12. Rud MV, Shepitko VI, Stetsuk YeV, Akimov OYe. Morphological changes in rat liver structure during central deprivation of lutheinizing hormone synthesis at 365th day of experiment. Visnyk problem biolohiyi i medytsyny. 2022; 2(2(165) :151–5. Doi 10.29254/2077-4214-2022-2-2-165-151-155.
13. Skotarenko TA, Shepitko VI, Stetsuk YeV, Boruta NV. Changes in corticosterocytes of the zona reticularis and chromaffin cells of the adrenal gland in rats during the inhibition testosterone synthesis. World of Medicine and Biology. 2022; 4(82) :241–245. doi 10.26724/2079-8334-2022-4-82-241-245.
14. Tzoupis H, Nteli A, Androutsou ME, Tselios T. Gonadotropin-Releasing Hormone and GnRH Receptor: Structure, Function and Drug Development. Curr Med Chem. 2020; 27(36) :6136–58. doi: 10.2174/0929867326666190712165444.
15. Voroshilova TA, Shepitko VI, Stetsuk YeV. Changes in nitric oxide production and development of oxidative stress in rats heart during prolonged triptorelin-induced central deprivation of luteinizing hormone synthesis. World of Medicine and Biology. 2022; 1(79) :179–183. doi 10.26724/2079-8334-2022-1-79-179-183.

Стаття надійшла 15.03.2022 р.

DOI 10.26724/2079-8334-2023-1-83-229-232
UDC 616.316-06:616.833-02:616.379.-008.64]-091/-092.9

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PATHOMORPHOLOGICAL CHANGES IN SALIVARY GLANDS OF RATS UNDER THE CONDITION OF DIABETIC NEUROPATHY AND CORRECTION

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The purpose of the study was to evaluate the effect of diabetic polyneuropathy on the development of pathological changes in the salivary glands of rats, as well as the feasibility of their correction by the drug Cocarnit. Immature white nonlinear rats of both sexes were simulated with streptozocin-induced diabetic neuropathy, which was confirmed by the Randall-Selitto tensoalgometric method. For correction, Cocarnit 1 mg/kg was administered intramuscularly for 9 days. The subjects were the submandibular salivary glands of rats. It was found that streptozocin-induced diabetic neuropathy leads to changes in the parenchymatous components in the lobules of submandibular salivary glands of rats, which was manifested by dystrophic and destructive changes in the epithelial cells of the terminal sections and ducts and blood perfusion in the hemomicrocirculatory vessels. Administration of Cocarnit reduces manifestations of periacinaric edema and improves trophism of epitheliocytes. Restoration of blood flow contributes to the normalization of secretory function of the glandular apparatus of submandibular salivary glands in rats.

Key words: diabetic peripheral neuropathy, streptozocin, salivary glands, Cocarnit, nicotinamide, cobalamin, thiamine diphosphate.

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ПАТОМОРФОЛОГІЧНІ ЗМІНИ У СЛИННИХ ЗАЛОЗАХ ЩУРІВ ЗА УМОВ ДІАБЕТИЧНОЇ НЕЙРОПАТІЇ ТА КОРЕКЦІЇ

Метою роботи було вивчити вплив діабетичної полінейропатії на розвиток патологічних змін в слинних залозах щурів, а також доцільність їхньої корекції препаратом Кокарніт. Статевозрілим білим нелінійним щурам моделювали стрептозоцин-індуковану діабетичну нейропатію, розвиток якої підтверджували тензоалгометричним методом Randall-Selitto. Для корекціїї виявлених змін щурам вводили Кокарніт. Об'єктами дослідження були піднижньощелепні слинні залози щурів. Виявлено, що стрептозоцин-індукована діабетична нейропатія призводить до змін паренхіматозних компонентів у часточках піднижньощелепних слинних залоз щурів, що проявлялось дистрофічними і деструктивними змінами епітеліоцитів кінцевих відділів і проток та перфузії крові у судинах гемомікроциркуляторного русла. Введення Кокарніту зменшує прояви периацинарного набряку і поліпшує трофіку епітеліоцитів. Відновлення кровотоку сприяє нормалізації секреторної функції залозистого апарату піднижньощелепних слинних залоз щурів.

Ключові слова: діабетична периферична полінейропатія, стрептозоцин, слинні залози, Кокарніт, нікотинамід, кобаламін, тіаміндифосфат.

