DOI 10 26724/2079-8334-2023-3-85-216-219
UDC 616.311.2-018.2:615.917.262:615.322-02:582.736.3]-092.9

K.S. Kazakova, G.A. Yeroshenko, O.V. Sheshukova, V.P. Trufanova, T.V. Polishchuk, A.I. Maksymenko, S.S. Bauman
Poltava State Medical University, Poltava

DYNAMICS OF EXPRESSION OF CARBOHYDRATE DETERMINANTS OF FUCOSE-SPECIFIC LECTIN OF THE BARK OF GOLDEN RAIN IN THE MUCOSA OF THE ATTACHED PART OF THE GUMS OF RATS UNDER CHRONIC ETHANOL INTOXICATION

e-mail: grem23062017@gmail.com

The purpose of the study was to determine the dynamics of expression of carbohydrate determinants of fucose-specific lectin of the bark of golden rain in the mucous membrane of the attached part of the gums of rats under chronic ethanol intoxication. The rats were administered 12 mg/kg of 40 % ethanol 4 times a day gastrointestinally. On days 5 and 12, the animals were removed from the experiment. Determination of carbohydrate components of the structural elements of the mucous membrane of the attached part of the gums was performed by lectin histochemistry. On the fifth day of observation, the study of the specificity of receptor binding to the fucose-specific lectin of the bark of the golden rain on the structural components of the epithelial plate of the attached part of the gums of rats revealed that the expression of receptors on the horny scales and basement membrane decreased from moderate to weak. The reaction of receptors to the lectin of the bark of the golden rain on the basal membranes of the vessels of the hemomicrocirculatory channel increased from moderate to strong. On the 12th day of observation, the components of the epithelial plate of the attached part of the gums of rats showed a stable weak degree of expression of receptors to the lectin of the bark of the golden rain in comparison with the previous period of the experiment.

Key words: lectins, mucous membrane, chronic ethanol intoxication, attached part of the gingiva.

К.С. Казакова, Г.А. Єрошенко, О.В. Шешукова, В.П. Труфанова, Поліщук Т.В., А.І. Максименко, С.С.Бауман

ДИНАМІКА ЕКСПРЕСІЇ ВУГЛЕВОДНИХ ДЕТЕРМІНАНТ ФУКОЗОСПЕЦИФІЧНОГО ЛЕКТИНУ КОРИ ЗОЛОТОГО ДОЩУ У СЛИЗОВІЙ ПРИРІПЛЕННОЇ ЧАСТИНИ ЯСЕН ЩУРІВ ЗА ХРОНІЧНОЇ ІНТОКСИКАЦІЇ ЕТАНОЛОМ

Метою дослідження було визначити динаміку експресії вуглеводних детермінант фукозоспецифічного лектину кори золотого дощу в слизовій оболонці прикріпленої частини ясен щурів за хронічної інтоксикації етанолом. Щурам шлунково-кишково вводили 12 мг/кг 40 % етанолу 4 рази на день. На 5 і 12 добу тварин вилучали з досліду. Визначення вуглеводних компонентів структурних елементів слизової оболонки прикріпленої частини ясен проводили методом лектинової гістохімії. На п'яту добу спостереження дослідження специфічності рецепторного зв'язування фукозоспецифічного лектину кори золотого дощу на структурних компонентах епітеліальної пластинки прикріпленої частини ясен щурів виявило, що експресія рецепторів на рогових лусочках і базальній мембрані знизилась від помірного до слабкого. Реакція рецепторів на лектин кори золотого дощу на базальних мембранах судин гемомікроциркуляторного русла посилювалася від помірної до сильної. На 12-ту добу спостереження компоненти епітеліальної пластинки прикріпленої частини ясен щурів демонстрували стійкий слабкий ступінь експресії рецепторів до лектину кори золотого дощу порівняно з попереднім терміном експерименту.

Ключові слова: лектини, слизова оболонка, хронічна інтоксикація етанолом, прикріплена частина ясен.

The study 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.

