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FEATURES OF GLYCOME OF STRUCTURAL COMPONENTS OF THE LUNGS OF FEMALE RATS OFFSPRING UNDER THE CONDITIONS OF THYROID DYSFUNCTION

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The study of the mechanism of the occurrence of thyroid pathology in patients with a pulmonological profile is poorly covered in the domestic literature. With the use of the lectin-peroxidase technique, the role of the glycome of the structural components of the lungs of the offspring of rats under conditions of thyroid dysfunction in the maternal organism was studied in the experiment. At long-term stages of development of the offspring of rats of the experimental group, the redistribution of receptors of lectins of various carbohydrate specificity used by us in the structural components of the bronchial tree, the air-blood barrier, and alveolar macrophages was declared. This can cause changes in their functional properties, slow down gas exchange processes, change immune properties, and be a trigger for starting pathological processes in the lungs.

Key words: rats, lungs, experimental hyperthyroidism, glycopolymers, lectins

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ОСОБЛИВОСТІ ГЛІКОМУ СТРУКТУРНИХ КОМПОНЕНТІВ ЛЕГЕНЬ ПОТОМСТВА САМОК ЩУРІВ ЗА УМОВ ДИСФУНКЦІЇ ЩИТОПОДІБНОЇ ЗАЛОЗИ

Дослідження механізму виникнення тироїдної патології у пацієнтів пульмонологічного профілю, у вітчизняній літературі мало висвітлені. З використанням методу лектин-пероксидазної техніки досліджували роль глікому структурних компонентів легень потомства щурів за умов дисфункції щитоподібної залози материнського організму в експерименті. На віддалених етапах розвитку потомства щурів дослідної групи задекларували перерозподіл рецепторів використаних нами лектинів різної вуглеводної специфічності у структурних компонентах бронхіального дерева, повітряно-кров'яного бар'єру, альвеолярних макрофагів, що може викликати зміну їх функціональних властивостей; сповільнювати процеси газообміну, змінювати імунні властивості та бути тригером запуску патологічних процесів у легенях.

Ключові слова: щури, легені, експериментальний гіпертироз, глікополімери, лектини

The study is a fragment of the research project "Morphofunctional and immunohistochemical features of tissues and organs in normal and pathological conditions", state registration No. 0122U000168.

The analysis of literary sources proved that in older age groups with bronchopulmonary pathology, thyroid dysfunction is often found, which significantly complicates the diagnosis and is often the cause of inadequate and ineffective treatment, especially in the case of lesions of the respiratory system, including in the case of Covid-19 [8, 12]. The study of the mechanism of the occurrence of thyroid pathology in patients with a pulmonological profile is poorly covered in the domestic literature.

In Ukraine, the quantitative incidence of various thyroid nosologies has increased fivefold over the past 10 years. This indicator depends on several factors: ecological features of the external environment, iodine provision of the body, adequate nutrition, lifestyle, presence of stress factors, concomitant diseases and their nosological forms, etc. [5].

According to the Ministry of Health of Ukraine [11], as of January 25, 2022, there were 19,118 new confirmed cases of the COVID-19 coronavirus disease in Ukraine (2,809 of them children, 594 medical workers). Endocrine disorders in patients can contribute to the spread of the COVID-19 pandemic and complications of the disease. For the most part, attention is focused mainly on diabetes mellitus. At the same time, thyroid pathology, which ranks second in prevalence after diabetes mellitus, is observed in more than 200 million people worldwide [9].

It is known from scientific sources that a significant number of glycoproteins are biomarkers of many diseases, including pulmonary pathology. For the study of glycopolymers, the methods of lectin histochemistry studies are widely used [6, 7]. Lectins are used to study carbohydrates and their derivatives, including the architecture of the cell surface and intracellular compartments and their changes in pathological processes. In particular, during tumor transformation, for studying blood groups, purification of lymphocyte subpopulations, biosynthesis and function of glycoconjugates of the cell surface, and the study of specific carbohydrate binding sites on glycoproteins [6, 13, 14]. Lectins as reagents are also used

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for targeted delivery of drugs to a certain type of cells or tissues, studying the chromosomal constitution of cells and detecting chromosomal disorders [7].

