11. Li SJ, Liang XY, Li HJ, Yang GZ, Li W, Li Z, Zhou L. et al. Low-dose irradiation inhibits proliferation of the p53null type human prostate cancer cells through the ATM/p21 pathway. Int J Mol Med. 2018; 41(1): 548–554. DOI: 10.3892/ijmm.2017.3237
12. Qin H, Zhang V, Bok RA, Santos RD, Cunha JA, Hsu IC, Santos Bs JD. et al. Simultaneous Metabolic and Perfusion Imaging Using Hyperpolarized 13C MRI Can Evaluate Early and Dose-Dependent Response to Radiation Therapy in a Prostate Cancer Mouse Model. Int J Radiat Oncol Biol Phys. 2020; 107(5): 887–896. DOI: 10.1016/j.ijrobp.2020.04.022
13. Tang FR, Loke WK, Khoo BC. Low-dose or low-dose-rate ionizing radiation-induced bioeffects in animal models. J Radiat Res. 2017; 58(2): 165–182. DOI: 10.1093/jrr/rrw120
14. Vaiserman A, Cuttler JM, Socol Y. Low-dose ionizing radiation as a hormetin: experimental observations and therapeutic perspective for age-related disorders. Biogerontology. 2021; 22(2): 145–164. DOI: /10.1007/s10522-020-09908-5
15. Zaporozhets T, Korovina L, Sanyk O. Effect of the Acute Total Gamma Radiation in a Sublethal Dose on the Biophysical Properties of Red Blood Cells, Lipid Peroxidation, Antioxidant Supply and Hemocoagulating Properties of Erythrocytes. Experimental and Applied Biomedical Research (EABR). 2023;24(2). DOI: 10.2478/sjecr-2022-0048

Стаття надійшла 17.03.2022 р.

DOI 10.26724/2079-8334-2023-2-84-238-241
UDC 616.315:599.323.4-072:612.08

Yu.V. Tymoshenko, G.A. Yeroshenko, O.O. Kulai, O.B. Ryabushko, K.V. Shevchenko, N.A. Ulanovska-Tsyba, L.V. Stotska
Poltava State Medical University, Poltava

METHACRYLIC ACID ETHER-RELATED CHANGES IN THE INTENSITY OF MARKING OF COMPONENTS OF THE RAT HARD PALATE MUCOSA REVEALED BY PROBING WITH SIALO-SPECIFIC SNA LECTIN FROM THE BARK OF *SAMBUCUS NIGRA*

The decrease in secretory activity of the salivary glands disrupts local homeostasis in the oral cavity, affects the functioning of the entire digestive system, inhibits processes of physiological regeneration and impairs the protective properties of the mucous membrane against antigens. We have found changes in the morphofunctional state of the hard palate mucosa during the experimental hyposalivation using lectin probing method.

Key words: hyposalivation, hard palate mucosa, methacrylic acid ether, elderberry bark lectin.

Ю.В. Тимошенко, Г.А. Єрошенко, О.О. Кулай, О.Б. Рябушко, К.В. Шевченко, Н.А. Улановська-Циба, Л.В. Стоцька

ЗМІНИ ІНТЕНСИВНОСТІ МАРКУВАННЯ КОМПОНЕНТІВ СЛИЗОВОЇ ОБОЛОНКИ ТВЕРДОГО ПІДНЕБІННЯ ЩУРІВ ПРИ ЗОНДУВАННІ СІАЛОСПЕЦИФІЧНИМ ЛЕКТИНОМ КОРИ БУЗИНИ ЧОРНОЇ (SNA) ТА ПІСЛЯ ДІЇ ЕФІРУ МЕТАКРИЛОВОЇ КИСЛОТИ

Зниження секреторної активності слинних залоз порушує місцевий гомеостаз в ротовій порожнині, відображається на роботі всієї системи травлення, гальмує процеси фізіологічної регенерації та порушує захисні властивості слизової оболонки до антигенів. Нами були визначені зміни морфофункціонального стану слизової оболонки твердого піднебіння за умов експериментальної гіпосалівації методом лектинового зондування.

Ключові слова: гіпосалівація, слизова оболонка твердого піднебіння, ефір метакрилової кислоти, лектин кори бузини чорної.

The is a fragment of the research project "Restructuring of the organs of the immune, respiratory and excretory systems under the effect of various exogenous factors (monosodium glutamate, sodium nitrite, ethanol, methacrylate)", state registration No. 0121U108234.