The study is a fragment of the research project "Features of the development of pathological changes in the organs of the digestive system under different conditions and the development of methods of their correction", state registration No. 0120U100502.

Diabetes mellitus is one of the top three diseases most often leading to disability and death. According to recent estimates by the International Diabetes Federation, 537 million adults live with diabetes, about 90 % of whom have type 2 diabetes. [6] Currently, there are 1 million 134 thousand registered diabetics in Ukraine. Diabetic peripheral polyneuropathy is the most common complication of diabetes, with high morbidity and reduced quality of life [4, 5, 7] impaired performance in a large number of patients and its development in 50 % of patients with both types of diabetes.

The pathogenesis of diabetic neuropathy is multifactorial and involves multiple interrelated mechanisms. Experimental evidence suggests that hyperglycemia, glucotoxicity and insulin deficiency act

together with other risk factors and activate several biochemical pathways affecting cellular metabolism [4]. A meta-analysis showed that patients with diabetes mellitus had a higher prevalence of oral mucosal disorders compared to patients without the disease [12]. Thus, hyposalivation has been shown to occur in diabetic patients compared with non-diabetic patients. The cause of these changes may be damage to the gland parenchyma, changes in salivary gland microcirculation, dehydration and impaired glycemic control [8].

Literature data indicate destructive changes in both parenchymatous and stromal elements of rat salivary glands and microcirculatory blood vessels caused by experimental diabetes mellitus [1]. Stable hyperglycemia has been shown to cause salivary gland dysfunction [2].

The oral cavity is often neglected in the management of diabetes because oral disease often goes unnoticed, as it does not necessarily cause pain, bleeding or other symptoms. Oral manifestations in poorly controlled diabetes that may be associated with diabetic neuropathy include xerostomia, glossodynia and dysgeusia, and possibly pain caused by trigeminal nerve damage and temporomandibular joint disorders [5].

The treatment of diabetic polyneuropathy is dominated by pathogenetic agents. Pathogenetic therapy consists of antioxidants and metabolic agents. According to the literature, Cocarnit (World Medicine) has a positive effect on metabolic and reparative processes, improves tissue trophism, has analgesic and vasodilating effects, improves nerve conduction. Cocarnit is used to treat various pathological conditions of the nervous system [3].

The purpose of the study was to evaluate the effect of streptozocin-induced diabetic polyneuropathy on the development of pathological changes in the salivary glands of rats, as well as the feasibility of their correction by Cocarnit.

Material and methods. During the experiments, the regulations of the 1997 Council of Europe Convention on Bioethics, the European Convention for the Protection of Vertebrate Animals used for experiments and other scientific purposes, the general ethical principles for experiments on animals, adopted at the First National Congress of Bioethics in Ukraine, were observed.

The study was performed on 42 sexually mature white nonlinear rats of both sexes, weighing 180-220 grams, which were divided into 4 groups. Group 1 was the control (intact rats). Diabetic neuropathy in group 2 and 3 rats was simulated by administration of a single injection of Streptozocin (Streptozocin, "Sigma", USA) at a dose of 65 mg/kg (Islam MS, 2007)**.** Glucose levels were monitored on days 14, 28 of the experiment. The development of neuropathy was recorded using a Randall-Selitto analgesimeter [10]. A Free Style Optium XEMV036-P0270 glucose meter and Free Style Optium H test strips were used to determine glucose concentration. On day 30 of the study a glucose tolerance test was performed to confirm the presence of diabetes mellitus in rats. For this purpose, after measuring basal glucose levels, a glucose solution of 3 g/kg was administered intragastrically to the rats. At 30, 60, 90 and 120 min after glucose administration, blood glucose concentration was determined. The next day, Group 3 and 4 rats were injected with Cocarnit (1 mg/kg, v/m) for 9 days, which contains 50 mg cocarboxylase, 20 mg nicotinamide, 500 μg cyancobalamin, 10 mg adenosintriphos dinatrium.

The animals were kept on a standard vivarium diet throughout the experiment. The subjects were submandibular salivary glands of rats.

After animals were euthanized, fragments of submandibular salivary glands were fixed in a 10 % solution of neutral formalin for three days. Submandibular salivary gland pieces fixed in formalin were then sealed in paraffin [9]. Sections 5-10 μm thick were obtained using a sled microtome and mounted on slides using a stencil technique. After staining with hematoxylin and eosin, the sections were placed in polystyrene and examined under a light microscope. Microphotography was performed using a DCM 900 digital microscope with software adapted for these studies.

