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IMPACT OF 1 % METHACRYLIC ACID METHYL ESTER ON LECTINOCHEMICAL CHARACTERISTICS OF THE GLANDULAR AREA OF THE RAT HARD PALATE IN HELIX POMATIA LECTIN PROBING

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The findings of the study have established that the effect of 1 % methacrylic acid methyl ester led to enhanced expression of receptors of the corneal layer of the epithelium throughout the entire experimental period, indicating the irritating effect of the ester, whereas the receptors of the granular, spinous and basal layers of the epithelium increased to the normal values only by day 14, demonstrating a compensatory adaptation mechanism to the action of methacrylate on the structural components of the epithelium of the glandular area of the rat hard palate. The lamina propria showed changes in fibroblasts and collagen fibers, characterized by an increased degree of conjugation with receptors to Helix pomatia lectin.

Key words: hyposalivation, mucous membrane, hard palate, methacrylic acid methyl ester, secretory activity, Helix pomatia lectin.

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ЛЕКТИНОХІМІЧНА ХАРАКТЕРИСТИКА ЗАЛОЗИСТОЇ ЗОНИ ТВЕРДОГО ПІДНЕБІННЯ ЩУРІВ ПРИ ЗОНДУВАННІ ЛЕКТИНОМ ВИНОГРАДНОГО РАВЛИКА ЗА УМОВ ДІЇ 1 % ЕФІРУ МЕТАКРИЛОВОЇ КИСЛОТИ

У роботі встановлено, що при дії 1 % розчину метилового ефіру метакрилової кислоти роговий шар епітелію мав посилену експресії рецепторів протягом всього терміну експерименту, що засвідчує подразнюючу дію ефіру. Тоді, як рецептори зернистого, шипуватого та базального шару підвищили показники норми лише на 14 добу - що доводить компенсаторний механізм адаптації до дії метакрилату структурних компонентів епітелію слизової оболонки залозистої зони твердого піднебіння щурів. Власна пластинка мала зміни фібробластів і колагенових волокон, які характеризувалися посиленням ступеня кон'югації з рецепторами до лектину виноградного равлика.

Ключові слова: гіпосалівація, слизова оболонка, тверде піднебіння, ефір метакрилової кислоти, секреторна активність, лектин виноградного равлика.

The paper has been written within the research scientific work "Restructuring of the organs of the immune, respiratory and excretory systems under the effect of various exogenous factors (monosodium glutamate, sodium nitrite, Ponceau-4R, ethanol, methacrylate)", State Registration No 0121U108234.

The secretory activity of salivary glands plays a very important role in maintaining the microbiota and homeostasis of the oral cavity. Saliva supports the physiological functioning of the organs of the oral cavity and the entire digestive tract. This affects both the occurrence of local dental pathology and overall human somatic health.

The functioning of salivary glands occurs differently. The major salivary glands secrete reflexively when stimulated by food-sensitive nerve endings in the oral cavity or under the influence of conditioned stimuli. The minor salivary glands continuously release secretions to moisten the oral mucosa. One of the main reasons for insufficient salivary gland functioning is the imbalance in regulation by the nervous system, primarily due to disturbances in vertebravisceral relationships, which lead to increased sympathetic nervous system tone [1]. Two types of saliva are distinguished: stimulated saliva (secreted upon stimulation) with a volume of 250–350 ml per day, and resting saliva (constantly secreted) with its volume of 350-450 ml per day [7].

The functions of maintaining the integrity of oral tissues are primarily performed through unstimulated (resting state) saliva secretion. Saliva functions related to digestion are performed by stimulated saliva [2, 6], which plays a particularly valuable role in conditions associated with its reduced production – hyposalivation [4, 13].

Its development is associated with endogenous or exogenous factors. It can have different origins: functional and organic. Functional hyposalivation can be reversible or irreversible, which is caused by the regeneration of glandular cells or complete absence of salivary glands, and in most cases, it is considered irreversible. Insufficient function or reduction in the number (thinning of the oral mucosa) of the minor salivary glands also contributes to the development of abovementioned condition [3, 11].

