activity of inducible NO synthase in the blood by 34.70 % and the activity of arginases by 52.17 % compared to the levels before treatment. The use of nanodispersed cerium oxide in the treatment of type 2 DM increases the activity of superoxide dismutase in the blood by 102.74 %, and the activity of catalase by 103.04 % compared to same indicators in the experimental group before therapy. Notably, blood malondialdehyde was significantly lower (by 2.35 times) compared to the same indicator before treatment.* ***Conclusions.*** *The use of NCO in patients with type 2 diabetes mellitus leads to an increase in antioxidant protection and a decrease in the intensity of lipid peroxidation in blood. NCO reduces the production of nitric oxide from the inducible NO synthase and weakens the competition between NO synthases and arginases for the reaction substrate. The findings of the study justify the need to include antioxidants in the pathogenetic therapy of diabetes mellitus and its complications.*

Keywords: *diabetes mellitus; nanodispersed cerium oxide; oxidative stress; nitric oxide*

Introduction

Diabetes mellitus (DM) is recognized by the WHO experts as a non-infectious epidemic and is the most frequent cause of disability and death of patients [1]. According to estimates made by the International Diabetes Federation, the global prevalence of DM will constitute 693 million in 2045 [1].

Achieving compensation of carbohydrate metabolism is a necessary condition for the prevention and treatment of all complications of DM. At the same time, the long-term course of DM, even along with stable compensation of the disease, contributes to the development and progression of its complications [2].

Contemporary diabetology have been focused on the search, creation and practical implementation of pathogenetic medications that can affect the main chains of the mechanisms of the development of DM and prevent its negative consequences [3, 4].

It is known that, in addition to glucotoxicity, the development of oxidative stress plays a leading role in the patho-

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genesis of complications of DM, therefore, in recent years, the attention of clinicians has been drawn to antioxidants, the introduction of which in the comprehensive treatment of diabetes improves its course and complications [5, 6]. At present, the positive effect of the use of antioxidants, in particular, tocopherol acetate, retinol, ascorbic acid, a complex of vitamins A, E, C, etc., has been proven in the treatment of DM [7, 8].

Cerium nanoparticles have been the issue of great interest in the field of biomedicine due to their self-regenerating antioxidant properties, which are widely studied as a promising drug for the treatment of diseases in which oxidative stress is a leading factor in their development [9, 10]. The antidiabetic potential of nanodispersed cerium (NDC), which was substantiated by A. Khurana A. et al. on the model of streptozotocin-induced type 1 diabetes mellitus in animals could be a novel strategy for the treatment of patients in the near future [11]. Y.H. Chen et al. demonstrated that hydrogel, containing nanocerium, significantly improved wound healing in diabetic rats by accelerating the formation of granulation tissue, collagen deposition, and angiogenesis [12].

Currently, the issue of substantiating the possibility of using NDC for the correction of endothelial dysfunction and oxidative-nitrosative stress in patients with type 2 DM is not studied sufficiently.

The aim of the study is to determine the effect of nanodispersed cerium oxide on the production of nitric oxide (NO), the activity of antioxidant enzymes and the intensity of lipid peroxidation in patients with type 2 diabetes mellitus.

The paper is the fragment of the research work, entitled "Peculiarities of the development of pathological changes in the organs of the digestive system under different conditions and the development of methods for their correction". State registration number 0120U100502. Duration 2019–2023.

Materials and methods

Seventy-two patients aged 36 to 66 years, average age 55.20 ± 6.82 years, who received treatment at the Municipal Clinical Hospital 2 in Poltava in the period from July to December 2022, have been involved into the study. The patients have been assigned into 2 groups: the control group (n = 35), which included patients without DM; experimental group (n = 37), which included patients diagnosed with type 2 DM.

The inclusion criteria for patients to be included into the clinical trial were men and women with confirmed diagnosis of type 2 DM. Prior to participation in the study, patients received oral hypoglycemic drugs, carbohydrate metabolism was in a state of subcompensation. All manipulations with the patients were carried out after obtaining the signed informed consent.

The exclusion criteria were: patients who experienced an acute coronary syndrome, stroke, vascular or cavity surgery in the 6 months preceding the study, as well as patients with: uncontrolled arterial hypertension; heart failure stage 2B and 3; arrhythmias, who required special antiarrhythmic treatment; renal (creatinine level more than 200 μmol/l) and liver (increase in the content of transaminases more than 2 times compared to the upper limit of the reference value) failure; acute diseases (infections, acute diseases or exacerbation of chronic diseases, injuries); decompensated DM and conditions that limit the use of therapy (dementia, alcohol addiction, drug addiction, cancer, mental disorders); anemia, pregnancy and lactation.

All manipulations with patients were carried out after obtaining the signed informed consent with the permission of the Biomedical Ethics Commission of the Poltava State Medical University (excerpt from the Minutes No. 212 as of 27.01.2023).