According to the European "Health for Everybody" Database, the standardized mortality rate from alcohol-related causes in Ukraine is one of the highest in Europe. According to the WHO in 2018, Ukraine ranks first in the world in terms of childhood alcoholism [8, 9].

Alcoholism is a serious chronic disease that is difficult to treat in most cases. The disease occurs as a result of regular and long-term alcohol consumption and is characterized by a special pathological condition of the body: an irrepressible craving for alcohol, changes in the degree of its tolerance, and degradation of the personality [7].

Being on the border with the external environment and constantly exposed to unfavorable factors, the epithelium of the oral mucosa is the first barrier. Reliability of protective functions is ensured not so much by mechanical properties as by the ability to quickly restore damaged structures and structural and functional connections with all organs and systems of the body. Therefore, the study of morphological changes in organs during chronic ethanol intoxication is an urgent problem in modern medicine [4].

The sensitivity of the gingival mucosa to various exogenous factors is very high and it is manifested by a violation of the process of epithelial keratinization and tension of the local protective barrier.

Diagnosis and treatment of the oral cavity diseases is based on the analysis of the histological structure and mechanisms of the oral cavity structures' functioning. Using the lectin chemistry method, it is possible to obtain information about the histofunctional state of organs and to establish its changes in pathological processes, under the influence of various exo- and endogenous factors [2, 3, 6].

The purpose of the study was to determine the dynamics of expression of carbohydrate determinants of fucose-specific lectin of the golden rain bark in the mucous membrane of the attached part of the rat gums under chronic ethanol intoxication.

Materials and methods. The study involved 20 white male rats (125±20) g. A constant temperature was maintained in the experimental biological clinic where the animals were kept, and the rats were properly cared for during the experiment [1].

Before the experiment, all animals were carefully examined, namely, their weight, sex, age, motor activity, and coat condition were taken into account.

The control group included 5 animals, the experimental group consisted of 15 animals. The rats were administered 12 mg/kg of 40 % ethanol (in terms of pure alcohol) 4 times a day gastrointestinally [6]. On days 5 and 12, the animals were removed from the experiment by an overdose of thiopental anesthesia. Determination of carbohydrate components of the structural elements of the attached part of the gums' mucous membrane was performed by lectin histochemistry.

After extraction, the material was fixed in a 10 % solution of neutral formalin, embedded in paraffin according to the conventional method. The histological sections were made 3-5 μm thick.

The material was prepared with standard kits of the Lectinost laboratory (Lviv) at a dilution of 1:50 lectin. Visualization of the reaction with lectin conjugates was performed by the semi-quantitative method in immersion magnifications of the Biorex-3 VM-500 microscope.

The animals were kept in the experiment following the "General Ethical Rules for Animal Experiments" adopted by the I National Congress on Bioethics and the requirements of the international principles of the "European Convention for the Protection of Animals Used for Experimental and Other Scientific Purposes"[10].

Results of the study and their discussion. Among modern methods of morphological research, lectin chemistry occupies an important place. This method provides recognition of the form of interaction between cells and their microenvironment, both in the process of embryonic development and in the mature body [3]. Methods of lectin histochemistry have been used in animal experiments to morphologically characterize the development of fibrosis of postinfarction heart muscle; remodeling of the left atrium against the background of hypertrophy and fibrosis; studying the relationship between endothelial cell glycoma of different localization and their permeability; and monitoring the restructuring of the heart muscle under conditions of using stem cells for its reparative regeneration. The role of lectin receptors in the process of embryonic cardiomorphogenesis in normal conditions has been studied in details.

Lectin chemistry can be used to identify the species characteristics of organs.

According to the literature, a very strong bonding reaction between carbohydrate residues on the structural components of protein acini of the human submandibular gland and the LABA lectin was established. In addition, the affinity determination for the fucose-specific golden rain bark lectin revealed a strong binding reaction on macrophages, proerythroblasts, and basophilic erythroblasts. A weakly positive reaction with LABA lectins was found on polychromatophilic erythroblasts. A weakly positive reaction with Con A, HPA, LABA lectins was also detected in orthochromic erythroblasts of the erythroblast insula.