Publications report [8, 9, 10] that prolonged wearing of removable acrylic dentures is associated with hyposalivation due to insufficient function of the minor salivary glands. The denture base exerts pressure on the mucous membrane of the hard palate, leading to its thinning, chronic inflammation and subsequent atrophy of salivary glands. The remaining salivary glands are more often replaced by connective or fatty tissue. After 5 years of wearing the denture, the minor salivary glands practically do not produce secretion in the denture bed area [14].

Acrylic plastics used for denture bases cannot be considered indifferent to the tissues of the oral cavity. Acrylic plastic contains free methyl ether of methacrylic acid, which is a cytotoxic substance that gradually diffuses and enters the oral cavity. The causes of pathology in most cases are due to the local factors. This depends directly on the removable denture and the material from which the base is made. A large number of patients using removable acrylic dentures suffer from "denture stomatitis." However, the causes of stomatitis are varied: colorants, residual monomer, opacifiers, plasticizers [5, 11].

The purpose of the study was to conduct a comparative analysis of changes in the mucous membrane of the glandular area of the rat hard palate during probing with Helix pomatia lectin under normal conditions and under the effect of 1 % methacrylic acid methyl ester.

Methods and Materials. The method of lectin histochemistry has been used to determine the carbohydrate components of the structural elements of the mucous membrane of the glandular area of the hard palate. The lectinochemical study was conducted at the "Lectinotest" laboratory of Danylo Halytsky Lviv National Medical University within the framework of scientific cooperation agreement. The lectin probing method, due to its sensitivity and selectivity in detecting the abovementioned molecular structures, significantly surpasses traditional methods of histochemical verification of carbohydrates [2]. A panel of peroxidase-labeled lectins was used to study the structural components of the mucous membrane of the glandular area of the hard palate. For the study, the material was fixed in a 10 % neutral formalin solution after removal, embedded in paraffin following standard procedures and histological sections of 5–6 μm thick were prepared. The visualization of lectin receptors was performed using a system of 3,3 - diaminobenzidine tetrahydrochloride in the presence of H2O2.

Table 1

Lectinochemical characteristics of the mucous membrane of the glandular area of the hard palate in rats during the probing with Helix pomatia lectin (HPA)

Structural components			Intact group	Day 14	Day 30
Epithelium	Corneal layer		2	3	3
	Granular layer		1	2	1
	Spinous layer		1	2	1
	Basal layer		1	2	1
	Basement membrane		1	1	1
Lamina propria	Fibroblasts		1	3	2
	Collagen fibers		1	3	2
	Vessels	Endothelial cells	1	2	1
		Basement membrane	1	1	1
		Elastic membrane	0	0	3
	Migrant cells	Mast cells	4	4	3
		Lymphocytes	1	1	0
		Macrophages	1	1	0
Glands	Acini	Basement membrane	3	2	1
		Plasmalemma	3	1	1
		Cytoplasm	1	1	1
		Myoepithelial cells	2	3	1
	Excretory ducts	Basement membrane	3	2	1
		Plasmalemma	3	1	1
		Cytoplasm	1	1	1
		Myoepithelial cells	2	3	1

For specificity control of histochemical reactions, the following methods were used: 1) exclusion of lectin-peroxidase conjugates from the staining protocol; 2) prior to the application of the lectin solution, pre-incubation of histological sections for 60 minutes in 1 % NaIO4 (Reanal, Budapest, Hungary) to oxidize the carbohydrate determinants of glycopolymers. In the first case, the histochemical reaction results were completely negative, while in the second case, they were significantly reduced. The findings of lectinochemical studies were incorporated into data tracking maps developed by the authors, which were implemented in the work of the morphological departments at PSMU.

Results of the study and their discussion. The intensity of labeling of components of the mucous membrane of the hard palate in intact rats using α-galactose-specific Helix pomatia lectin (HPA) revealed a moderate level of expression with receptors on the corneal layer. The reaction of cells in the granular, spinous, basal layers and basement membrane was weak (Table 1).

The intensity of receptor expression in fibroblasts and collagen fibers in the lamina propria of intact rats was weak. The reaction of receptors on vascular endothelial cells and the basement membrane was also weak. Negative reaction on the elastic membranes of arterioles was detected.

Macrophages and lymphocytes showed weak receptor exposure, while the mast cells showed a very strong reaction (Figure 1a).

