DOI 10.26724/2079-8334-2022-1-79-200-204
UDC 616-091

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FEATURES OF THE STRUCTURE OF THE EPITHELIUM OF THE MUCOUS MEMBRANE OF THE TONGUE UNDER THE EFFECT OF COMPLEX OF FOOD ADDITIVES IN THE EXPERIMENT

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The paper was aimed at the study of the features of the structural organization of the covering epithelium of the mucous membrane of dorsal surface of the tongue of albino rats under the conditions of consumption of complex of food additives, supplemented into the ration, for 4 weeks. The study was performed on 30 outbred albino rats, both male and female, weighing 204±0.67 g. The standard ration of the experimental animals was supplemented with a complex of food additives: monosodium glutamate, Ponceau 4R and sodium nitrite for 1 and 4 weeks. The analysis of the structure of the epithelium of the mucous membrane of the tongue was performed on the conventional histological specimens and semi-thin sections. It has been established that consumption of complex of food additives supplemented into the standard ration of white rats for 4 weeks leads to a progressive thickening of the stratified squamous epithelium of the entire dorsal surface of the tongue and is accompanied by hydropic dystrophy, acanthosis, hyperkeratosis, focal stratification disorder.

Key words: food additives, mucous membrane, stratified squamous epithelium, the tongue, albino rats.

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ОСОБЛИВОСТІ СТРУКТУРИ ЕПІТЕЛІЮ СЛИЗОВОЇ ОБОЛОНКИ ЯЗИКА ПІД ВПЛИВОМ КОМПЛЕКСУ ХАРЧОВИХ ДОБАВОК В ЕКСПЕРИМЕНТІ

Метою нашого дослідження було вивчення особливостей структурної організації покривного епітелію слизової оболонки спинки язика білих щурів за умов введення в раціон комплексу харчових добавок впродовж 4 тижнів. Дослідження виконано на 30 безпородних білих щурах обох статей, масою 204±0,67 г. Піддослідні тварини додатково до стандартного раціону отримували комплекс харчових добавок: глютамат натрію, понсо 4R та нітрат натрію впродовж 1 і 4 тижнів. Дослідження будови епітелію слизової оболонки язика проводили на традиційних гістологічних препаратах і напівтонких зрізах. Встановлено, що додаткове введення в стандартний раціон білих щурів комплексу харчових добавок впродовж 4 тижнів, призводить до прогресивного збільшення товщини багатошарового плоского епітелію спинки язика в усіх відділах і супроводжується розвитком гідропічної дистрофії, акантозу, гіперкератозу, вогнищевого порушення стратифікації.

Ключові слова: харчові добавки, слизова оболонка, багатошаровий плоский епітелій, язик, білі щури.

The paper is a fragment of a research project "Patterns of morphogenesis of organs, tissues and neurovascular formations in normal condition, pathology and under the influence of exogenous factors", state registration No. 0118U004457.

One of the main functions of the oral mucosa is to ensure the interaction between the internal environment of the body and various external factors. In this regard, a significant number of publications of both domestic [4, 7, 9] and foreign researchers [10, 11, 14] are devoted to the peculiarities of the structure of the mucous membrane of the tongue under the influence of certain external factors. At the same time, the changes in the mucous membrane of the tongue that occur when using different combinations of food additives supplemented to the food ration, are not fully elucidated in the contemporary publications [9, 6, 15]. However, it has been found that consumption of supplemented complex of food additives (monosodium glutamate, Ponceau 4R and sodium nitrite) in the food ration leads to morphological changes in the muscles of the tongue in experimental animals [5, 12, 13].

The purpose of the study was to investigate in the experiment the features of the structural organization of the covering epithelium of the mucous membrane of dorsal surface of the tongue of albino rats under the conditions of consumption of complex of food additives, supplemented into the ration for 4 weeks.

Materials and methods. 30 outbred albino rats, both male and female, were involved into the experiment. All studies were conducted according to the rules of humane treatment of laboratory animals in accordance with the requirements of the World Medical Association Declaration of Tokyo and the general ethical principles of working with experimental animals, approved by the First National Congress on Bioethics [8]. The animals were assigned into three groups (n=10 each). Group I animals (control group) received standard ration; animals of the Group II and III (experimental groups) received standard ration, supplemented with complex of food additives: monosodium glutamate, Ponceau 4R and sodium nitrite for 1 and 4 weeks, respectively.