Taking into account the important role of glycoconjugates of the surface of cells and intracellular compartments in the processes of glycosylation, we proposed a study of the glycome (carbohydrate determinants) of the structural components of the lungs of the offspring of hyperthyroid female rats at distant stages of postnatal development.

The purpose of the study was to establish the features of the glycome structural components of the lungs of rat's offspring under the conditions of dysfunction of the thyroid gland of the maternal organism in the experiment.

Materials and methods. The experimental study was performed on the offspring of 20 female (10 control, 10 experimental) rats of the Wistar line. For 20 days before mating, female rats of the study group received L-thyroxine (levothyroxine sodium) Berlin-Chemie at a dose of 150 μg/kg of body weight. After euthanasia, through an overdose of ether anesthesia, lungs were taken from the offspring of control (52) and experimental (48) animals on the 40th and 60th day of postnatal development. When working with animals, we were guided by the provisions of the national "General Ethical Principles of Animal Experiments" (Ukraine, 2001), consistent with the requirements of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986), Law of Ukraine No. 3447-IV dated 21.02.2006 "On the Protection of Animals from Cruelty Treatment" and Helsinki Declaration.

Histological material (thyroid and lungs) was fixed in 4 % neutral formalin, compacted and embedded in paraffin blocks, and sections 5–7 μm thick were made. The drugs were stained with hematoxylin and eosin using the standard method [10]. Control of thyroid function was carried out by studying its morphology after staining histological preparations with hematoxylin and eosin, as well as determining the level of hormones T_3, T_4 by radioimmunological method using standard kits "Total T3 RIA KIT" and "Total T4 RIA KIT" (manufactured by Immunotech, Czech Republic).

Glycoreceptors were detected by the lectin-peroxidase technique using 5 lectins of different carbohydrate specificity (Table 1).

Table 1

Lectins and their carbohydrate specificity

Name of lectin, its abbreviation	Specific monosaccharide	Complementary oligosaccharide residue
Galanthus nivalis agglutinin, GNA	αDMan	Man(α1-3)Man, oligomannose N-glycans
Arachis hypogaea, PNA*	DGal	DGal(β1-3)GalNAc
Triticum vulgare, WGA	DGlcNAc > NeuNAc	NeuNAc(α2-6)Gal(β1-4)GlcNAc, Man(β1-4)GlcNAc(β1-4)GlcNAc
Glycine hispada (Moenh), Maxin, SBA	αDGalNAc > βDGalNAc	GalNAc(α1-3)Gal(β1-3)GalNAc
Laburnum anagyroides, LABA	LFuc	Gal(β1-4)Fuc(β1-3)Glc

Note 1. * – Arachis hypogaea conjugate with peroxidase manufactured by Sigma.

A chromogen solution containing 0.05 % 3,3-diaminobenzidine tetrahydrochloride and 0.015 % H_2O_2 in a buffered solution was applied to the sections to visualise the lectin binding sites. The intensity of the lectin-receptor reaction was evaluated semiquantitatively in pluses: – no binding, + weak binding, + + moderate binding, + + + intense binding.

Lectins were made from raw materials from the Carpathian region in the Danylo Halytsky Lviv National Medical University laboratory. The concentration of lectins for incubation was first tested on histological preparations of control animals. We found that the optimal concentration is 50 μl of lectin per 1 ml of BS.

To control the specificity of histochemical reactions, lectin was applied to the sections in the presence of 0.5...1.0 mmol/L of the corresponding carbohydrate inhibitor, excluding one of the components (lectin, peroxidase, diaminobenzidine) from the drug treatment regimen or treated histological sections with lectins after their pre-incubation in 1 % NIO_4 solution for 30 min, during which oxidation of glycoconjugates occurs. The specificity of the reactions was confirmed by a negative staining result when the lectin was excluded from the drug treatment procedure or after oxidation of carbohydrate residues of glycoconjugates with HIO_4, according to Lutsyk AD (1989).