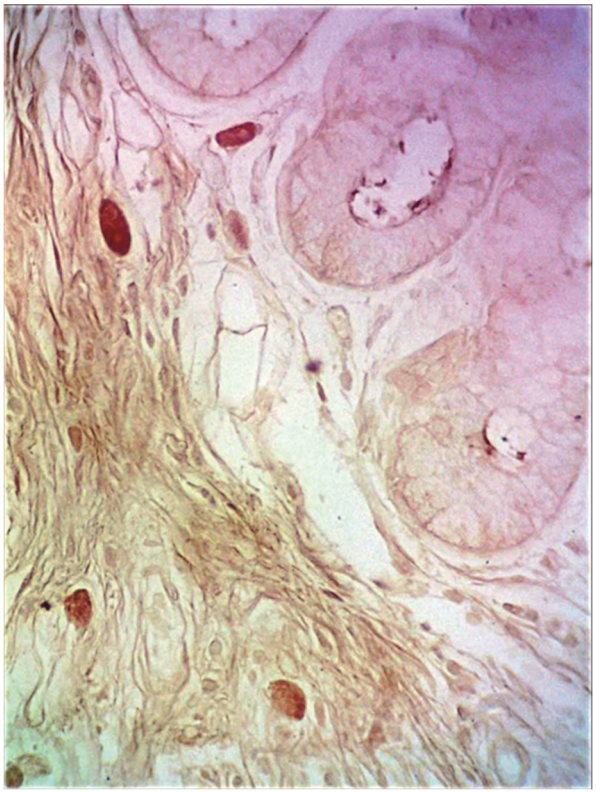

Fig. 1a. A very strong expression of α-galactose-specific Helix pomatia lectin on the mast cells in the lamina propria of the glandular area of the hard palate of intact rats. HPA labeling. Magnification: Obj. x 100, Oc. x 10.

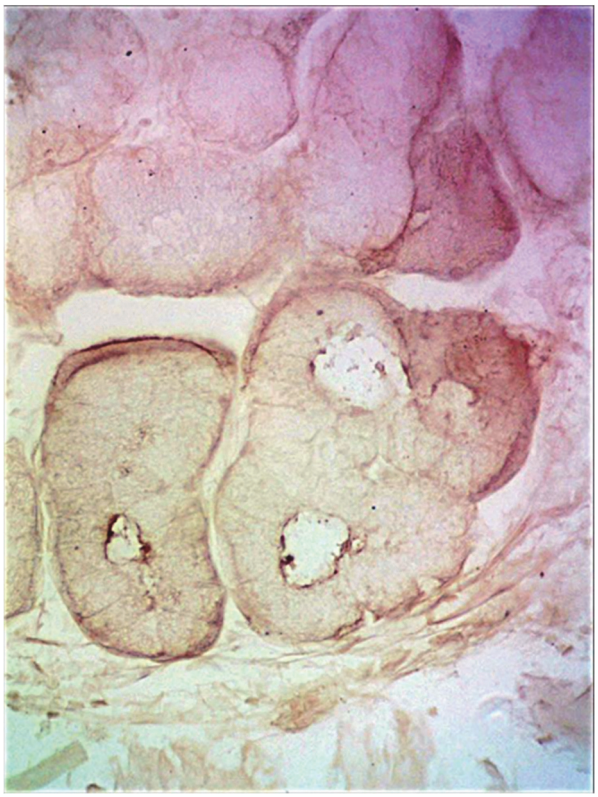

Fig. 1b. A strong expression of α-galactose-specific Helix pomatia lectin on myoepithelial cells and on the apical plasmalemma of epithelial cells in the acini of salivary glands in the glandular area of the hard palate of intact rats. HPA labeling. Magnification.: Obj. x 100, Oc. x 10.

The affinity of cytoplasmic components of epithelial cells of the palatine glands of intact rats to α-galactose-specific Helix pomatia lectin was weak. A strong reaction was observed towards the basement membrane and apical plasmalemma of the acinar epithelial cells in the intact group of animals (Table).

The intensity of labeling of the cell surface and cytoplasm of myoepithelial cells was moderate (Figure 1b).

The affinity between the basement membrane and plasmalemma of ductal epithelial cells in intact rats was strong. The cytoplasm showed weak expression of receptors for α-galactose, whereas myoepithelial cells of the excretory ducts reveled moderate expression (Table).