The secretory activity of the salivary glands plays a crucial role in maintaining oral microbial balance and homeostasis. Decreased salivary secretion is exacerbated in concomitant somatic pathologies, use of medications and wearing of removable dentures [3, 5]. This affects both the local manifestations of dental pathology and the overall human somatic health.

The functions of maintaining the integrity of the oral tissues are primarily performed by unstimulated (at rest) salivary secretion. Salivary functions related to digestion are performed by stimulated saliva [4], which plays a particularly important role in conditions associated with decreased saliva production, also known as hyposalivation [2, 12]. At this point, patients with hyposalivation experience constant dryness in the oral cavity, called xerostomia, which leads to the rapid onset of inflammatory processes and the active progression of dental caries [5, 11]. Aggregations of leukocytes, which provide a physiological barrier against infection [1, 7, 8, 15], are localized in the connective tissue of the mucous membrane, which serves as the stroma for the minor salivary glands, and this barrier is disrupted when secretion is reduced. Hyposalivation not only disrupts local homeostasis in the oral cavity but also affects

the functioning of the entire digestive system, inhibits processes of physiological regeneration and impairs the protective properties of the mucous membrane against antigens. Furthermore, patients experience physical and psychological distress because they are unable to carry out various physiological functions [6].

The purpose of the study was to determine the transformation of marking of the intensity of components of the hard palate mucosa using the sialo-specific lectin from the bark of elderberry (*Sambucus nigra*).

Material and methods. The study involved identification of carbohydrate determinants in the structural elements of the glandular zone of the hard palate mucosa in control group of rats using the lectin from the bark of *S.nigra*. The experiment was conducted during the adaptation period to the wearing of removable dentures on day 14 and day 30 of the experiment. A total of 20 male outbred albino rats were involved in the experiment and assigned in to control group (n=5) and experimental group (n=15). Hyposalivation was induced by exogenously treating the rat oral mucosa with a 1 % methyl ether methacrylate solution for 30 days [14]. The animals were sacrificed on day 14 and day 30 through overdose of thiopental anesthesia. The fragments of the hard palate were fixed in 10 % neutral formalin solution, embedded in paraffin following the standard techniques and histological sections with a thickness of 5-6 μm were prepared. Lectin receptor visualization was performed using the system of 3,3'-diaminobenzidine tetrahydrochloride-H_2O_2. The localization and intensity of lectin-histochemical reaction were studied on the sections, evaluated semi-quantitatively as follows: 0 – no reaction; 1 – weak reaction (light brown staining); 2 – moderate reaction (yellow-brown staining); 3 – strong reaction (brown staining); 4 – very strong reaction (dark brown staining). Photodocumentation was performed using the Biorex-3 BM-500T microscope with a DCM 900 digital photomicrographic attachment and adapted software for the study.

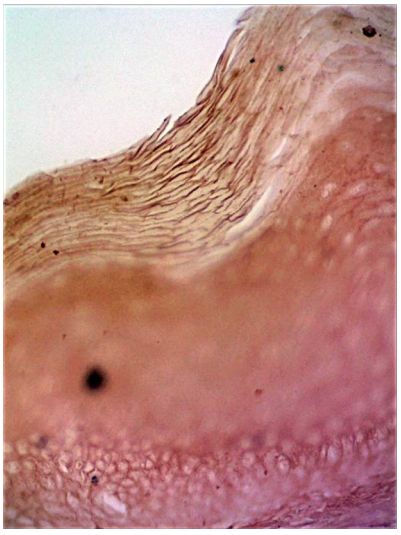

Fig. 1. Strong expression of sialospecific lectin from the bark of *S. nigra* on the keratinocytes of the epithelial layer of the mucous membrane in the glandular zone of the intact rat's hard palate. SNA marking. Objective lens: 100×magnification; Ocular lens: 10×magnification.

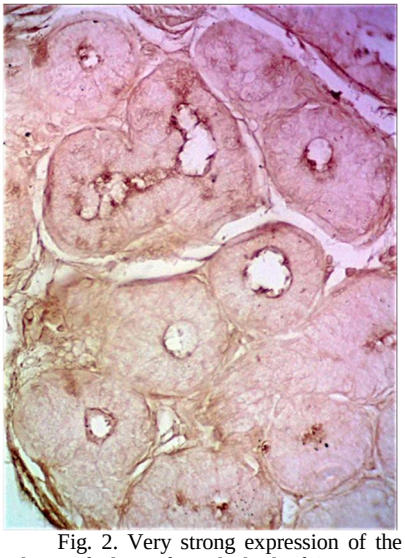

Fig. 2. Very strong expression of the sialospecific lectin from the bark of *S.nigra* on the apical plasmolemma of the epithelial cells of the acini and the ducts of the salivary glands of the mucous membrane of the glandular zone of the intact rat's hard palate. SNA marking. Objective lens: 100×magnification; Ocular lens: 10×magnification.