Clinical, instrumental and laboratory examination included collection of complaints, anamnesis, objective examination, registration of anthropometric parameters, calculation of the body mass index, determination of blood pressure level, electrocardiography.

All patients underwent a standard laboratory examination by standard methods, which included complete blood count and biochemical blood tests, urinalysis, blood glucose test, urine glucose test, glycated hemoglobin test, microalbuminuria test.

The patients of the experimental group were offered treatment with NDC (Cerera vitamin-mineral supplement) at a dose of 20 drops of the solution once a day in the morning with water (30–50 ml) 30 minutes before meal for 20 days; the manufacturer is the D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine; PJSC "NVK DiaProph-Med".

All subjects underwent blood biochemistry. In the control group, blood test was made once and in the experimental group, blood test was made twice (once before treatment and once after treatment). The blood was tested on the activity of inducible (iNOS) and constitutive isoforms of NO synthase (cNOS) [13], arginase activity, superoxide dismutase (SOD) activity, catalase and the concentration of free malondialdehyde (MDA) [14].

The results of biochemical studies were subjected to statistical processing using the Mann-Whitney U test to determine the statistical significance of differences between the indicators in the control group and the experimental group before treatment. Comparison of the results between the experimental group before treatment and after treatment was carried out using the Wilcoxon test. The difference between indicators was considered statistically significant at $p < 0.05$.

Results

We have found that the activity of iNOS in patients before treatment was by 2.5 times higher compared to the same indicator in the control group. The activity of arginase in the blood in this group of patients was also increased by 72.5 % as compared to the control group. Statistically, cNOS activity did not change significantly (Table 1).

The activity of antioxidant enzymes in the blood serum of patients with type 2 DM before treatment significantly decreases, namely, SOD decreases by 39.78 %, and catalase activity by 39.26 % compared to the same indicators in the control group. The concentration of MDA in the blood of patients with type 2 DM before treatment significantly increases by 3.51 times compared to the control group (Table 1). Thus, the pro-antioxidant balance in the blood of patients with type 2 DM changes in a decompensatory manner, as evidenced by the activation of lipid peroxidation along with the decrease in antiradical protection.

Table 1. Biochemical changes in the blood of patients of the control and experimental groups (M ± m)

Biochemical parameters	Groups		
	Control, n = 35	Experimental, n = 37	
		Before treatment	After treatment
iNOS activity, μmol/min per 1 g protein	0.88 ± 0.26	2.19 ± 0.16*	1.43 ± 0.30**
cNOS activity, μmol/min per 1 g protein	0.049 ± 0.013	0.031 ± 0.007	0.044 ± 0.010
Arginase activity, μmol/min per 1 g protein	0.80 ± 0.02	1.38 ± 0.04*	0.66 ± 0.03**
SOD activity, U	12.72 ± 0.99	7.66 ± 0.29*	15.53 ± 1.48**
Catalase activity, μkat/g	2.70 ± 0.11	1.64 ± 0.19*	3.33 ± 0.33**
MDA concentration, μmol/L	7.58 ± 0.68	26.62 ± 1.61*	11.34 ± 0.39**

***Notes:** * — the difference is statistically significant when compared with the control group ($p < 0.05$); ** — the difference is statistically significant when compared with the experimental group before treatment ($p < 0.05$).*

The use of NDC in patients with type 2 DM significantly reduces the activity of iNOS in the blood by 34.70 % and the activity of arginases by 52.17 % compared to the levels before treatment. The use of nanodispersed cerium oxide does not have a statistically significant effect on cNOS activity (Table 1).

Nanodispersed cerium oxide, used for treatment of type 2 DM, increases the activity of SOD in the blood of patients by 102.74 %, and the activity of catalase by 103.04 %, compared to the same indicators in the experimental group before treatment. Moreover, blood MDA in patients is by 2.35 times significantly lower compared to the same indicator before treatment (Table 1).

The simultaneous increase in the activity of iNOS and arginase, which is observed in patients with type 2 DM before treatment, can lead to the development of endothelial dysfunction. On the one hand, the increased activity of arginases will lead to the enhanced competition between NO synthases and arginases for the substrate, which can lead to the dissociation of the constitutive NOS isoforms from their substrate and their transition to the production of reactive oxygen species.

On the other hand, excessive activity of iNOS along with increased production of reactive oxygen species in type 2 DM can lead to the formation of toxic peroxynitrite, which can also contribute to the dissociation of constitutive NOS isoforms with their substrate. Under the conditions of dissociation of the constitutive NOS isoforms (and especially the endothelial isoform) with the substrate of the reaction, the production of oxide from them will be significantly reduced, which will lead to the inability of the endothelium to control the lumen of the vessels.

Discussion

A decrease in the activity of antioxidant enzymes and an increase in the concentration of MDA indicates the development of oxidative stress in patients of the experimental group before treatment. The development of oxidative stress can be associated both with an increase in the production of reactive oxygen species in type 2 DM, and with the activation of redox-sensitive transcription factors, such as nuclear factor kappa B (NF-κB) [15, 16].

