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CORRECTION OF METABOLIC DISORDERS IN PARODONTAL TISSUES OF RATS CAUSED BY STREPTOZOCININ-INDUCED DIABETIC NEUROPATHY

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According to the WHO, in 2019, diabetes was the direct cause of 1.5 million deaths. A common consequence of uncontrolled diabetes is hyperglycemia, which over time leads to complications, especially of the nervous system and blood vessels. The prevalence of diabetic polyneuropathy, as the most common form of complications of diabetes, is high and, according to various authors, ranges from 15.5 to 77.6%. The search for effective means of correcting complications of diabetes, including polyneuropathy, remains relevant.

The aim of our study is to study the effectiveness of Cocarnit in the correction of periodontal syndrome in rats with diabetic polyneuropathy.

Diabetic polyneuropathy was simulated in experimental animals by a single injection of streptozocin (Streptozocin Sigma, USA). On the 30th day of the experiment, performed a glucose tolerance test was to confirm the presence of diabetes mellitus. Confirmation of the development of neuropathy was assessed by measuring the pain sensitivity threshold using the Randall-Selitto strain-algometric test. The objects of the study were periodontal tissues of rats in the homogenate which determined the activity of catalase, total proteolytic and antitryptic activity, the content of TBA-active products, oxidatively modified proteins, free fucose, glycosaminoglycans.

We found that with the development of diabetic neuropathy, the total proteolytic activity probably increases against the background of a significant increase in total antitryptic activity, increases the content of free fucose and glycosaminoglycans. The development of oxidative stress in periodontal tissues of animals is confirmed by a significant increase in the content of TBA-active products, oxidatively modified proteins, and molecules of medium weight. Thus, streptozocin-induced diabetic neuropathy contributes to the development of periodontal syndrome in rats. The injection of Cocarnit prevents the development of pathological changes in the periodontal tissues of rats in diabetic neuropathy as evidenced by the prevention of catabolism of glycoproteins and proteoglycans, the development of oxidative stress, inhibition of activation of proteolytic processes.

Key words: *diabetic neuropathy, periodontium, proteinase-inhibitory potential, oxidative stress, Cocarnit.*

Relationship of the publication with the planned research works. This work is a fragment of the research "Features of the development of pathological changes in the digestive system under different conditions and the development of methods for their correction" (state registration number 0120U100502).

Introduction. The number of people with diabetes in 2021 reached 537 million, and this number is projected to increase to 643 million in 2030, and to 783 million in 2045 [1]. Diabetic polyneuropathy is one of the most common chronic complications of diabetes diagnosed in almost half of patients. Clinical manifestations of diabetic polyneuropathy (DN) are quite diverse and occur in the practice of specialists in various fields. The prevalence of diabetic polyneuropathy, as the most common form of complications of diabetes, is high and, according to various authors, ranges from 15.5 to 77.6% [2].

The mechanism of development of diabetic polyneuropathy is described by various theories, including edema and degeneration of nerve fibers; development of oxidative stress due to excessive formation of free radicals [3]; strengthening the processes of lipid peroxidation, posttranslational and oxidative modification of proteins; enhancing the activity of the polyol pathway and, as a consequence, the accumulation of sorbitol [2]; increased non-enzymatic glycosylation of proteins due to chronic hyperglycemia, in particular peripheral nerve proteins, tubulin, which leads to disruption of their functions [4]. Believe that glucotoxicity is a leading factor in the pathogenesis of diabetic complications and causes structural and metabolic changes in the central and peripheral nervous systems.

The sustainability of normoglycemia is the main condition for the prevention and treatment of diabetic complications. Unfortunately, a small number of patients manage to maintain normal blood glucose levels, which dictates the need to find drugs that prevent glucotoxicity. Metabolic changes mainly affect sensory nerve fibers, resulting in paresthesias and pain. Many authors see some prospects for the treatment of DN not only in the fight against hyperglycemia but also in therapy aimed at improving the metabolism of nervous tissue. As a rule, metabolic drugs use as a part of combination pharmacotherapy in various diseases and have a secondary importance, but due to neuropathies their role increases, because metabolic disorders in this case are an important part of the pathogenesis. The main advantage in the treatment of diabetic polyneuropathy is given to the means of pathogenetic orientation. Pathogenetic therapy involves the administration of antioxidants and metabolic agents [5]. Some effectiveness of B vitamins in the treatment of diabetic polyneuropathy show both with the introduction of one vitamin [6] and with the use of vitamin B complex [7]. However, the correct combination of B vitamins, dose, duration of treatment is not fully understood. Among some drugs, Cocarnit (World Medicine) attracts attention, which, according to the literature, has a positive effect on metabolic, reparative processes, improves nerve tissue trophism, has analgesic, vasodilating effect [8, 9] and contains not only B vitamins but macroergic substances that improve nerve conduction.

