

G.A. Yeroshenko, Yu.V. Tymoshenko, O.O. Kulai, K.V. Shevchenko, I.M. Zviabilska,
L.M. Kopantseva, T.V. Khilinska
Poltava State Medical University, Poltava

COMPARATIVE DESCRIPTION OF THE BINDING SPECIFICITY OF RECEPTORS OF STRUCTURAL COMPONENTS OF THE GLANDULAR ZONE MUCOSA OF THE RAT HARD PALATE USING α -GALACTOSE-SPECIFIC SOYBEAN LECTIN

e-mail: gala_umsa@ukr.net

The paper presents the data of a lectinochemical study of the components of the mucous membrane of the glandular zone of the dura mater of rats with the help of α -galactose-specific soybean lectin. It was found that increased expression of receptors for α -galactose-specific lectin of soybean seeds was determined in the spiky, granular layers to a strong degree, in the basal layer the degree of binding remained constant, compared to the 14th day of observation. It increased from weak to moderate on the part of the basement membrane. The reaction from the mast cells remained strong. Therefore, the changes that occur under the influence of a 1 % solution of methyl ether of methacrylic acid on the mucous membrane of the hard palate should indicate changes in the structural components of this zone, which leads to a violation of the function of the minor salivary glands and the development of a pathological process. The α -galactose-specific lectin of soybean seeds can be a marker of these processes.

Key words: oral mucosa, secretory activity, salivary glands, hard palate, methyl methacrylate, soybean lectin (SBA).

Г.А. Єрошенко, Ю.В. Тимошенко, О.О. Кулай, К.В. Шевченко, І.М. Звягільська,
Л.М. Копанцева, Т.В. Хілінська

ПОРІВНЯЛЬНА ХАРАКТЕРИСТИКА СПЕЦИФІЧНОСТІ ЗВ'ЯЗУВАННЯ РЕЦЕПТОРІВ СТРУКТУРНИХ КОМПОНЕНТІВ СЛИЗОВОЇ ОБОЛОНКИ ЗАЛОЗИСТОЇ ЗОНИ ТВЕРДОГО ПІДНЕБІННЯ ЩУРІВ ЗА ДОПОМОГОЮ α -ГАЛАКТОЗОСПЕЦИФІЧНОГО ЛЕКТИНУ НАСІННЯ СОЇ

В роботі представлені дані лектинохімічного дослідження компонентів слизової оболонки залозистої зони твердого щурів за допомогою α -галактозоспецифічного лектину насіння сої. Встановлено, що посилення експресії рецепторів до α -галактозоспецифічного лектину насіння сої визначено в шпигуватому, зернистому шарі до сильного, в базальному шарі ступінь зв'язування залишився сталим, порівняно з 14-ою добою спостереження. З боку базальної мембрани, зі слабого посилюється до помірного. Реакція з боку мастоцитів залишилась сталою сильною. Отже, зміни які відбуваються під впливом дії 1 % розчину метилового ефіру метакрилової кислоти на слизовій оболонці твердого піднебіння мають свідчити про зміни структурних компонентів даної зони, що призводить до порушення функції малих слинних залоз та розвитку патологічного процесу. А α -галактозоспецифічний лектин насіння сої може виступати маркером цих процесів.

Ключові слова: слизова оболонка порожнини рота, секреторна активність, слинні залози, тверде піднебіння, ефір метакрилової кислоти, лектин насіння сої (SBA).

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A consistent prevalence of pathological changes in the human oral mucosa is observed, requiring timely diagnosis and treatment. Among the factors provoking these pathological changes is the reduced secretory activity of the salivary glands. This pathology remains a relevant medical and biological issue, necessitating the search for novel, adequate, and accessible correction methods [8, 10, 11].

The current development of morphological research offers a wide range of diagnostic studies. Lectin-histochemical methods are promising for differentiating carbohydrate determinants in biological macromolecules [1, 2, 3, 4, 7]. However, all procedures for correcting pathological processes require preclinical trials involving laboratory animals. Identifying the species of experimental animals most similar to the human body opens new horizons for advancing comparative morphology. The available scientific literature lacks comprehensive studies on the changes occurring in the tissues of the minor salivary glands of the hard palate under the influence of exogenous hyposalivation.