Results of the study and their discussion. When using the sialo-specific SNA lectin from the bark of *S.nigra* as a marker, probing of the glandular zone of the mucous membrane of the intact rats' hard palate revealed a strong degree of binding to the receptors of the horny scales, granular, spinous, basal layers and basal lamina (Fig. 1). The expression level of fibroblast receptors in the lamina propria of the mucous membrane of the glandular zone of the hard palate of intact rats was moderate, while the expression of collagen fibers was strong.

The receptors of endotheliocytes of the vessels of the microcirculatory bed showed a strong reaction. The degree of marking of the basement membrane of the vessels and the elastic membranes of arterioles in the intact animals was weak. Macrophages showed a strong degree of binding, lymphocytes exhibited moderate receptor exposure and the mast cells showed a very strong reaction (Table 1).

The expression of receptors for the sialospecific lectin from the bark of *S. nigra* on the components of the secretory glandular complexes of the acini and ductal system of the palatine glands was homogenous. The affinity of the basement membrane was weak. The cytoplasmic components of the epithelial cells and myoepithelial cells exhibited a moderate reaction towards the sialospecific SNA lectin. The apical plasmolemma of the epithelial cells of the acini and ductal system showed a very strong reaction in the intact group of animals (Fig. 2).

By day 14 of the observation, the intensity of the marking of the receptors on the horny scales, cells of the granular, spinous and basal layers increased to a very strong level in response to soybean lectin. The expression of receptors on the basement membrane decreased from strong to moderate (Fig. 3).

On day 14 after exposure to 1 % methacrylic acid ether, the determination of specificity and degree of binding of components of the lamina propria of the mucous membrane of the glandular zone of the rat hard palate revealed an increase in receptor expression to sialospecific lectin from the bark of *S. nigra*. In the intact group of animals, the expression of receptors in the fibroblasts increased from moderate to very

strong on day 14 of the observation. Intensification of binding on the collagen fibers from a strong to a very strong was also noted in the intact rats.

Table 1

Lectin-histochemistry characterization of the mucous membrane of the glandular zone of the rat hard palate in probing with SNA lectin from the bark of *Sambucus nigra*

Structural components			Intact group	day 14	day 30
Epithelium	Corneal layer		3	4	3
	Granular layer		3	4	3
	Spinous layer		3	4	2
	Basal layer		3	4	2
	Basement membrane		3	2	1
Lamina propria	Fibroblasts		2	4	3
	Collagen fibers		3	4	3
	Vessels	Endotheliocytes	3	2	2
		Basement membrane	1	2	2
		Elastic membrane	1	1	1
	Migrant cells	Mast cells	4	4	4
		Lymphocytes	2	2	1
		Macrophages	3	2	1
Glands	Acini	Basement membrane	1	3	3
		Plasmolemma	4	4	2
		Cytoplasm	2	2	2
		Myoepithelial cells	2	3	3
	Excretory ducts	Basement membrane	1	3	3
		Plasmolemma	4	4	2
		Cytoplasm	2	2	2
		Myoepithelial cells	2	3	3

On day 14 of the experiment, the reaction from the components of the vascular wall remained stable. The binding of sialospecific lectin from the bark of *S. nigra* to the elastic membranes of arterioles in the lamina propria of the mucous membrane of the glandular zone of the rat hard palate remained consistently weak compared to the intact group of animals. A decrease in the degree of receptor expression for the lectin from the bark of *S. nigra* from strong to moderate was observed on the endothelial cells of the microcirculatory vascular bed.