Results of the study and their discussion. According to the general principles of structural organization, the rat submandibular gland corresponds to that in humans and has a lobular structure. The parenchyma consisted of numerous terminal sections producing seromucosal secretion and a system of discharge ducts. The stroma was formed by loose connective tissue. The particle hemocirculatory bed was represented by arterioles, capillaries and venules. Intraepithelial lymphocytes were detected in the ductal epithelium, lymphocytes, macrophages, plasmocytes and mastocytes in the loose periductal connective tissue, and plasmocytes periacinarly (fig. 1).

Cocarnit administration did not significantly affect the structural organization of the submandibular salivary glands in rats. The epitheliocytes of the terminal sections adhered tightly to the basal membrane. The secretory granules evenly filled the cytoplasm.

The nuclei of ductal epitheliocytes were placed in the centre of the cells, had a rounded shape and clearly visualised nuclei. In the striated ducts, the basal striation was preserved. The blood vessels of the haemocirculatory bed had normal blood flow (fig. 2).

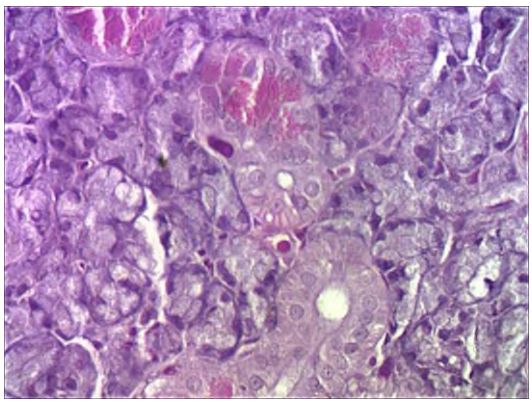

Fig. 1. Submandibular salivary gland of the control rat. Paraffin section. Staining: hematoxylin-eosin. Magnification: Ocular 10, Lens 40.

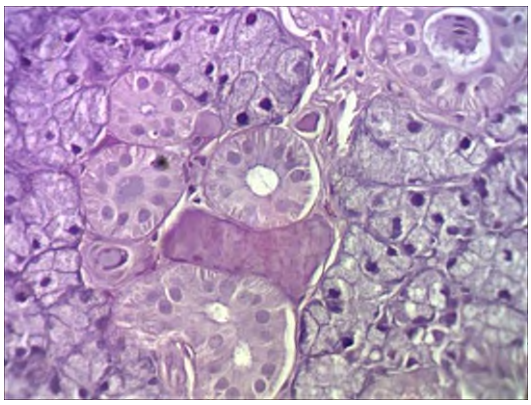

Fig. 2. Submandibular salivary gland of the rat after Cocarnit injection. Paraffin section. Staining: hematoxylin-eosin. Magnification: Ocular 10, Lens 40.

In streptozocin-induced diabetic neuropathy, periacinar edema, desquamation of epithelial cells, and thickening of the cytoplasm of individual cells were observed in the salivary gland tissues of rats (Fig. 3a).

Dystrophic and destructive changes were detected in the ductal system. Periductal connective tissue was swollen and there was an increase in the number of leucocytic cells and the appearance of intraepithelial leucocytes (fig. 3b).

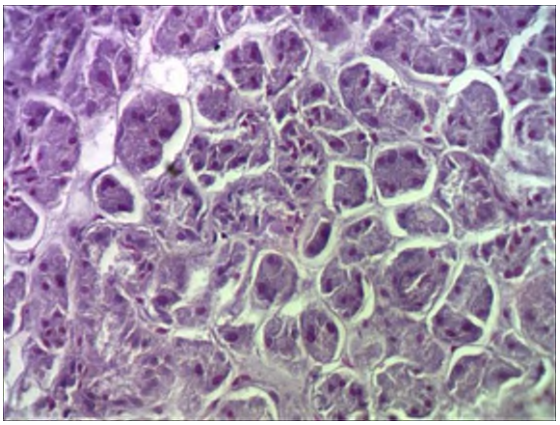

Fig. 3a. Periacinar edema and dystrophic changes of epitheliocytes in the rat submandibular salivary gland in streptozocin-induced diabetic neuropathy. Paraffin slice. Staining: hematoxylin-eosin. Magnification: Ocular 10, Lens 40.