Upon euthanasia of the animals under thiopentone anesthesia overdose (200 mg/kg body weight), the tongue was removed. Under the control of binocular loupe, the tongue was cut in two halves along the

midline with a safety razor blade. One half was fixed in neutral 10 % formalin during 24-hour, the other half of the tongue was fixed in 2.5 % glutaraldehyde solution during 24-hour. The material fixed in formalin after dehydration according to the conventional technique was embedded into liquid paraffin using a "Microm" station for paraffin blocks embedment. Sections of 5-7 μm thick were made from the paraffin blocks on the "Leica" rotary microtome, which were stained with hematoxylin and eosin according to conventional technique [3]. Fragments of the tongue, fixed with glutaraldehyde, after dehydration in alcohols and acetone were embedded into EPON-812 according to conventional technique, followed by the manufacturing of semi-thin sections. Semi-thin sections were stained with methylene blue and polychrome method [1].

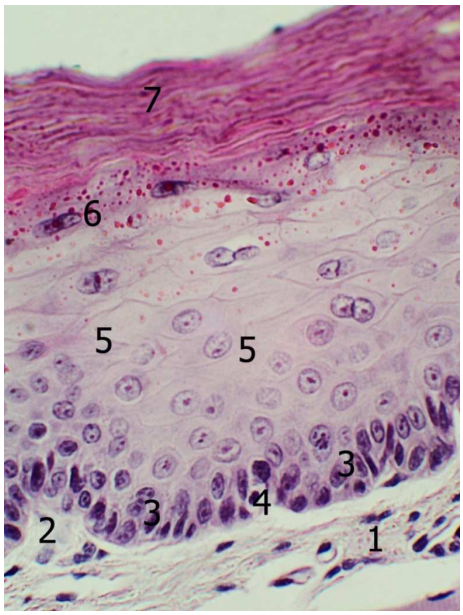

Fig. 1. The structure of the mucous membrane of the tongue of an albino rat. H&E stain. Objective lens: magnification ×40, ocular lens: magnification ×10. 1 – lamina propria of the mucous membrane; 2 – connective tissue papillae; 3 – basal epitheliocytes; 4 – intraepithelial lymphocyte; 5 – spinous epitheliocytes; 6 – granular epitheliocytes; 7 – corneal epithelium.

The study of the micropreparations and determination of morphometric parameters was performed using the Olimpus BX 41 microscope, with a digital microphoto attachment and a package of the licensed software.

Results of the study and their discussion. The findings of the studies have shown that in the intact animals, the dorsal surface the tongue in the area of the tip, body and root was covered with mucous membrane comprised of the stratified squamous keratinized epithelium and lamina propria. The greatest thickness of the mucous membrane was in the area of the root of the tongue (186±5.08 μm); in the body the thickness was 175±7.60 μm, and in the area of the tip the thickness of the mucous membrane was the smallest (166.1±5.08 μm).

The thickness of the stratified squamous epithelium did not differ significantly in different parts of the tongue and accounted for 130.5±6.54 μm on the tip, 128.7±6.48 μm on the body and 131.4±7.23 μm on the root.

Targeted study of the epithelial layer of the dorsum of the tongue provided with identification of the three layers, the cellular elements of which had pronounced morphological differences (fig. 1).

Thus, directly on the basement membrane, the basal layer was identified, represented by the single-layer of cellular elements of elongated and prismatic shape, which were oriented by the long axes or at a small angle, or perpendicular to the basement membrane. Occasionally, among the basal epitheliocytes, isolated intraepithelial lymphocytes were detected. Its small amount should not be regarded as the manifestation of pathological process.

In addition to the cells described above, Langerhans cells, melanocytes and Merkel cells were sometimes found in the basal layer of the epithelium of the mucous membrane of the tongue (Fig. 2). It should be noted that for a detailed study of the above cellular elements, it is necessary to use additional techniques, which is not the objective of the present study.

Spinous layer was located directly above the basal layer, consisting on the average of 3–4 layers of cells. Generally, the cells of the spinous layer are slightly larger than the basal ones and stained less intensely, and their shape is quite diverse. Namely, epitheliocytes of both elongated and prismatic shape are often found; less often – cells whose shape resembles hexagon.

The granular layer was located above the spinous layer, represented by the squamous cells arranged in one or two layers, which with their long axes were oriented parallel to the surface of the tongue. In the cytoplasm of these cells inclusions in the form of intensely colored grains and lumps of different sizes were detected, which were keratohyalin and light, poor in chromatin, nuclei.

The most superficial position was occupied by the corneal layer, represented by homogeneous disintegrated, intensely colored lamellar masses.

