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DYNAMICS OF EXPRESSION OF CARBOHYDRATE DETERMINANTS OF MANNOSE-SPECIFIC LECTINS IN THE MUCOUS MEMBRANE OF THE RAT ATTACHED GINGIVA IN CHRONIC ETHANOL INTOXICATION

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The study found that concanavalin A lectin is specific to the horny scales of the mucous membrane of the attached part of the gums. On day 5 and day 12 of the experiment the ethanol effect showed weakening of the intensity of marking on the keratinocytes, which was morphologically manifested by the phenomena of hyperkeratosis. The expression of receptors on fibroblasts and collagen fibers to the resident structural components of the lamina propria was reduced at all times of observation. The mast cells were characterized by an increase in degree on day 5 and day 12 of the experiment.

Key words: attached part of gums, rats, lectin concanavalin A, sounding.

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ДИНАМІКА ЕКСПРЕСІЇ ВУГЛЕВОДНИХ ДЕТЕРМІНАНТ МАНОЗОСПЕЦИФІЧНИХ ЛЕКТИНІВ У СЛИЗОВІЙ ОБОЛОНЦІ ПРИКРІПЛЕНОЇ ЧАСТИНИ ЯСЕН ЩУРІВ ПРИ ХРОНІЧНІЙ ІНТОКСИКАЦІЇ ЕТАНОЛОМ

У роботі встановлено, що лектин конканаваліну А є специфічними до рогових лусочок слизової оболонки прикріпленої частини ясен. При дії етанолу на п’яту та дванадцяту доби встановлено зниження інтенсивності маркування на кератиноцитах, що морфологічно проявлялось явищами гіперкератозу. До резидентних структурних компонентів власної пластинки експресія рецепторів на фібробластах і колагенових волокнах зменшилася на всіх термінах спостереження. Мастоцити характеризувалися збільшенням ступеня на 5-у та 12-ту доби.

Ключові слова: прикріплена частина ясен, щури, лектин конканаваліну А, зондування.

The work is a fragment of the research project “Experimental morphological study of the effect of cryopreserved cord blood products and embriofetoplacental complex (EFPC), diphereline, ethanol and 1 % methacrylate on the morphofunctional condition of several internal organs”, state registration No. 0119U102925.

The problem of alcohol use is extremely relevant to date: the global consumption of alcoholic beverages is accounted for huge numbers. Alcoholism is a severe chronic disease, in most cases it is difficult to cure. The disease occurs due to regular and prolonged alcohol consumption and is characterized by a special pathological condition of the body: uncontrollable craving for alcohol, changes in its tolerability and personality degradation [15].

The growing of alcoholic drinks market in modern Ukraine, its diversity and ease of purchase, obsessive advertising, as well as a very common tradition of alcohol consumption among the population are the causes of early alcohol consumption and alcoholism of children and youth.

The gingival mucosa is very sensitive to various exogenous factors, which is manifested by epithelium keratinization disorder and the tension of the local protective barrier [7].

The diagnosis and treatment of various oral diseases are based on understanding the histological structure and mechanisms of functioning of the structures of the oral cavity. The method of lectin sounding with its sensitivity and selectivity for the detection of the specified molecular structures is more preferable over the traditional methods of histochemical verification of carbohydrates [9].

The purpose of the study was to determining the dynamics of expression of carbohydrate determinants of mannose-specific Concanavalin A lectin in the mucous membrane of the rat attached gingiva in chronic ethanol intoxication.

Materials and Methods. 15 albino outbred male rats were involved into the study. 5 animals were assigned into control group and 10 animals were assigned into experimental group. The rats were administered with 12 mg/kg 40° pure ethanol 4 times a day directly into the stomach. [6]. Animals were sacrificed under thiopental anesthesia overdose on day 5 and day 12 of the experiment. The method of lectinohistochemistry was used to determine the carbohydrate components of the structural elements of the mucous membrane of the attached gingiva. [10, 11]. The obtained material was fixed in 10 % neutral buffered formalin, subsequently embedded into paraffin according to standard procedure, [1] and histological sections of 3-5 µm thick were made. Mannose carbohydrate residues were detected by Concanavalin A lectin (Con A). The material was processed by the "Lectinost" (Lviv) standard sets of the laboratory at a lectin dilution of 1:50. Visualization of the reaction with lectin conjugates was performed by the method of semi-quantitative method in immersion magnifications of the Biorex-3 VM-500 microscope. Animal housing and experiments on them have been carried out in compliance with the "General Ethic Rules for Conducting Experiments on Animals", adopted by the I National Congress on Bioethics [13] and the requirements of international principles of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes".