The aim of the study was to investigate the effectiveness of Cocarnit in the correction of the periodontal syndrome in rats with diabetic polyneuropathy.

Object and methods of research. Experimental research was performed on the basis of the scientific laboratory of the Department of Bioorganic and Biological Chemistry Poltava state medical university and the research laboratory of the training and research center of the Institute of Biology and Medicine of Taras Shevchenko National University of Kyiv.

The study was performed on 41 adult white rats of both sexes weighing 180-200g. The experiments complied with the norms of the Convention on Bioethics of the Council of Europe 1997, The European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes, and the general ethical principles of animal experiments adopted by the First National Congress of Bioethics of Ukraine.

Diabetic polyneuropathy (DN) was simulated in experimental animals by a single intraperitoneal injection of streptozocin (Streptozocin Sigma, USA) at a rate of 65 mg/kg. On days 7, 14, 21, and 28 of the experiment, glucose levels were determined using a Free Style Optium XEMV036-P0270 glucometer and Free Style Optium H test strips. Blood was collected from the tail vein using an intravenous catheter for measurement. On the 30th day of the experiment, a glucose tolerance test was performed. Confirmation of the development of DN was assessed by measuring the threshold of pain sensitivity (PBC) using strain-algometric test Randall-Selitto.

After confirmation of the development of polyneuropathy on day 31 of the experiment, Cocarnit (World Medicine) was administered intramuscularly for 9 days at a rate of 1 mg/kg dissolved in 0.5% lidocaine hydrochloride [10]. The drug contains 20 mg of nicotinamide, 50 mg of cocarboxylase, 500 mcg of cyanocobalamin, 10 mg of disodium adenosine triphosphate trihydrate.

Slaughter of animals was carried out under thiopental anesthesia, by bloodletting, followed by a sampling of biological material. The objects of the study were periodontal soft tissues of rats in the homogenate which determined the total proteolytic activity [11], total antitryptic activity [12], the content of TBA-active products [13], the content of oxidatively modified proteins [14], and catalase activity [15], the content of free fucose [16] and glycosaminoglycans (GAG) [17].

The obtained results were analyzed using the methods of variation statistics. The calculation of the Shapiro-Wilk test was used to check the distribution for normality. If the data corresponded to the normal distribution, the significance of their difference when comparing arithmetic means were determined using the Student's T-Test for independent samples. When the data series were not subject to the normal distribution, statistical processing was performed using a nonparametric method – the Mann-Whitney test.

Table 1 – The content of free fucose and GAG in the periodontal tissues of rats under the conditions of DN and correction by Cocarnit

Groups of animals	GAG content, μmol/g	Fucose content, μmol/g
1. Control (n=10)	0,61±0,05	7,93±0,19
2. Diabetic neuropathy (n=12)	0,99±0,03	10,61±0,38
3. Diabetic neuropathy + Cocarnit (n=21)	0,76±0,01	7,8±0,16
4. Control + Cocarnit (n=8)	0,34±0,01	2,47±0,11
Statistical indicator	$P_{1-2}<0,05$; $P_{2-3}<0,05$; $P_{1-3}<0,05$	$P_{1-2}<0,05$; $P_{2-3}<0,05$; $P_{1-3}<0,05$

Note: n is the number of animals.

Research results and their discussion. We found that streptozocin in rats caused diabetes mellitus with the development of DN, which was manifested by an increase in PST, which was measured by the strain-algometric method. As a result of the conducted research was established that in control animals the initial PST was 100,1±3,4% and on the 14th, 28th day of PST measurement fluctuated slightly within the initial level, which is evidence of normal functioning of the neuromuscular complex in rats. In animals simulated DN PST increased significantly on all days of measurement compared with the initial value: on the 14th day after administration of streptozocin PST increased by 22,4±8,4% ($p<0,05$), and on the 28th day – by 100,9±15,3% ($p<0,001$). And in the group of rats injected with Cocarnit for 9 days, PST was lower by 109,2±3,4% ($p<0,001$) compared with the group of rats with diabetic polyneuropathy without correction and did not differ from the level of PST in the control.