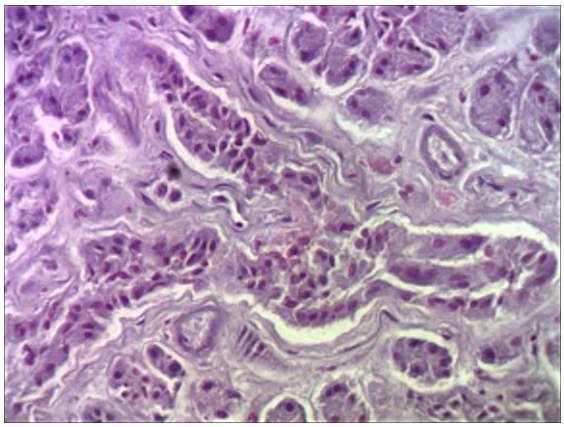

Fig. 3b. Epithelial cell desquamation and capillary confusion in the rat submandibular salivary gland in streptozocin-induced diabetic neuropathy. Paraffin section. Staining: hematoxylin-eosin. Magnification: Ocular 10, Lens 40.

Blood perfusion in the arterioles was maintained. There were no formular elements detected in the lumens. The lumen of the venules was irregularly shaped due to compression by edematous fluid. No erythrocytes were detected in the lumen of the capillaries (fig. 3b).

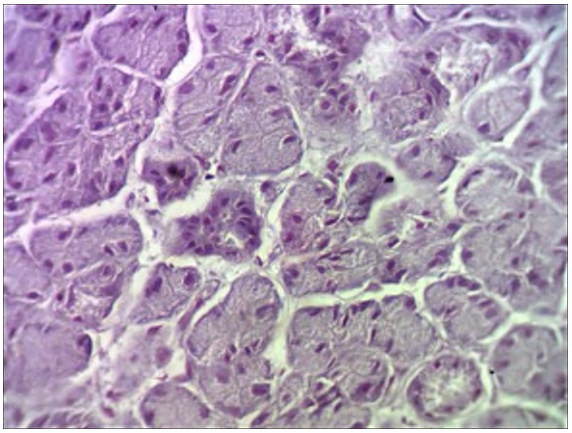

Fig. 4a. Minor periacinar edema in the submandibular salivary gland of the rat during correction of streptozocin-induced diabetic neuropathy with Cocarnit. Paraffin section. Staining: hematoxylin-eosin. Magnification: Ocular 10, Lens 40.

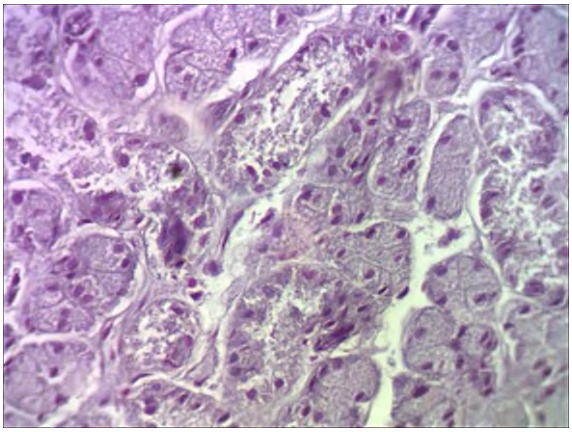

Fig. 4b. Local destruction and desquamation of granular duct epitheliocytes in the rat submandibular salivary gland during correction of streptozocin-induced diabetic neuropathy with Cocarnit. Paraffin section. Staining: hematoxylin-eosin. Magnification: Ocular 10, Lens 40.

The phenomena of periacinaric oedema were markedly less than in the salivary glands of animals with diabetic neuropathy when corrected with Cocarnit. The epitheliocytes of the terminal sections were preserved. Their structure was undisturbed (fig. 4a).

In the ducts locally, predominantly granular, there were dystrophic changes in the epitheliocytes as well as their destruction and desquamation into the lumen (fig. 4b). Blood perfusion in the blood vessels of the haemomicrocirculatory bed in both periprotochial and periacinarial vessels did not differ from that of the control rats.

The data we obtained indicate that Cocarnit, which is widely used for the treatment of neurological pathological conditions [3]. This is due to its positive effect on metabolic processes, improves trophic nervous tissue, which has a positive effect on the functioning of the parenchymatous organs. The revealed changes in salivary glands are stereotypic for the response to the action of pathogenic factors and are consistent with the results of other researchers [11, 13]. Similar destructive and dystrophic changes were found in the submandibular salivary glands of rats during chronic ethanol intoxication.