Determining the changes in the structural components of the mucous membrane of the glandular zone of the hard palate in rats during the modeling of exogenous hyposalivation will help to understand the patterns of pathology development, improve the quality of monitoring during the correction process, and develop a set of prophylactic methods to prevent possible complications [1, 2, 13].

In a state of physiological rest, the oral mucosa must remain constantly hydrated. This function is ensured by the secretion of the minor salivary glands, whose primary function is exocrine. This involves

the secretion of organic components of saliva, followed by hydration within the ductal system [8, 12, 14]. Disruption of saliva production negatively impacts the morphofunctional state of the digestive system organs and leads to the development of diseases [10, 12].

A separate group consists of exogenous hyposalivation, which arises under the influence of the prosthetic bed. A common cause of this, according to the literature [9], is the use of acrylic removable dentures. It has been shown that acrylic removable laminar dentures have a toxic, allergic, and traumatic effect on the tissues of the prosthetic bed [5]. However, the reduction in salivary gland function is caused by several factors, including the action of residual monomer from the acrylic prosthesis (free methyl methacrylate) and the pressure exerted by the prosthetic base on the bed [6, 9].

The effect of methyl methacrylate on the structural components of the mucous membrane in humans and laboratory animals is having a similar impact, making this area of research a relevant direction in the overall development of morphological science.

The purpose of the study was to compare the changes in the structural components of the glandular zone of the hard palate using soybean lectin probing under normal conditions and during the modeling of experimental exogenous hyposalivation.

Material and methods. The modeling of changes in the mucous membrane of the hard palate was performed by treating this area with a 1 % solution of methyl methacrylate for 30 days [1, 2]. A solution of the Ftoraks monomer was prepared by dissolving 1 g in 100 ml of distilled water. The oral cavity of the rats was irrigated with 1 % solution of methyl methacrylate, followed by excluding access to water for the next 2 hours. The animals were euthanized at 14- and 30 days post-treatment by overdosing with thiopental anesthesia.

To determine the carbohydrate components of the structural elements of the glandular zone mucosa of the hard palate, we utilized the method of lectin histochemistry. The study was conducted at the "LectinTest" laboratory of the Danylo Halytsky Lviv National Medical University [13, 15]. For examining the structural components of the glandular zone mucosa of the hard palate, a panel of peroxidase-labeled lectins was used. The visualization of lectin receptors was performed using a 3,3-diaminobenzidine tetrahydrochloride in the presence of H₂O₂.

The specificity of histochemical reactions was controlled by excluding lectin–peroxidase conjugates from the staining protocol. Before applying the lectin solution, a preincubation of histological sections was conducted for 60 minutes in 1 % HIO₄ (Reanal, Budapest, Hungary) to oxidize the carbohydrate determinants of glycopolymers. In the first case, the results of the histochemical reaction were completely negative, while in the second case, they were significantly reduced.

We developed data accounting sheets to record the results of the studies, implemented in the work of the morphological departments at Poltava State Medical University.

Results of the study and their discussion. Following the probing of the glandular zone the hard palate mucosa in the intact group of rats using α -galactose-specific soybean lectin (SBA), a moderate degree of expression with the receptors of the horny scales of the epithelial plate was established (Fig. 1).

The reaction of the granular, spinous, and basal cell layers and the basement membrane was weak (Table 1).

The degree of receptor expression was strong in the fibroblasts in the lamina propria of the intact group of rats, while collagen fibers showed weak expression. The reaction of receptors on the endothelial cells of the vessels was strong.

The degree of labelling of the basement membrane of the vessels and elastic membranes of arterioles was weak in the intact group of animals. Moderate receptor expression was noted on macrophages and lymphocytes, while the mast cells exhibited a strong reaction.

The affinity of the cytoplasmic components of the epithelial cells, as well as the surface and cytoplasm of the myoepithelial cells of the palatine glands in the intact group of rats, to the α -galactose-specific soybean lectin was weak. A strong reaction was observed at the basement membrane. At the same time, the apical plasmalemma of the epithelial cells in the acini exhibited a very strong response in the intact group of animals (Fig. 2).

The intensity of labelling of the basement membrane of ductal epithelial cells was strong in the intact group of rats, while the plasmalemmas exhibited very strong labelling. The expression of receptors for α -galactose was weak in the cytoplasm and myoepithelial cells of the excretory ducts.