Results of the study and their discussion. Sounding of the mucous membrane of the attached gingiva with mannose-specific Concanavalin A lectin in control rats showed a weak conjugation with cells of the granular, spinous and basal layers and moderate conjugation in the basement membrane of the epithelial plate (table 1).

Table 1

Lectinochemical characteristics of the mucous membrane of the rats gums in sounding with mannose-specific Concanavalin A lectin (Con A)

Structural components			Control group	Day 5	Day 12
Epithelium	horny layer		3	2	2
	granular layer		1	1	1
	spinous layer		1	1	1
	basal layer		1	1	1
	basement membrane		2	1	1
Lamina propria	fibroblasts		1	1	1
	collagen fibers		3	1	3
	vessels	endothelial	1	1	1
		basal membrane	2	1	1
		elastic membrane	0	0	0
	migratory cells	mast cells	0	2	2
		lymphocytes	0	0	0
		macrophages	0	0	0

Note: 0 - no reaction; 1 - weak reaction (light brown stain); 2 - moderate reaction (yellow-brown stain); 3 - strong reaction (brown stain); 4 - very strong reaction (dark brown stain).

The study of the specificity of the conjugation of mannose-specific Concanavalin A lectin with receptors on the structural components of the mucous membrane of the attached gingiva in rats of the control group in the epithelial plate showed a very strong sensitivity of receptors on the horny scales (fig. 1).

Fibroblasts showed very weak intensity of marking of the components of the lamina propria. Collagen fibers revealed strong conjugation (fig. 2).

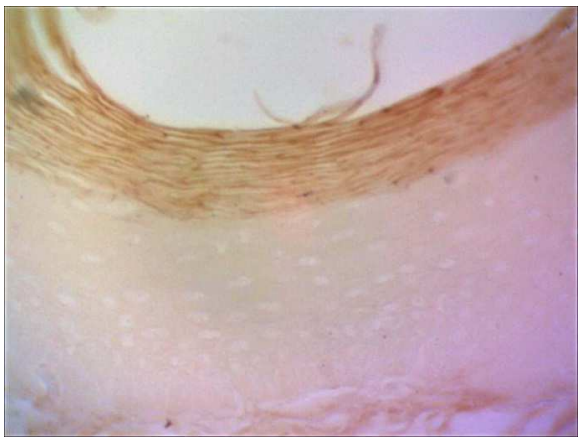

Fig. 1. Strong expression of the mannose-specific Concanavalin A lectin on the horny scales of the epithelial plate of the attached gingiva in a rat of control group. Con A marking. Lens: 40× magnification; Ocular lens: 10× magnification.

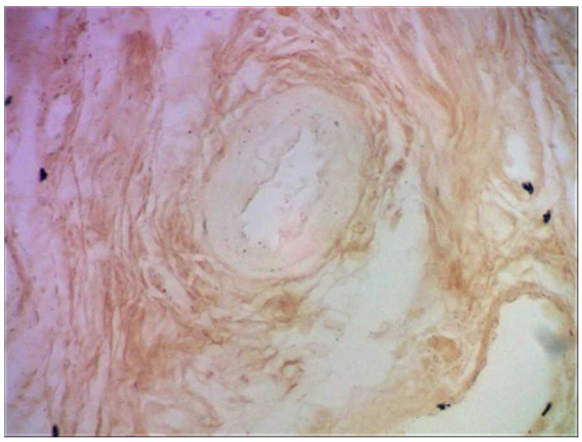

Fig. 2. Strong expression of the mannose-specific Concanavalin A lectin on the collagen fibers and moderate expression on the venular endothelial cells of the attached gingival in a rat of control group. Con A marking. Lens: 100×magnification; Ocular lens: 10× magnification.

Sensitivity to Concanavalin A lectin was negative on the inner elastic membranes of arterioles; endothelial cells of the blood microvasculature showed a weak reaction and the basement membranes showed a moderate degree of conjugation.

Mannose-specific lectin sounding of the specificity of conjugation of migratory cells of the connective tissue of the lamina propria of the mucous membrane of the attached gingiva of rats of the control group revealed a negative reaction of the mast cells, lymphocytes and macrophages.