By day 14 of the observation, the intensity of labeling of receptors of the horny scales to Helix pomatia lectin increased to strong one. The expression of receptors on the cells in the granular, spinous and basal layers raised from weak to moderate, while the labeling of the basement membrane remained constant at a weak level (Figure 2a).

The investigation of the specificity of lamina propria components' binding on day 14 of the observation revealed an increase in the expression of receptors for α-galactose on fibroblasts and collagen fibers from weak to strong.

The reaction of vascular wall components on day 14 of the experiment remained constant, except for the enhanced reaction of the endothelial cell receptors from weak to moderate.

The affinity of receptors for α-galactose in migrant cells of connective tissue of macrophages (weak), lymphocytes (weak) and mast cells (very strong), remained at the level of the intact group (Figure 2b).

On day14 of the experiment, an increase in the receptor expression to LABA on myoepithelial cells to strong one (moderate in the intact group) was noted (Figure 3a).

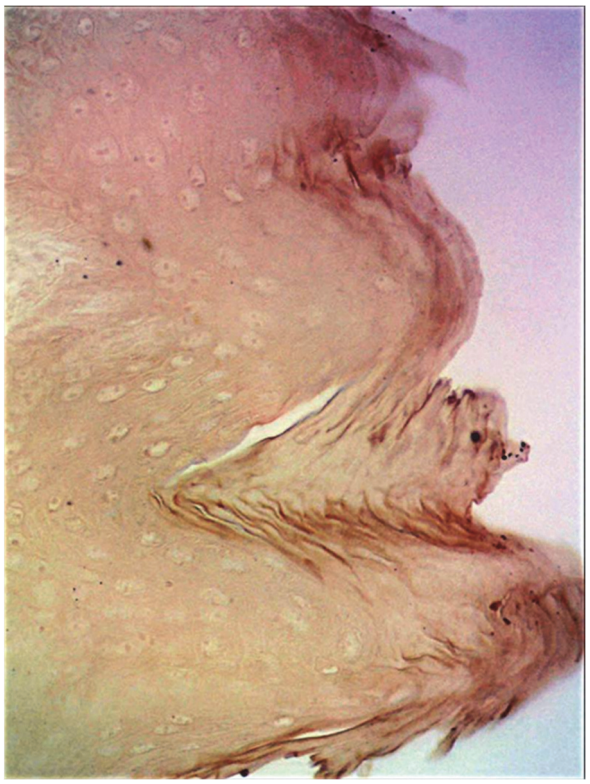

Fig. 2a. Enhancement of expression of α-galactose-specific Helix pomatia lectin on keratinocytes of the epithelial plate of the glandular area of the rat hard palate on day 14 of the observation. HPA labeling. Magnification.: Obj. x 100, Oc. x 10.

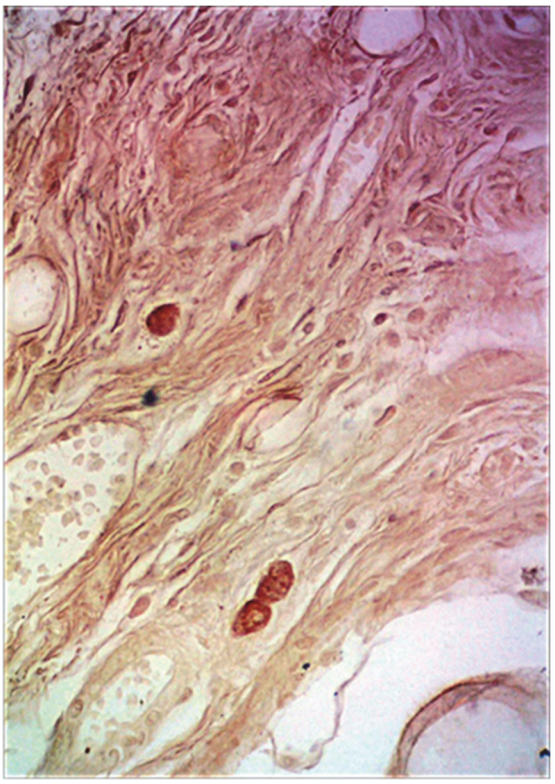

Figure 2b. Enhancement of expression to strong with Helix pomatia lectin on collagen fibers and very strong on mast cells in the lamina propria of the glandular area of the rat hard palate on day 14 of the observation. HPA labeling. Magnification: Obj. x 100, Oc. x 10.