Within one week of consumption of the complex of food additives, supplemented to the ration of laboratory animals, the thickening of the mucous membrane in the all parts of the tongue was noted. The studied index was 194.1±3.20 μm on the tip, 217.0±2.44 μm on the body and 197.82±4.26 μm on the root. The increase in the thickness of the mucous membrane occurred primarily due to the thickening of the epithelial layer. The thickness of the latter was 138.7±2.15 μm on the tip, 170.9±1.47 μm on the body and 152.7±3.29 μm on the root.

The detailed study of the structure of the epithelial layer showed a significant thickening of the corneal layer in all parts of the tongue that indicated the activation of the process of keratohyalin formation,

leading to hyperkeratosis. In turn, hyperkeratosis may be the result of the formation of a protective mechanism that prevents the penetration of harmful external factors into the lamina propria of the mucous membrane, which may well be food additives. At the same time, excessive keratinization may indicate the development of corneal dystrophy in the covering epithelium, the cause of which may be both direct contact with an aggressive substance and some disorders of homeostasis.

No significant differences in the granular layer of the covering epithelium in the experimental animals were noted, compared to the intact animals. However, in the latter, the presence of small areas in which a significant number of squamous cells, containing rod-shaped pyknotic nuclei, was noteworthy. Additionally, thinning of the granular layer, in which epitheliocytes were located in 2–3 layers, was observed.

In the literature this phenomenon is called parakeratosis and is a special type of keratinization. This fact indicates the activation of the direct path of keratinization, called orthokeratosis, in the animals of the experimental group. In turn, orthokeratosis can be regarded as the manifestation of the protective reaction of the mucous membrane in response to adverse external factors.

In the spinous layer, we observed a significant number of epitheliocytes with the phenomena of hydropic dystrophy. It should be noted that isolated cells with similar changes were found in the intact covering epithelium, which is not considered the pathology. However, we observed that in some areas the amount of dystrophically altered epitheliocytes reached 20–30 % of the total cell population, which should be regarded as the manifestation of damage.

Additional evidence in favor of the development of the pathological process in the epithelial layer of the mucous membrane of the tongue should also be considered as a disrupted stratification. This is manifested by the lack of clear boundaries between individual epitheliocytes and disruption of the order of cellular elements.

We did not detect any pronounced changes in the basal layer of the epithelial lamina. However, the presence of small areas in which basal epitheliocytes were located in several layers, forming the solid, acanthotic structures that penetrated into the connective tissue of the lamina propria, was noteworthy in the root and body of the tongue (fig. 3).

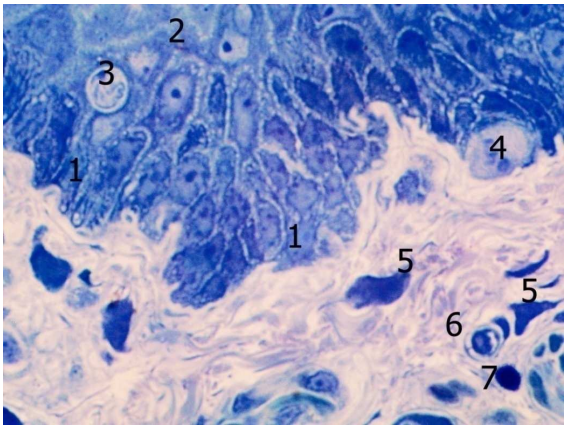

Fig. 2. The structure of the mucous membrane of the tongue of an albino rat. Semi-thin section. Polychrome stain. Objective lens: 100×magnification, ocular lens: 10×magnification. 1 – basal epitheliocytes; 2 – spinous epitheliocytes; 3 – Langerhans cell; 4 – Merkel cell; 5 – fibroblasts; 6 – blood vessel; 7 – mast cell.

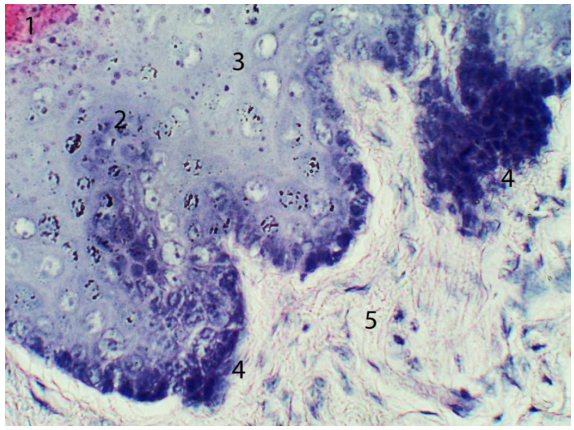

Fig. 3. The structure of the mucous membrane of albino rats (combined effect of food additives for one week). H&E stain. Objective lens: magnification ×40, ocular lens: magnification ×10. 1 – corneal layer of the squamous epithelium; 2 – the focus of disrupted stratification in the epithelial layer; 3 – epitheliocytes with dystrophic changes; 4 – areas of increased proliferative activity of the basal epithelium; 5 – connective tissue of the lamina propria of the mucous membrane.