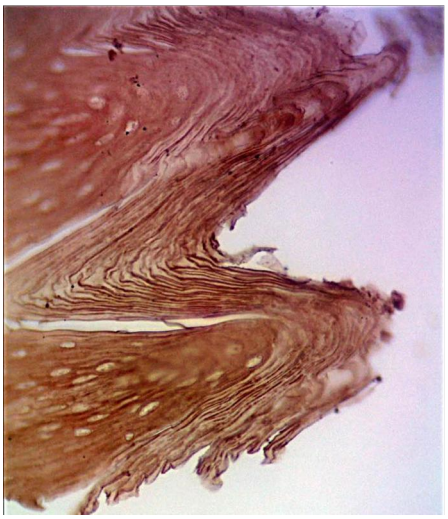

Fig. 3. Increase in the expression of sialospecific lectin from the bark of *S. nigra* on the keratinocytes of the epithelial layer of the mucous membrane of the glandular zone of the rat hard palate on day 14 of the observation. SNA marking. Objective lens:100×magnification; Ocular lens: 10×magnification.

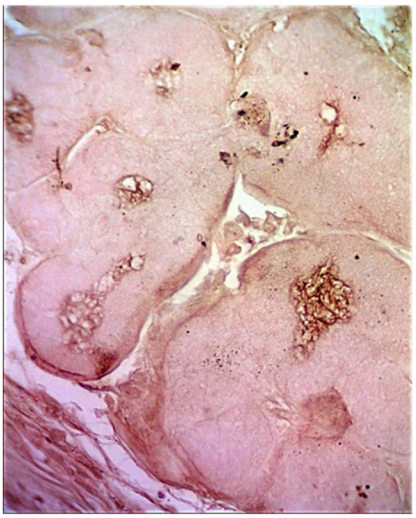

Fig. 4. Increase in the expression of the sialospecific lectin from the bark of *S.nigra* on the myoepithelial cells and the basement membrane of the acini and ducts of the salivary glands in the mucous membrane of the glandular zone of the rat hard palate on day 14 of the observation. SNA marking. Objective lens: 100×magnification; Ocular lens: 10×magnification.

The affinity of receptors for neuraminic acid in migrant cells of connective tissue, such as mast cells (very strong) and lymphocytes (moderate), remained constant at the level of the intact group. However, macrophages showed a shift from a strong reaction to a moderate one.

By day 14 of the observation, an increase in the expression of the receptors to the SNA lectin on the basement membrane of the acini and excretory ducts was noted. The reaction of the apical plasmolemma of the acinar and ductal epithelial cells remained at a very high level (Fig. 4).

The reaction of the myoepithelial cells of the acini and ductal system intensified from moderate to strong in the intact group of animals, while the cytoplasmic response remained constant.

By day 30 of the observation, a decrease in the degree of receptor expression to the sialospecific lectin from the bark of *S. nigra* was detected in all layers of the epithelial lamina of the salivary glandular zone of the rat hard palate mucosa. In the corneal and granular layers it decreased from very strong to strong,

while in the spinous and basal layers it decreased from very strong to moderate compared to the previous observation period. The degree of marking in the basement membrane progressively decreased from strong to moderate in the intact group of animals on day14 of observation and weak on day 30 of the experiment.

On day 30 of the observation, in the lamina propria of the glandular zone of the rat hard palate, the expression of receptors to the lectin from the bark of *S. nigra* on fibroblasts and collagen fibers, as the resident components of the loose connective tissue, decreased from very strong to strong compared to day 14 of the experiment. The reaction of the structural components of the microcirculatory bed remained constant compared to the previous observation period.

According to our results, migratory cells of connective tissue - lymphocytes and macrophages reduced the degree of expression of receptors to a weak one, which is consistent with the data obtained by us earlier and by other researchers' migrant cells of the connective tissue, namely, lymphocytes and macrophages, decreased the degree of receptor expression to weak [2, 13]. Mast cells exhibited a very strong reaction throughout the entire observation period. On day 30 of the experiment, the acini and ductal system of the palatine glands maintained the strong intensity of receptor exposure on the basement membrane (weak in the intact group and strong on day 14 of the observation). A constant reaction was observed in the cytoplasm of epitheliocytes at a moderate level compared to the previous observation period and intact group. The reaction of the apical plasmolemma decreased from very strong to moderate. On day 30 of the observation, the expression of receptors to the sialospecific lectin from the bark of *S. nigra* on myoepithelial cells of the acini and excretory ducts decreased from strong to moderate, which indicated exhaustion of the glandular epithelium and a decrease in the functional activity of the palatine glands [11].

Conclusion

1 % methyl ether methacrylate solution induced hyperexpression of receptors to the lectin from the bark of *S. nigra* in the cells of the basal layer. Fibroblasts and collagen fibers of the lamina propria exhibited an increased degree of conjugation with receptors to the lectin from the bark of *S. nigra*.