The expression of lectin, golden rain bark, peanut, soybean, black elderberry, mistletoe, and wheat germ receptors on the cell surfaces of macrophages, proerythroblasts, and basophilic erythroblasts has been increased. This can be used as a selective marker.

The study determined the degree of expression of carbohydrate determinants in the structural elements of the mucous membrane of the attached part of the rat gums of the control group, as well as at the phase of formation of a severe craving for alcohol (fifth day of observation) and in the development of physical alcohol dependence (12th day of the experiment).

Determination of the affinity for fucose-specific lectin of the golden rain bark revealed a moderate degree of expression on the horny scales and basal membrane of the epithelial plate of the gums' attached part of the control group rats (Fig. 1).

The similarity to the fucose-specific lectin of the golden rain cortex from the components of the lamina propria was very weak for fibroblasts and collagen fibers in the control rats (Figure 2).

The intensity of labeling was negative on the internal elastic membranes of arterioles, endothelial cells of hemomicrocirculatory vessels. Thus, basal membranes showed a moderate reaction.

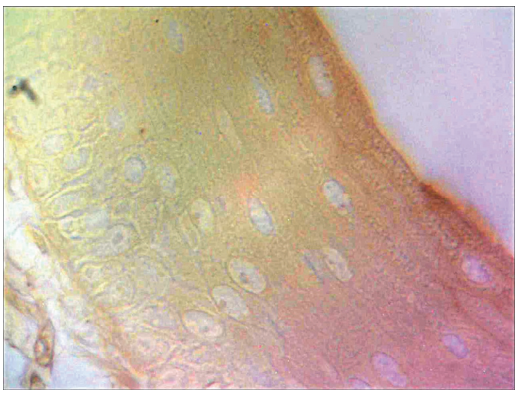

Fig.1. Moderate expression of fucose-specific golden rain cortex lectin on keratinocytes of the epithelial plate of the attached part of the gingiva of the control group rat. LABA marking. Magn: obj. x 100, eyepiece x 10.

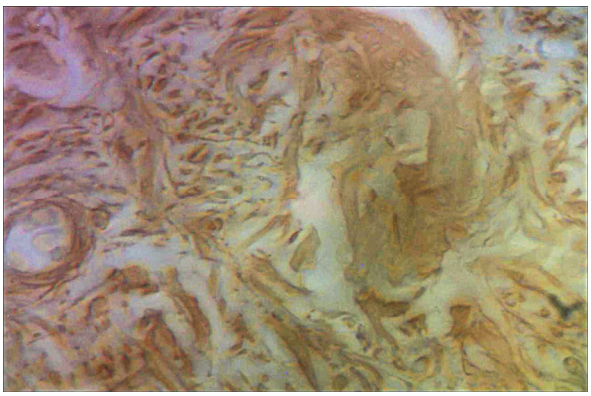

Fig. 2. Strong expression of fucose-specific golden rain cortex lectin on collagen fibers and moderate expression on the basal membrane and hemomicrovascular endothelial cells of the own plate of the attached part of the gingiva of the control group. LABA marking. Magn: obj. x 100, eyepiece x 10.

Probing of the specificity of binding of migratory connective tissue cells of the own plate of the mucous membrane of the attached part of the gums of the control group rats revealed a negative reaction from lymphocytes and macrophages. Mast cells in the control group of animals showed a high level of expression (Fig. 3).

The expression of receptors on the stratum corneum and basal membrane decreased from moderate to weak on the fifth day of observation of the specificity of receptor binding to the fucose-specific lectin of the golden rain bark on the structural components of the epithelial lamina propria of the attached part of the rat gingiva.

The reaction with the cells of the granular, stratum corneum, and basal layers remained consistently weak.

The intensity of labeling of the lamina propria components did not change and was weak for fibroblasts and collagen fibers, as in the control rats.

The reaction of receptors to the golden rain bark lectin on the basal membranes of the vessels of the hemomicrocirculatory system increased from moderate to strong. Vascular endothelial cells and elastic membranes showed a weak degree of cohesion. This did not differ from the results in the control group of animals.