Under the conditions of DN development in the periodontal tissues of rats, the content of free fucose increased 1,3 times and 1,6 times the content of glycosaminoglycans compared to the control **(table 1)**. This suggests that DN causes increased catabolism of rat periodontal connective tissue polymers.

The introduction of Cocarnit reduces the catabolism of periodontal biopolymers under conditions of diabetic neuropathy, as evidenced by the likely reduction in fucose and GAG in comparison with these values in rats, which simulated diabetic neuropathy without correction.

Analyzing the proteinase-inhibitory balance in rat periodontal tissues, we found that under conditions of DN development, the total proteolytic activity probably increases against the background of a significant in-

Table 2 – Proteinase-inhibitory balance of periodontal tissues under the conditions of DN and correction by Cocarnit

Groups of animals	Total antitryptic activity, g/kg	Total proteolytic activity, μg/g•min
1. Control (n=10)	32,56±1,31	3,17±0,01
2. Diabetic neuropathy (n=12)	53,85±0,71	3,41±0,03
3. Diabetic neuropathy + Cocarnit (n=21)	38,4±1,03	3,21±0,07
4. Control + Cocarnit (n=8)	16,72±0,98	2,46±0,08
Statistical indicator	$P_{1-2}<0,05$; $P_{2-3}<0,05$; $P_{1-3}<0,05$	$P_{1-2}<0,05$; $P_{2-3}<0,05$; $P_{1-3}<0,05$

Note: n is the number of animals.

Table 3 – Indicators of oxidative stress in periodontal tissues under the conditions of DN and correction by Cocarnit

Groups of animals	Catalase activity, μcat /g min	The content of OMP, conventional units	The content of TBA reactants, μmol/g	The content of molecules of medium mass, conventional units
1. Control (n=10)	0,27±0,04	1,35±0,01	2,41±0,13	0,29±0,02
2. Diabetic neuropathy (n=12)	0,11±0,01	1,76±0,03	4,45±0,03	0,33±0,01
3. Diabetic neuropathy + Cocarnit (n=21)	0,05±0,01	1,44±0,04	3,17±0,12	0,29±0,02
4. Control + Cocarnit (n=8)	0,68±0,04	0,92±0,04	4,15±0,11	0,26±0,02
Statistical indicator	P_{1-2}<0,05; P_{2-3}<0,05; P_{1-3}<0,05	P_{1-2}<0,05; P_{2-3}<0,05	P_{1-2}<0,05; P_{2-3}<0,05; P_{1-3}<0,05	P_{1-2}<0,05; P_{2-3}<0,05

Note: n is the number of animals.

crease in total antitryptic activity compared to control animals **(table 2)**.

Under the conditions of experimental correction by Cocarnit, the total activity of proteases in periodontal tissues of rats probably decreased, which indicates a protective effect of the drug on the development of proteolytic processes in streptozotocin-induced diabetic neuropathy.

Under the conditions of DN, on the 30th day of the experiment, we found the development of oxidative stress in periodontal tissues of animals, as evidenced by a significant increase in the content of TBA-active products, oxidatively modified proteins, and medium weight molecules **(table 3)**. Under the conditions of correction by Cocarnit, the content of TBA-active products, medium-weight molecules, and OMP in periodontal tissues was significantly reduced compared to animals that were simulated diabetic neuropathy without correction.

Conclusions. Streptozocin-induced diabetic neuropathy causes periodontal syndrome in rats. Metabolic correction by Cocarnit prevents depolymerization of proteoglycans and glycoproteins, prevents the development of proteolysis, and helps to suppress free radical oxidation in the periodontal tissues of rats in diabetic neuropathy.

Prospects for further research. Based on the received biochemical data for confirmation of the efficiency of use of Cocarnit at a diabetic neuropathy it is planned to carry out morphological and histologic research of periodontal tissues.