On day 14 of the experiment, the intensity of receptor labelling of the horny scales for the soybean lectin increased to a strong one. The expression of receptors in the granular, spinous, and basal layer cells increased from weak to moderate, while the basal membrane remained constant at a weak level (Fig. 3). On day 14 of the experiment, the determination of specificity and binding degree of components in the

lamina propria revealed a decrease in receptor expression for α -galactose from strong in the intact group of animals to moderate in the fibroblasts, while the expression in collagen fibers increased from weak to moderate.

Table 1

Lectin-histochemistry characterization of the glandular zone mucosa of the rat hard palate in probing with soybean lectin (SBA)

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

The reaction of the components of the vascular wall remained stable on day 14 of the experiment, except for a decrease in the degree of expression of soybean lectin receptors from strong to weak one on the endothelial cells of the microvessels.

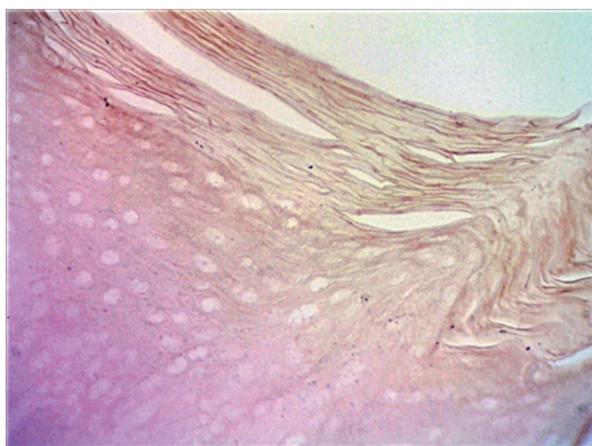


Fig. 1. Moderate expression of α -galactose-specific soybean lectin on keratinocytes of the epithelial plate of the glandular zone mucosa of the hard palate in the intact group of rats. SBA labelling. Objective lens:100 \times magnification; Ocular lens: 10 \times magnification.

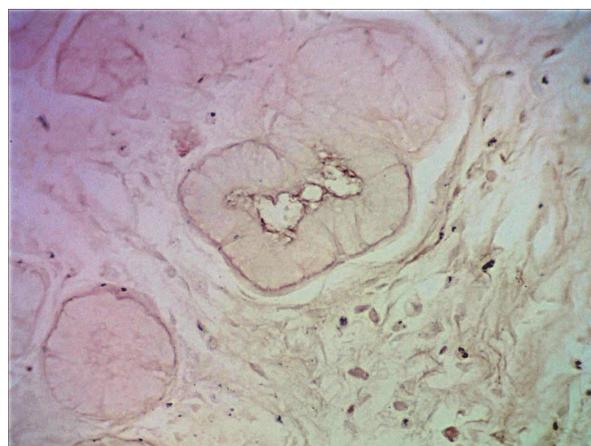


Fig. 2. Strong expression of α -galactose-specific soybean lectin on the basement membrane of the acini and excretory ducts, and very strong expression on the apical plasmalemma of the epithelial cells of the salivary glands in the glandular zone of the hard palate of rats in the intact group. SBA labelling. Objective lens:100 \times magnification; Ocular lens: 10 \times magnification.

The affinity of the receptors for α -galactose in the migrant cells of the connective tissue of mast cells (strong) and lymphocytes (moderate) remained stable at the level of the intact group. At the same time, macrophages changed from weak to negative reactions. By day 14 of the experiment, an increase in the expression of receptors to HPA on myoepithelial cells was established, reaching a very strong level (weak in the intact group) (Fig. 4).

The basement membrane of the acini showed a decreased reaction from strong to weak, while the basement plasmalemma remained stable, and the cytoplasm reaction intensified from weak to moderate.

Regarding the degree of expression of α -galactose-specific soybean lectin on the structural components of the excretory ducts of the rat palatine glands on day 14 of the experiment, similar changes were found as those established for the acini.

On day 30 of the experiment, the reaction of the epithelial layer of the glandular of rat hard palate zone mucosa remained consistently strong regarding the horny scales compared to the previous observation period. An increase in the expression of receptors to α -galactose-specific soybean lectin was determined in the spinous and granular layers to strong. In contrast, the binding degree in the basal layer remained stable compared to day 14 of the observation. The reaction from the basal membrane increased from weak to moderate compared to day 14 of the observation.