Presumably, the above morphological picture indicates an increase in the proliferative activity of basal epitheliocytes, as evidenced by the increase in the number of mitotic figures in the basal layer. At the same time, similar structural phenomena could be the consequence of disrupted differentiation of basal epitheliocytes. This is confirmed by the presence of foci of disrupted stratification in the immediate vicinity of the described structures.

Within four weeks of consumption of the complex of food additives, supplemented to the ration of laboratory animals the progressing thickening of the mucous membrane in the all parts of the dorsum of the tongue was noted. The studied index was 249.7±2.45 μm in the area of the root, 290.3±2.53 μm in the body, and 217.6±3.1 μm in the area of the tip of the tongue. Similar to the previous experimental group, the thickening of the mucous membrane occurred due to an increase in the thickness of the stratified squamous epithelium. This parameter was 192.92±3.22 μm in the area of the root of the tongue, 228.56±3.43 μm in the area of the body, and 155.4±8.23 μm in the area of the tip of the tongue.

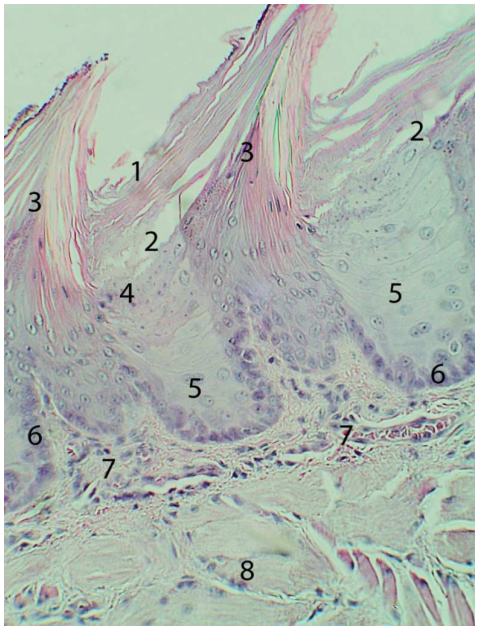

Fig. 4. The structure of the tongue of albino rats (combined effect of food additives for four weeks). H&E stain. Objective lens: 10×magnification, ocular lens: 10×magnification. 1 – corneal layer with the phenomena of keratolysis; 2 – subcorneal cavities; 3 – filamentous papillae; 4 – granular layer of the stratified squamous epithelium; 5 – spinous layer of the stratified squamous epithelium; 6 – basal layer of the stratified squamous epithelium; 7 – lamina propria of the mucous membrane; 8 – muscles of the tongue.

Similarly, in all observations, a pronounced thickening of the corneal layer was detected. The phenomena of keratolysis, a detachment of the corneal layer from the granular one, with the formation of subcorneal cavities of varying prominence was observed (fig. 4).

In the granular layer, we quite often observed the phenomena of parakeratosis, the morphological features of which were described in detail above. Epitheliocytes with the phenomena of hydropic dystrophy were detected in the spinous layer, and their amount did not differ significantly from the indices of the previous experimental group. Epithelial cells with dystrophic changes were found in different parts of the tongue with almost the same frequency, with the largest number of them observed in the upper cellular layers of the spinous layer.

The areas of increased proliferative activity of the basal epithelium mentioned above were much less common, and the mitotic activity of basal epitheliocytes was less pronounced. This may indicate a decrease in the activity of proliferative processes in the epithelial layer.

Thus, the structure of the covering epithelium of the mucous membrane of the tongue of intact rats is generally consistent with the findings reported in the publications on this topic [9]. However, the findings of our study suggest that the cellular composition and metric parameters of the epithelium of the mucous membrane of the dorsum of the tongue of intact albino rats have a fundamentally similar structure on the tip, body and root of the tongue. The thickening of the covering epithelium of the tongue, induced by consumption of the complex of food additives, supplemented into the food ration, may have compensatory and adaptive value, creating an additional barrier to penetration of adverse exogenous factors into the lamina propria of the mucous membrane of the tongue. At the same time, it should be taken into account that the above processes may be a manifestation of alternative changes associated with both the direct influence of aggressive exogenous factors and the disrupted trophism of the stratified squamous epithelium [2, 15].