On day 5 of the experiment, the specificity of the conjugation of receptors with the Concanavalin A lectin on the structural components of the rat attached gingiva revealed that the expression of receptors on the horny scales reduced from moderate to weak.

Cells of the granular, horny, basal layers and the basement membrane showed weak reaction.

The intensity of marking of the resident cells of the lamina propria did not change and was weak. The expression of mannose-specific receptors of the collagen fibers reduced from strong to weak (fig. 3).

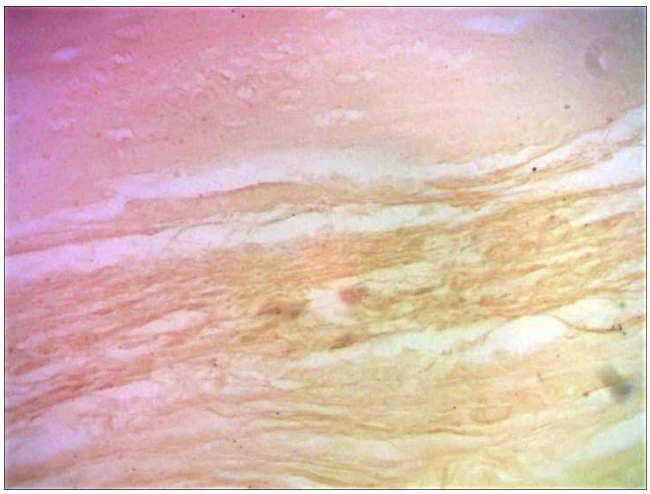

Fig. 3. Weakened expression of mannose-specific Concanavalin A lectin on the collagen fibers in the lamina propria of the rat attached gingiva on day 5 of the experiment. Con A marking. Lens: 40× magnification; Ocular lens: 10×magnification.

On day 5 of the experiment, the sensitivity of the endothelial cells of the blood microvasculature to mannose was steadily weak and on the basement membranes it reduced from moderate to weak; elastic membranes of arterioles showed a negative reaction, similarly to rats from control group (table 1).

The sensitivity to Concanavalin A lectin increased on the mast cells from negative to moderate, which indicated the activation of the processes of accumulation of secretory granules at this time of observation. The reaction with lymphocyte and macrophage receptors was negative, similarly to the animals of control group.

On day 12 of the experiment, the components of the epithelial plate of the rat attached gingiva showed a constant degree of expression of receptors to Concanavalin A lectin in contrast to the previous term of the experiment, which was morphologically confirmed by impaired differentiation of gingival epithelium in the form of parakeratosis and acanthosis. In the lamina propria, enhanced expression on the fibroblasts was established, similarly to control rats; collagen fibers steadily maintained a weak intensity of marking (table 1). Endothelial cells of the sections of the blood microvasculature and basement membranes retained the degree of conjugation, compared to the previous term of the experiment; elastic membranes of arterioles showed no expression (table 1). Among the migratory cells of the connective tissue, a negative reaction to mannose-specific Concanavalin A lectin by lymphocytes and macrophages has been established; the mast cells maintained a moderate intensity of marking, similarly to the previous period of the experiment.

Varzhapetyan S.D. et al has found that histotopography of glycoproteins in various forms of stomatogenous maxillary sinusitis can be used for development of methods of local therapy of the affected

structures of Schneiderian membrane [4]. Lutsyk O.D. confirmed that mannose-specific PSA, GNA and Con A lectins can be recommended as the selective histochemical markers of the fetal Leydig cells [12].

In his publications, Bilash S.M. has shown the ways to detect species characteristics of organs by lectinochemistry. A very strong reaction of conjugation between carbohydrate residues on the structural components of the protein acini of the human submandibular gland and Con A lectin with carbohydrate determinants of the secretory granules of serocytes and myoepitheliocytes has been established [3].

A strong reaction of conjugation on macrophages, proerythroblasts and basophilic erythroblasts with ConA, LABA, PNA lectins was established [2].

Conclusion

Concanavalin A lectin is specific to horny scales. On day 5 and day 12 of the experiment the ethanol effect showed weakening of the intensity of marking on the keratinocytes, which was morphologically manifested by the phenomena of hyperkeratosis. The expression of receptors on fibroblasts and collagen fibers to the resident structural components of the lamina propria was reduced at all times of observation. The mast cells were characterized by an increase in degree on day 5 and day 12 of the experiment.

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