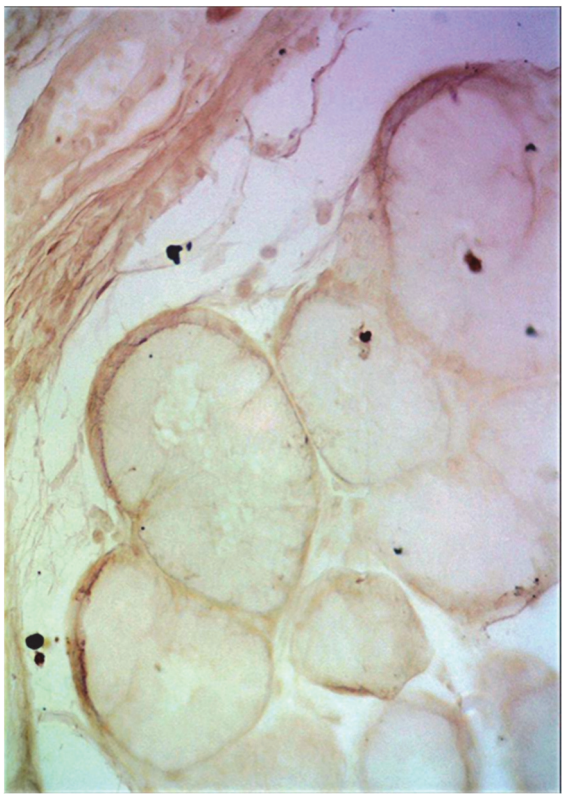

Fig. 3a. Enhancement of expression to strong one of α-galactose-specific Helix pomatia lectin on the myoepithelial cells in the acini of rat palatine glands on day 14 day of the observation. HPA labeling. Magnification: Obj. x 100, Oc. x 10.

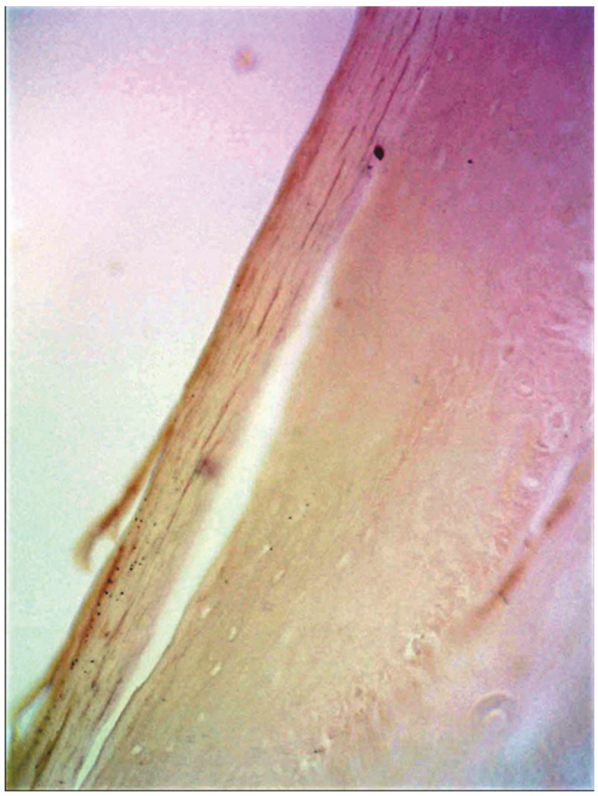

Fig. 3b. Decreased intensity of labeling of receptors for α-galactose-specific Helix pomatia lectin on keratinocytes of the granular, spinous and basal layers of the epithelial plate of the glandular area of the hard palate of rats on day 30 of the observation. HPA labeling. Magnification: Obj. x 100, Oc. x 10.

The reaction of the acinar basement membrane decreased from strong to moderate; the reaction of the basal plasmalemma decreased from strong to weak, and the reaction of cytoplasm remained consistently weak. Similar changes were observed regarding the structural components of the excretory ducts of palatine glands in rats on day 14 of 1 % methacrylic acid methyl ester administration.

On day 30 of the observation, in the epithelial plate of the glandular area of rat hard palate, the reaction of the horny scales remained consistently strong compared to the previous observation period. A decrease in the binding degree of receptors to Helix pomatia lectin was observed in the spinous, granular and basal layers, decreasing to weak one, compared to day 14 of the observation. The reaction of the basement membrane remained consistently weak throughout the experiment (Figure 3b).