References

1. Kazakova KS, Yeroshenko AI, Lysachenko OD. Zminy predstavnytstva mihrantnykh klityn slyzovoi obolonky yasen shchuriv pry khronichnii intoksykatsii etanolom. Svit medytsyny ta biolohii. 2015;3(52):103-106. [in Ukrainian]

2. Kazakova KS. Dynamika ekspresii vuhlevodnykh determinant strukturnykh komponentiv slyzovoi obolonky prykriplenoi chastyny yasen shchuriv za umov khronichnoi intoksykatsii etanolom. Visnyk problem biolohii ta medytsyny. 2018;3 (145):294 – 297. [in Ukrainian]

3. Senchakovych YuV, Kazakova KS, Yeroshenko GA. Suchasni pohliady na prychyny dysfunktsii slynnykh zaloz. Svit biolohii i medytsyny. 2013;4 (41):112–116. [in Ukrainian]

4. Sliuna. Yeyo znachenye dlia zdorovia y rol pry zabolevanyiakh [Internet]. Dostupno: http://www.zubki.ru/index.php/2017/08/11/slyuna-ee-znachenie/. [in Russian]

5. Yakymenko DO. Osoblyvosti profilaktyky i likuvannia proteznykh stomatytiv u khvorykh z metabolichnym syndromom. Odesa: Derzh. ustanova "In-t stomatolohii Nats. akad. med. nauk Ukrainy"; 2012. 20 s. [in Ukrainian]

6. Cavalcante RB, Nonaka CF, Rabenhorst SH, da Costa Miguel MC, Pinto LP, de Souza LB. Pleomorphic adenoma and adenoid cystic carcinoma of salivary glands: E-cadherin immunoexpression and analysis of the CDH1-160C/A polymorphism. Arch Oral Biol. 2017 Jan;73:48-54.

7. Dawes C, Pedersen AM, Villa A, Ekström J, Proctor GB, Vissink A, et al.The functions of human saliva: A review sponsored by the World Workshop on Oral Medicine VI. Arch Oral Biol. 2015 Jun; 60(6):863-74.

8. Fragoulis GE, Fragkioudaki S, Reilly JH, Kerr SC, McInnes IB, Moutsopoulos HM. Analysis of the cell populations composing the mononuclear cell infiltrates in the labial minor salivaryglands from patients with rheumatoid arthritis and sicca syndrome. J Autoimmun. 2016 Sep;73:85-91.

9. Medeiros RJ, de Almeida OP, Leão JC, Gueiros LA. Lymphoepithelial infiltrate of palatal minor salivary glands: implications for diagnostic work-up. Int J Oral Maxillofac Surg. 2016 Dec;45(12):1626-9.

10. Wang Z, Li W, Hong X, Su JZ, Hua H, Peng X, et al. Minor salivary glands function is decreased in hyposalivation-related diseases. Arch Oral Biol. 2016 Sep;69:63-70.

11. Yeroshenko GA, Shevchenko KV, 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. Wiadomosci lekarskie. 2019; LXXII, 3:323-326.

12. Kazakova KS, Senchakovich YuV, Borzykh OA, Yeroshenko AI, Comparative analysis of metric changes in the sections of hemo microcirculatory stream of rat mucous membrane of gums and hard palate after exposure to ethanol and methacrylate. In: Fundamental and Applied Studies in EU and CIS Countries: The VI International Academic Congress; 2016 May 24-26; Proceedings of the Congress. UK, Cambridge; 2016:600-5.

13. Timoshenko Yu, Kazakova KS, Yeroshenko AI. Lectinochemical characteristics of rat normal masticatory oral mucosa. In: Fundamental and Applied Studies in the Modern World: Proceedings of the XV International Academic Congress; 2016 Sept 6-8; UK, Oxford; 2016;XV:207-11.

14. Yeroshenko GA, Senchakovich YuV, Yeroshenko AI. Methacrylate-induced changes in metric parameters of rat palatine glands. Eur Int JSciTechn. 2015;4(3):132-5.

15. Yeroshenko GA, Tymoshenko YuV. The dynamics of expression of the carbohydrate determinants of structural components of mucous membrane of glandular zone of hard palate in experimental hyposalivation. World of medicine and biology. 2018;1(63):123-126.

Стаття надійшла 24.02.2022 р.