The binding affinity to fucose decreased from strong to negative on mastocytes. The reaction with lymphocyte and macrophage receptors was negative.

On the 12th day of observation, the components of the epithelial plate of the attached part of the rat gums showed a stable low degree of expression of receptors to the golden rain bark lectin compared with the previous period of the experiment (Fig. 4). This was morphologically confirmed by impaired gingival epithelial differentiation in the form of parakeratosis and acanthosis.

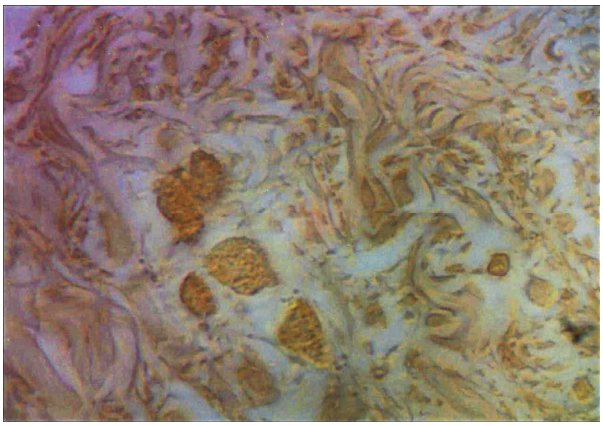

Fig. 3. Strong expression of fucose-specific golden rain cortex lectin on mastocyte granules in the own plate of the attached part of the gingiva of the control group rat. LABA marking. Magn: obj. x 100, eyepiece x 10.

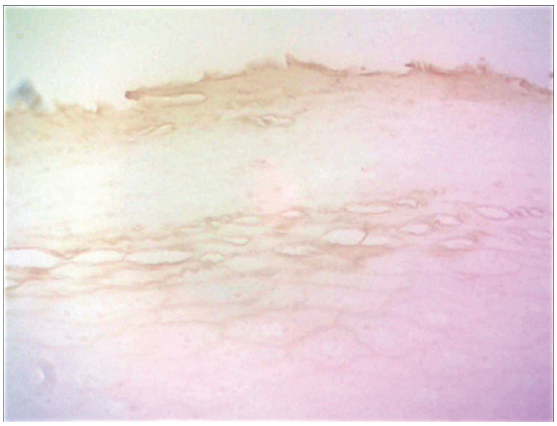

Fig. 4. Weak expression of fucose-specific golden rain bark lectin on keratinocytes of the epithelial plate of the attached part of the rat gingiva on 12th day 12 of observation. LABA marking. Magn: obj. x 100, eyepiece x 10.

A stable weak intensity of labeling on fibroblasts and collagen fibers was found in the lamina propria.

The endothelial cells of the hemomicrocirculatory system reduced the degree of bonding from moderate to weak compared to the previous period of the experiment. Basement membranes decreased their compatibility with fucose from strong to weak compared to the previous experiment.

The elastic membranes of arterioles showed no expression during the entire observation period.

A negative reaction was found for migratory connective tissue cells.

On the 12th day of observation, the components of the epithelial lamina of the attached part of the rat gingiva showed a stable low degree of expression of receptors to the golden rain bark lectin compared with the previous period of the experiment, which was morphologically confirmed by impaired differentiation of the gingival epithelium in the form of parakeratosis and acanthosis.

In the lamina propria, a stable weak intensity of labeling on fibroblasts and collagen fibers was found.

The endothelial cells of the hemomicrocirculatory system reduced the degree of binding from moderate to weak compared to the previous period of the experiment. Basement membranes decreased their affinity for fucose from strong to weak compared to the previous experiment. The elastic membranes of arterioles showed no expression during the entire observation period [2, 3].

Mast cells and macrophages on the 12th day of the experiment were characterized by a decrease in the degree of conjugation with receptors to lectins of peanuts, the bark of common golden rain, wheat germ and black elder, which reflected the processes of active extrusion of secretory granules and a decrease in the amount of substrate for fixing lectins [5].

In the end of experiment migratory connective tissue cells showed an absence of expression, which is due to the exhaustion of the local protective barrier and is consistent with the data of other researchers [4, 10].