Conclusion

Streptozocin-induced diabetic neuropathy leads to changes of parenchymatous components in the lobules of submandibular salivary glands of rats, which was manifested by dystrophic and destructive changes of terminal and duct epitheliocytes and blood perfusion in the hemimicrocirculatory vessels. Cocarnit injection reduces the manifestations of periacinaric edema and improves the trophism of epitheliocytes. Restoration of blood flow contributes to the normalization of secretory function of the glandular apparatus of submandibular salivary glands in rats.

Referenses

1. Blyshchak NB, Borys RY, Haliuk UM. Morfolohichna perebudova pidnyzhnoshchelepnoi zalozy shchuriv cherez 2 ta 4 tyzhni perebihu eksperymentalnoho tsukrovoho diabetu.. Klinichna anatomiia ta operatyvna khirurhiia. 2018. 3(17): 29–37. [in Ukrainian]

2. Kotyk TL. Kharakterystyka perebudovy vyvidnykh protok pidnyzhnoshchelepnoi zalozy za umovy rozvytku eksperymentalnoho tsukrovoho diabetu z vykorystanniam analizu holovnykh component. Halytskyi likarskyi visnyk. 2015; 3(22): 108–112. [in Ukrainian]

3. Nikitina N, Berehovyi S, Stepanova L, Kabanov O. Vyznachennia optymalnoi skhemy vvedennia Kokarnitu shchuram iz diabetychnoiu polineiropatiieiu za dopomohoiu tenzoalhometrychnoho metodu.. Visnyk Kyivskoho natsionalnoho universytetu imeni Tarasa Shevchenka. Problemy rehuliatsii fiziolohichnykh funktsii. 2017; 2(23):37–42. [in Ukrainian]

4. Albers JW, Pop-Busui R. Diabetic neuropathy: mechanisms, emerging treatments, and subtypes. Curr Neurol Neurosci Rep. 2014;14:473. doi:10.1007/s11910-014-0473-5.

5. Borgnakke WS, Anderson PF, Shannon C, Jivanescu A. Is there a relationship between oral health and diabetic neuropathy? Curr Diab Rep. 2015 Nov;15(11):93. doi: 10.1007/s11892-015-0673-7. PMID: 26374570.

6. International Diabetes Federation. IDF Diabetes Atlas [Internet]. Available from: https://diabetesatlas.org/.

7. Juster-Switlyk K and Smith AG. Updates in diabetic peripheral neuropathy [version 1; peer review: 3 approved]. F1000Research 2016, 5(F1000 Faculty Rev):738 (https://doi.org/10.12688/f1000research.7898.1)

8. López-Pintor R, Casañas E, González-Serrano J, Serrano J, Ramírez L, Arriba L, Hernández G. (2016). Xerostomia, Hyposalivation, and Salivary Flow in Diabetes Patients. Journal of Diabetes Research. 2016. 1–15. 10.1155/2016/4372852.

9. Pronina OM, Koptev MM, Bilash SM. Response of hemomicrocirculatory bed of internal organs on various external factors exposure based on the morphological research data, Svit medytsyny ta biologiyi. 2018; 1(63): 153–57. http://dx.doi.org/10.26.724/2079-8334-2018-1-63-153-157

10. Santos-Nogueira E, Redondo Castro E, Mancuso R, Navarro X. Randall-Selitto Test: A New Approach for the Detection of Neuropathic Pain after Spinal Cord Injury. J Neurotrauma. 2012; 29(5): 898–904. doi: 10.1089/neu.2010.1700

11. Shevchenko KV, Yeroshenko GA, Yakushko OS, Kazakova KS, Kramarenko DR. Morphometric description of the exchange segment of microvasculature of rats' salivary glands in normal conditions and chronic ethanol intoxication. Wiadomości Lekarskie. 2019; 72(3): 323–26.,

12. Velasco-Ortega E, Delgado-Ruiz RA, López-López J. Dentistry and Diabetes: The Influence of Diabetes in Oral Diseases and Dental Treatments. J Diabetes Res. 2016;2016:6073190. doi:10.1155/2016/6073190

13. Yeroshenko GA, Shevchenko KV, Yakushko OS. Morphometric characteristics of rat salivary glands hemomicrovasculature capacity component under normal conditions and in ethanol chronic intoxication. World of medicine and biology. 2018;3(65):149–152.

Стаття надійшла 24.02.2022 р.