Conclusions

1. Consumption of complex of food additives (monosodium glutamate, Ponceau 4R and sodium nitrite), supplemented to the standard ration of albino rats, for four weeks leads to a progressive increase in the thickness of the stratified squamous epithelium of the entire dorsal surface of the tongue, accompanied by the development of the phenomena of hydropic dystrophy, acanthosis, focal disrupted stratification.

2. The greatest thickening of the stratified squamous epithelium, induced by the effect of the complex of food additives for 4 week, is observed in the body of the tongue (128.7±6.48 μm to 228.56±3.43 μm).

Prospects for further research will encompass the follow-up study of the cellular composition of the mucous membrane of the tongue, under the condition of supplement of the standard ration of albino rats with the complex of food additives, using immunohistochemical methods of research.

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Стаття надійшла 24.12.2020 р.

DOI 10.26724/2079-8334-2022-1-79-
UDC 616.33:615.24:599.323.41

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LECTINS IN THE INVESTIGATION OF RENAL PATHOLOGIES

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Carbohydrate-rich biopolymers – glycoproteins and proteoglycans – play an extremely important role in renal histophysiology. In particular, glycoproteins podoplanin and podocalyxin of podocytes maintain the morpho-functional status of these cellular elements: formation of pedicels, slit diaphragms, and, together with the glomerular membrane – a negative electrical charge and selective permeability of the filtration barrier. Proximal tubules brush border glycoproteins megalin and kubilin are in charge of endocytosis and reabsorption of macromolecules from the ultrafiltrate. Glycoproteins of extracellular matrix – fibronectin, laminin, tenascin, nidogen, various types of collagen, heparan-sulfate proteoglycans perlecan and agrin, dermatan-sulfate proteoglycans versican, biglycan and decorin provide adhesive, mechanical support and inductive properties of renal micro- and ultrastructures. Therefore, lectins as reagents capable of selective recognition of specific glycoepitopes proved to be a valuable tool in the investigation of both normal renal morphogenesis and pathogenesis of nephropathies. Special attention is paid to modern trends in lectinology – investigation of endogenous lectins of humans and animals and their role in health and disease. Practical examples of lectins application as selective histological markers of renal structures are depicted.

Key words: lectins, glycoconjugates, kidney, histopathology.

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ЛЕКТИНИ У ДОСЛІДЖЕННІ ГІСТОПАТОЛОГІЇ НИРКИ

У гістофізіології нирок виключно важлива роль належить високомолекулярним вуглеводовмісним біополімерам – глікопротеїнам і протеогліканам. Зокрема, глікопротеїни подоцитів подопланін і подокаліксин забезпечують підтримання морфо-функціонального статусу означених клітинних елементів: формування цитоподій, щілинних діафрагм, та, спільно з мембраною ниркового клубочка – негативний електричний потенціал і селективну проникність фільтраційного бар'єру. Глікопротеїни щіточкової облямівки епітеліоцитів проксимальних трубочок нефронів мегалін і кубілін відіграють провідну роль у механізмах ендоцитозу та реабсорбції макромолекул з ультрафільтрату. Глікопротеїни екстрацелюлярного матриксу – фібронектин, ламінін, тенасцин, нідоген, колаген IV типу, гепаран-сульфат протеоглікани перлекан та агрин, дерматан-сульфат протеоглікани версикан, біглікан та декорин забезпечують адгезивні, опорно-механічні та індуктивні властивості ниркових мікроструктур. Лектини як реагенти здатні до вибіркового розпізнавання глікополімерів у залежності від складу та конфігурації їхніх кінцевих вуглеводних детермінант представляють собою цінний інструмент у дослідженні як нормального морфогенезу нирок, так і етіопатогенезу нефропатій. Особлива увага приділена сучасним тенденціям у лектинології – дослідженню ендогенних лектинів людини і тварин та їхній ролі за фізіологічних умов та у патогенезі ниркових хвороб. Наведено приклади практичного застосування лектинів як селективних гістологічних маркерів ниркових структур.

Ключові слова: лектини, глікокон'югати, нирка, гістопатологія.

The study is an initiative.

Carbohydrate-rich biopolymers – glycoproteins and proteoglycans – play an extremely important role in renal histophysiology. In particular, glycoproteins podoplanin and podocalyxin maintain the morpho-functional status of podocytes: formation of pedicells, slit diaphragms, and, combined with glomerular membrane – a negative electrical charge and selective permeability of the filtration barrier [18, 20]. Proteoglycans possess dual nature: from one hand, some of them expose glycosidic moieties (e.g.