On day 30 of the observation, in the lamina propria of the glandular area of the rat hard palate, the expression of receptors to Helix pomatia lectin decreased to moderate in the cytoplasm of fibroblasts and collagen fibers. The reaction of receptors to HPA on the endotheliocytes of hemomicrovessels decreased to weak, while the reaction on the basement membrane of vessels remained consistently weak. A change from negative to strong in the reaction of the internal elastic membranes of arterioles in the lamina propria was noted.

The reaction of mast cells decreased from very strong to strong, while lymphocytes and macrophages showed a negative reaction (Figure 4a).

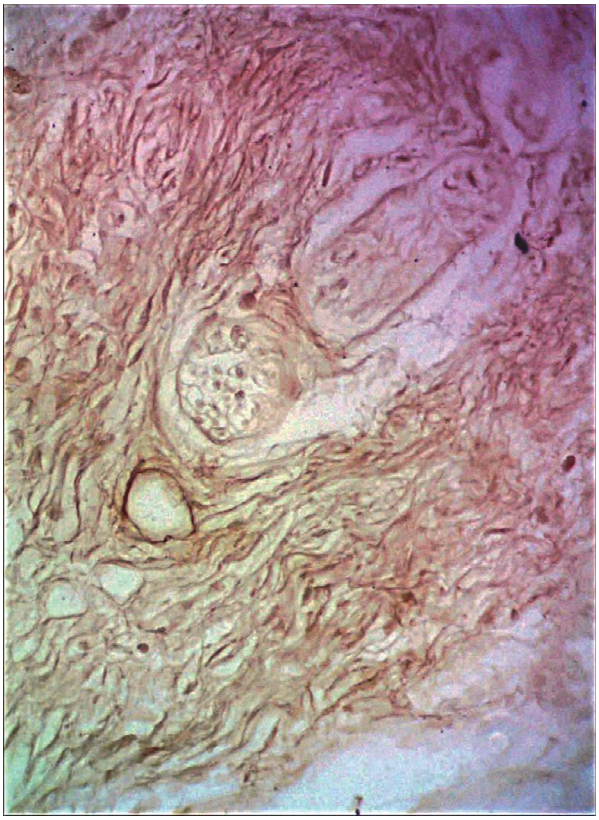

Fig. 4a. Enhancement of expression to strong of α-galactose-specific Helix pomatia lectin on arteriolar elastic membranes and reduced expression on collagen fibers and fibroblasts in the lamina propria of the glandular area of the rat hard palate on day 30 of the observation. HPA labeling. Magnification: Obj. x100, Oc. x10.

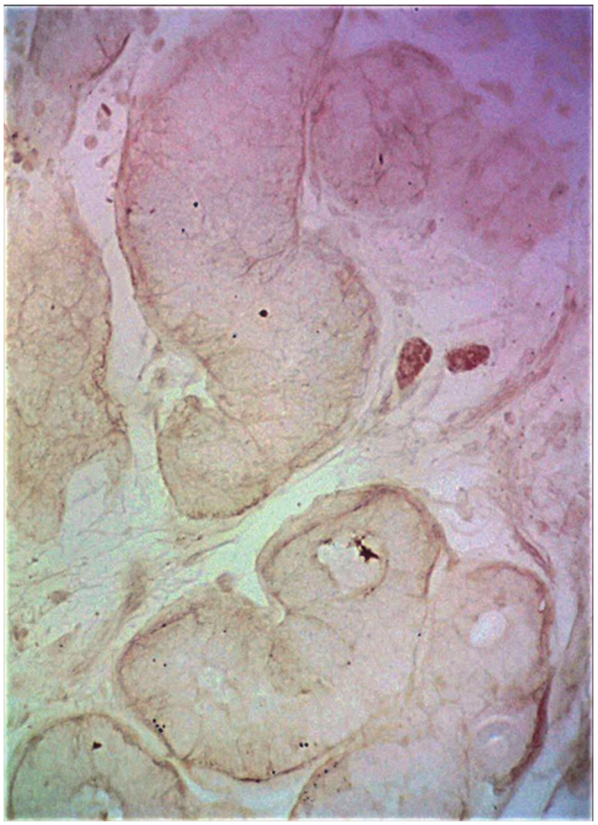

Fig. 4b. Reduction in the expression of α-galactose-specific Helix pomatia lectin on myoepitheliocytes of the acini of salivary glands and mast cells in the lamina propria of the glandular area of the rat hard palate on day 30 of the observation. HPA labeling. Magnification: Obj. x100, Oc. x10.