Fig. 3. Increase in the expression of α -galactose-specific soybean lectin on the horny scales of the epithelial layer of the glandular zone mucosa of the rat hard palate on day 14 of the experiment. SBA labelling. Objective lens:100 \times magnification; Ocular lens: 10 \times magnification.

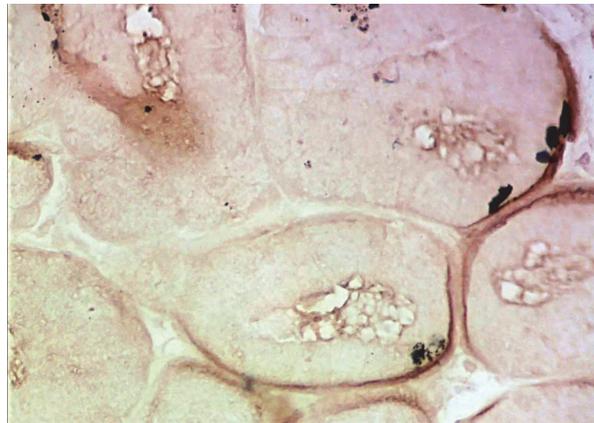


Fig. 4. Decrease in the intensity of labelling of α -galactose-specific soybean lectin on the basal membrane of the acini and excretory ducts of the salivary glands in the glandular zone of the rat hard palate on day 14 of the experiment. SBA labelling. Objective lens:100 \times magnification; Ocular lens: 10 \times magnification.

On day 30 of the observation, no changes in the expression of receptors to soybean lectin were noted in the lamina propria of the glandular zone of the rat hard palate of all studied structural components, compared to day 14 of the experiment. However, the reaction of receptors to SBA on fibroblasts increased to strong one. The response of the mast cells remained consistently strong.

On day 30 of the experiment, the acini of the palatine glands showed a slight recovery to moderate intensity of receptor expression on the basal membrane (strong in the intact group and weak 14 of the observation). In the cytoplasm of epithelial cells, a stable reaction was established at a moderate level compared to the previous observation period. The reaction on the apical plasmolemma decreased from very strong to strong. On day 30 of the observation, the expression of receptors for α -galactose-specific soybean lectin on the myoepithelial cells of the acini decreased from very strong to strong.

On day 30 of the observation, the changes in the labelling of receptors of the structural elements of the excretory ducts of the palatine glands were similar to the changes in the acini.

The analysis of the findings of the study on changes in the degree of expression of receptors for α -galactose-specific soybean lectin in the rat hard palate mucosa showed that on day 14 of the experiment, the intensity of receptors' labelling on the horny scales for the soybean lectin increased to a strong level compared to the intact group of animals. The expression of receptors on the granular, spinous and basal layers increased from weak to moderate, while the basal membrane remained constant at a weak level. The determination of specificity and the degree of binding of components in the lamina propria indicated a decrease in receptor expression for α -galactose in fibroblasts from strong levels in the intact group of animals to moderate levels. An increase in expression from weak to moderate was established on the collagen fibers. The response from the basal membrane of the acini decreased from strong to weak. In contrast, the response from the basal plasmalemma remained constant, and the cytoplasmic response increased from weak to moderate.

On day 30 of the observation, the reaction of the horny scales in the epithelial layer of the glandular zone of the hard palate mucosa in rats remained consistently strong compared to the previous observation period. An increase in the expression of receptors for α -galactose-specific soybean lectin was noted in the spinous and granular layers, reaching strong levels. In the basal layer, the degree of binding remained constant compared to day 14 of the observation, while the reaction from the basal membrane increased from weak to moderate. On day 30 of the observation, the reaction of the horny scales in the epithelial layer of the glandular zone of the mucous membrane of the hard palate in rats remained consistently strong

compared to the previous observation period. An increase in the expression of receptors for α -galactose-specific soybean lectin was noted in the spinous and granular layers, reaching strong levels. In the basal layer, the degree of binding remained constant compared to day 14 of the observation, while the reaction from the basal membrane increased from weak to moderate compared to day 14. The response of the mast cells remained consistently strong.

Conclusion

Thus, the changes occurring under the effect of a 1 % solution of methyl methacrylate on the mucous membrane of the hard palate indicate alterations in the structural components of the basal zone, leading to the impairment of the function of minor salivary glands and the development of a pathological process. Additionally, α -galactose-specific soybean lectin may serve as a marker for these processes.

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