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КОРЕКЦІЯ МЕТАБОЛІЧНИХ ПОРУШЕНЬ У ТКАНИНАХ ПАРОДОНТА ЩУРІВ СПРИЧИНЕНИХ СТРЕПТОЗОЦИНІНДУКОВАНОЮ ДІАБЕТИЧНОЮ НЕЙРОПАТІЄЮ

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Резюме. Протягом останніх років значно зросла поширеність цукрового діабету. Останні дані видання Diabetes Atlas IDF показують, що 0,5 млрд дорослих людей у всьому світі живуть з цукровим діабетом. Найпоширенішим ускладненням цукрового діабету є периферична діабетична нейропатія, яка призводить до розвитку патологічних змін нервової системи і кровоносних судин та значно знижує якість життя хворих.

Актуальною проблемою на сьогоднішній день є пошук ефективних препаратів корекції полінейропатії та інших ускладнень цукрового діабету. Нашу увагу привернув препарат метаболічної дії Кокарніт, до складу

якого входить 20 мг нікотинаміду, 50 мг кокарбоксилази, 500 мкг ціанкобаламіну, 10 мг динатрію аденозинтрифосфату тригідрату.

Метою нашої роботи є дослідити ефективність препарату Кокарніт при корекції пародонтального синдрому у щурів за умов діабетичної полінейропатії.

Експериментальну діабетичну полінейропатію дослідним тваринам моделювали шляхом одноразового введення стрептозоцину (Streptozocin Sigma, USA). Для підтвердження розвитку цукрового діабету на 30 добу експерименту проводили глюкозотолерантний тест. Розвиток діабетичної нейропатії оцінювали вимірюючи поріг больової чутливості за допомогою тензоалгометричного тесту Randall-Selitto. Об'єктами дослідження були тканини пародонта щурів у гомогенаті яких визначали вміст ТБК-активних продуктів, окисно-модифікованих білків, вільної фукози, глікозаміногліканів, активність каталази, загальну протеолітичну та антитриптичну активність.

Встановлено, що діабетична нейропатія викликає розвиток пародонтального синдрому про що свідчить вірогідне зростання загальної протеолітичної активності на тлі достовірного збільшення загальної антитриптичної активності, збільшення вмісту вільної фукози та глікозаміногліканів, та інтенсифікація вільно-радикального окиснення. Введення Кокарніту запобігає розвитку патологічних змін у тканинах пародонта щурів за умов діабетичної нейропатії.

Ключові слова: діабетична нейропатія, пародонт, протеїназно-інгібіторний потенціал, оксидативний стрес, Кокарніт.

CORRECTION OF METABOLIC DISORDERS IN PARODONTAL TISSUES OF RATS CAUSED BY STREPTOZOCININDUCED DIABETIC NEUROPATHY

Kotvytska A. A., Kryvoruchko T. D., Neporada K. S., Berehovyi S. M.

Abstract. The prevalence of diabetes has increased significantly in recent years. The latest Diabetes Atlas IDF data shows that 0.5 billion adults worldwide live with diabetes. The most common complication of diabetes is peripheral diabetic neuropathy, which leads to the development of pathological changes in the nervous system and blood vessels and significantly reduces the quality of life of patients. An urgent problem today is the search for effective drugs to correct polyneuropathy and other complications of diabetes. Our attention was drawn to the metabolic drug Cocarnit, which contains 20 mg of nicotinamide, 50 mg of cocarboxylase, 500 mcg of cyanocobalamin, 10 mg of disodium adenosine triphosphate trihydrate.

The aim of our study was to investigate the effectiveness of Cocarnit in the correction of periodontal syndrome in rats with diabetic polyneuropathy.

Experimental diabetic polyneuropathy was simulated in experimental animals by a single injection of streptozocin (Streptozocin Sigma, USA). To confirm the development of diabetes on the 30th day of the experiment, a glucose tolerance test was performed. The development of diabetic neuropathy was assessed by measuring the pain sensitivity threshold using the Randall-Selitto strain-algometric test. The subjects of the study were periodontal tissues of rats in the homogenate which determined the content of TBA-active products, oxidatively modified proteins, free fucose, glycosaminoglycans, catalase activity, total proteolytic and antitryptic activity.

Diabetic neuropathy has been shown to cause periodontal syndrome, as evidenced by a probable increase in total proteolytic activity with a significant increase in total antitryptic activity, increased free fucose and glycosaminoglycans, and intensification of free radical oxidation. The introduction of Cocarnit prevents the development of pathological changes in the periodontal tissues of rats in diabetic neuropathy.

Key words: diabetic neuropathy, periodontium, proteinase-inhibitory potential, oxidative stress, Cocarnit.

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Conflict of interest.
The authors confirm that there is no conflict of interest in this article.

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