Conclusion

The lectinochemical studies have shown that the lectin of the bark of the golden rain is specific for migratory cells in animals of the control group. Mastocytes and macrophages were characterized by a decrease in the degree of conjugation with receptors for goldenrod bark lectins on the 12^{th} day of the experiment. It reflected the processes of active extrusion of secretory granules and a decrease in the amount of substrate for lectin fixation.

That is why, it is possible to obtain information about the histological and functional state of the organs of the oral cavity and to establish its changes in pathological processes under the influence of various exo- and endogenous factors using the method of lectinochemistry.

References

1. Bagriy M, Dibrova VA, Popadinets OG, Grishchuk MI. Metodyky morfolohichnykh doslidzhen: Monografiya. Vinnytsya: Nova knyha, 2016. 328 s. [in Ukrainian]

2. Bilash VP. Porivnyalna kharakterystyka vuhlevodnykh zalyshkiv na strukturnykh komponentakh kintsevykh sekretornykh viddiliv pidnyzhnyoshchelepnykh slynnykh zaloz lyudyny ta deyakykh laboratornykh tvaryn. Svit medytsyny ta biolohiyi. 2017; 1 (59): 99–103. [in Ukrainian]

3. Bilash SM, Boruta NV, Starchenko II. Lektynokhimichna kharakterystyka klitynnykh elementiv erytroblastnoho ostrivtsia chervonoho kistkovoho mozku u shchuriv pry vvedeni kriokonservovanoyi platsenty. Svit medytsyny ta biologiyi. 2017; 3(61): 83–5.doi:10.26724/2079-8334-2017-3-61-83-85 [in Ukrainian]

4. Varzhapetian SD, Huliuk AH. Vyvchennia rozpodilu hliukokonyuhativ vperspektyvi rozrobok mistsevoyi terapiyi stomatohennykh verkhnoshchelepnykh synusytiv. Svit medytsyny ta biologiyi. 2018; 2 (64): 126–31. doi: 10.26724/2079-8334-2018-2-64-126-131 [in Ukrainian]

5. Yeroshenko GA, Tymoshenko YuV. Dynamika ekspresiyi vuhlevodnykh determinant strukturnykh komponentiv slyzovoyi obolonky zalozystoyi zony tverdoho pidnebinnia za umov eksperymentalnoyi hiposalivatsiyi. Svit medytsyny ta biologiyi. 2018; 1 (63): 123–26. doi: 10.26.724/2079-8334-2018-1-63-123-126 [in Ukrainian]

6. Ivanochko VM. Morfologichniy stan strukturnykh komponentiv filtratsiyno-reabsorbtsiynoho baryeru nyrok u normi i pry khronichniy alkoholizatsiyi napoyamy riznoyi yakosti i mitsnosti [dysertatsiya]. Ternopil, 2003.19 s. [in Ukrainian]

7. Kazakova KS, Yeroshenko GA, Shevchenko KV. Toksychnyi vplyv etanolu na slyzovi obolonky. Svit medytsyny ta biologiyi. 2017; 3 (61):169–73. doi: 10.26724/2079-8334-2017-3-61-169-173 [in Ukrainian]

8. Kazakov OA. Suchasnyi stan problemy pidlitkovoho alkoholizmu v Ukraini. Svit medytsyny ta biologiyi. 2017;2(60):54–6. [in Ukrainian]

9. Chemerys NM. Medyko-sotsialni aspekty problemy vzhyvannia psykhoaktyvnykh rechovyn moloddiu (ohliad naukovoi literatury). Ukraina. Zdorovia natsii. 2017; 3: 285–291. [in Ukrainian]

10. Shehedin AYu, Yashchenko AM, Lutsyk OD. Eksponuvannia hlikokonyuhativ u postnatalnomu morfohenezi yayechka shchura za danymy pasreaktsiyi ta lektynovoyi histokhimiyi. Svit medytsyny ta biologiyi. 2017; 2(60):156–60. [in Ukrainian]

Стаття надійшла 2.08.2022 р.