A decrease in the concentration of MDA and an increase in the activity of antioxidant enzymes in the blood of patients of the experimental group after treatment indicates the ability of NDC to prevent the development of oxidative stress. This property may be related to the ability of nanodispersed cerium oxide to exhibit a direct antioxidant effect, which was shown in many studies [17, 18]. The increase in the activity of antioxidant enzymes can be explained by the stimulating effect of nanodispersed cerium oxide on the transcription nuclear factor, nuclear factor erythroid 2-related factor 2, which, through interaction with the antioxidant responsive element, enhances the expression of SOD and catalase [19].

A decrease in the activity of iNOS and arginase in patients of the experimental group after treatment indicates a decrease in the risk of developing endothelial dysfunction in the use of NDC in patients with type 2 diabetes mellitus. A decrease in iNOS activity is associated with the ability of nanodispersed cerium oxide to reduce the intensity of activation of the transcription factor NF-κB [20]. The decrease in arginase activity in the use of nanodispersed cerium oxide in patients with type 2 DM can be explained by the ability of cerium to increase the expression of p53, which is a powerful repressor of arginase activity [21].

Thus, the use of NDC in the comprehensive treatment of patients with type 2 DM leads to a statistically significant increase in the antioxidant protection of the body and inhibition of the development of oxidative stress, which prevents the development of complications.

Conclusions

The course of type 2 diabetes mellitus in patients is accompanied by a decrease in antioxidant protection and an increase in the peroxidation of lipids in the blood. Type 2 diabetes mellitus leads to increased production of nitric oxide from the inducible isoform of NO synthase and increases the competition between NO synthases and arginases for the reaction substrate.

The use of nanodispersed cerium oxide in patients with type 2 diabetes mellitus leads to increased antioxidant protection and reduces the intensity of lipid peroxidation in the blood. Nanodispersed cerium oxide reduces the production of nitric oxide from the inducible isoform of NO synthase and weakens the competition between NO synthases and arginases for the reaction substrate. The findings of the study justify the need to include antioxidants in the pathogenetic therapy of diabetes mellitus and its complications.

Nanodispersed cerium oxide is an effective means of correcting the increased production of nitric oxide from the inducible isoform of NO synthase, preventing the development of oxidative stress in patients with type 2 diabetes mellitus.

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Вплив нанодисперсного оксиду церію на розвиток оксидативного стресу та продукцію оксиду азоту в крові хворих на цукровий діабет 2-го типу

Резюме. ***Актуальність.*** У патогенезі ускладнень цукрового діабету (ЦД) провідну роль, окрім глюкотоксичності, відіграє розвиток оксидативного стресу. З огляду на те, що оксидативний стрес є одним із ключових механізмів виникнення ЦД та призводить до метаболічних порушень в організмі, розглядаються різні підходи до його корекції. Важливими завданнями сучасної діабетології залишаються пошук та впровадження в практичну діяльність патогенетичних лікарських засобів, що можуть впливати на основні ланки ЦД та попереджати його негативні наслідки. ***Мета дослідження:*** визначити вплив нанодисперсного оксиду церію (НОЦ) на продукцію оксиду азоту, активність антиоксидантних ферментів та інтенсивність перекисного окиснення ліпідів у крові хворих на цукровий діабет 2-го типу. ***Матеріали та методи.*** Дослідження проведене за участі 72 осіб віком від 36 до 66 років, середній вік 55,20 ± 6,82 року, які проходили лікування в КП «Друга міська клінічна лікарня» м. Полтави в період з липня по грудень 2022 року. Пацієнти були розподілені на дві групи: контрольну (n = 35), до якої увійшли особи без цукрового діабету, та експериментальну (n = 37), що включала хворих із діагностованим ЦД 2-го типу. ***Результати.*** Використання НОЦ у пацієнтів із ЦД 2-го типу вірогідно знижує активність iNOS у крові на 34,70 % та активність аргіназ на 52,17 % порівняно з рівнем до лікування. Активність супероксиддисмутази за умов застосування нанодисперсного оксиду церію для лікування ЦД 2-го типу зростає на 102,74 %, а активність каталази — на 103,04 % порівняно з цими показниками в експериментальній групі до терапії. Уміст малонового діальдегіду в крові пацієнтів за цих умов вірогідно зменшується (у 2,35 раза) порівняно з показником до лікування. ***Висновки.*** Застосування НОЦ у хворих на ЦД 2-го типу приводить до підвищення антиоксидантного захисту та зниження інтенсивності перекисного окиснення ліпідів у крові. НОЦ зменшує продукцію оксиду азоту з індуцибельної ізоформи NO-синтази та послаблює конкуренцію між NO-синтазами й аргіназами за субстрат реакції. Отримані результати дослідження обґрунтовують необхідність включення антиоксидантів у патогенетичну терапію цукрового діабету та його ускладнень.

Ключові слова: цукровий діабет; нанодисперсний оксид церію; оксидативний стрес; оксид азоту