On day 30 of the experiment, the acini of the palatine glands showed the weak intensity of carbohydrate determinants' exposure to Helix pomatia lectin in the cytoplasm of epithelial cells. Reduction in receptor exposure on the basement membrane (from strong in the intact group and moderate on day 14 of the observation) to weak was noted.

The reaction remained constant at a weak level in the cytoplasm of the acinar epithelial cells throughout the experiment, compared to the previous observation period. The expression of receptors to α-galactose-specific Helix pomatia lectin on the acinar myoepitheliocytes decreased from strong to weak (Fig. 4b).

The analysis of the specificity of binding of the α-galactose-specific Helix pomatia lectin to receptors of structural components of the excretory ducts of the palatine glands on day 30 of the observation

revealed weak labeling of all studied components: basement membrane, plasmalemma, cytoplasm of myoepitheliocytes and cytoplasm.

This indicates an indirect effect of the methacrylic acid methyl ester on the acini of the minor salivary glands, where the dynamics of expression of carbohydrate determinants of structural components showed low values throughout the stimulus action, leading to changes in their secretory activity [14, 15].

Conclusion

The effect of 1 % methacrylic acid methyl ester led to enhanced receptor expression of the corneal layer of the epithelium throughout the experiment, indicating the irritant effect of the ester. However, receptors in the granular, spinous and basal layers increased to normal levels only by day 14, demonstrating a compensatory adaptation mechanism to the methacrylate's action on the structural components of the epithelium of the mucous membrane of the glandular area of the rat hard palate. The lamina propria had changes in fibroblasts and collagen fibers, characterized by an increased degree of conjugation with receptors to Helix pomatia lectin.

References

1. Alhareb AO, Akil HBM, Ahmad ZAB. Poly (methyl methacrylate) denture base composites enhancement by various combinations of nitrile butadiene rubber/treated ceramic fillers. J Thermopl Comp Mat. 2017 Aug 1;30(8):1069–90.
2. Bilash VP, Grygorenko AS, Yeroshenko GA, Shevchenko KV, Tumoshenko YuV, Lysachenko OD, et al. The impact of the complex food additives on the glandular apparatus of the rat's duodenal mucosa. World of medicine and biology. 2021; 78, 4, 196–202.
3. 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.
4. 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.
5. Dvornyk VM, Kuz VS, Kuz GM. Characteristics of modern basic dental materials and their effect on the tissues of the oral cavity. Actual. problem modern honey. Visn. Ukraine honey. stomatal Acad. 2014; 2(46):179–84.
6. 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.
7. 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.
8. Orlandi E, Iacovelli NA, Bonora M. Salivary Gland. Photon beam and particle radiotherapy: Present and future. Oral oncology. 2016; 60: 146–56.
9. Pedersen AML, Sørensen CE, Proctor GB, Carpenter GH, Ekström J. Salivary secretion in health and disease. J Oral Rehabil. 2018; 45(9): 730–46.
10. Sliuna. Yiyi znachennia dlia zdorovia i rol pry zakhvoriuvanniakh [Internet]. Dostupno: http://www.zubki.ru/index.php/2017/08/11/slyuna-ee-znachenie.
11. Tanasiewicz M, Hildebrandt T, Obersztyn I. Xerostomia of Various Etiologies: A Review of the Literature. Adv Clin Exp Med. 2016; 25(1):199–206.
12. 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.
13. Wu CB, Xi H, Zhou Q, Zhang LM. The diagnostic value of technetium 99m pertechnetate salivary gland scintigraphy in patients with certain salivary gland diseases. J Oral Maxillofac Surg. 2015; 73 (3): 443–50.
14. 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; 63, 1. 123–126.
15. Yeroshenko GA, Senchakovich YuV, Yeroshenko AI. Methacrylate-induced changes in metric parameters of rat palatine glands. Eur Int JSciTechn. 2015;4(3):132–5.

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