Microscopy and photographing of preparations were carried out using a microscope "Swift Instruments International", equipped with a digital camera "Echoo-Imager 502000" using the computer program "TopViev 3.2".

Results of the study and their discussion. On the 40th and 60th days of postnatal development, structural components characteristic of the lungs of adult rats were presented. Terminal bronchioles were dichotomously divided into alveolar bronchioles. Their lumens were visually wider than at previous stages of postnatal development. Folds were present in the small-caliber bronchi. The epithelium of the mucous membrane of the terminal bronchioles was simple cuboidal. Areas of connective tissue with cellular elements and fibrous structures with a predominance of elastic fibers were observed around the bronchial tree. In the lungs of rats of the experimental group for this period of development, thinning of the wall of the components of the pulmonary acinus, expansion of the lumen of the vessels of the microcirculatory channel, and an increase in the amount of connective tissue around the structures of the bronchial tree were noted.

Semi-quantitative results of lectin histochemistry studies of the lungs of the offspring of control and experimental females are presented in Table 2.

Table 2

The specificity of binding of lectins to the structural components of the lungs in normal conditions and under conditions of experimental hyperthyroidism

Lectin	Animal group	Structural components of the lungs		
		Bronchial tree		Alveolar wall
		Small bronchi	Terminal bronchioles	
WGA	C	Epithelium +++ Lamina propria	Apical surface of epithelial cells + + +	+ + +
	E	Epithelium +++ Lamina propria +	Apical surface of epithelial cells + + +	+ + +
LABA	C	Epithelium + + +	Apical surface of epithelial cells + + +	Epithelium + + Macrophages + + +
	E	Epithelium + + +	Apical surface of epithelial cells + +	Epithelium + + + Macrophages +
GNA	C	Epithelium +++ Lamina propria + +	Epithelium + +	Epithelium + +
	E	Epithelium + + + Lamina propria –	Epithelium + +	Epithelium + Type II pneumocytes + + +
PNA	C	Homogeneous binding	Homogeneous binding	Homogeneous binding
	E	Homogeneous binding	Homogeneous binding	Homogeneous binding
SBA	C	Epithelium + +	Epithelium + + +	Epithelium + + Type II pneumocytes + + +
	E	Apical surface of epithelial cells + +	Epithelium + +	Epithelium + +

Note: + + + intense binding; + + moderate binding; + weak binding; – no binding; C – control; E – experiment.

Microscopically, the specificity of the binding of the used lectins to the epithelium of bronchial tubes of various calibers, the basal membrane of alveoli, pneumocytes, and the endothelium of the vessel wall (components of the aerogematic barrier) is shown. Thus, exposure of fucozoglycans identified by LABA lectin was observed on the apical surface of Clara cells (Fig. 1 A). Instead, αDGalNAc-specific SBA lectin receptors were characteristic of type II pneumocytes (secretory alveolar cells) (Fig. 2B). Along with other lectins, the exposure of αDMan receptors of the specific GNA lectin was ascertained in the components of the bronchial tree, with the most intense exposure in the composition of the epithelium of the mucous membrane on the apical surface, especially in ciliated epithelial cells and to a lesser extent in the lamina propria of the mucous membrane (Fig. 2A). Exposure of WGA lectin receptors specific to DGlcNAc>NeuNAc was ascertained both on the apical surface of epithelial cells of the bronchial tree and in the cytoplasm and on the surface of type I respiratory pneumocytes in the wall of the alveoli. It should be noted that sialoglycans were present in alveolar macrophages both on the surface and in their cytoplasm. At the same time, exposure of both LFuc-specific LABA lectin receptors (Fig. 1 B) and DGlcNAc > NeuNAc-specific WGA lectin receptors was observed in alveolar macrophages. Since alveolar macrophages showed greater affinity for the fucose-specific LABA lectin, it can be considered as their marker. As for the galactosyl components detected by the PNA lectin, their homogeneous binding was observed both in the structural components of the airways and in the elements of the respiratory department of the lungs.

The comparative lectin-histochemical studies of the lungs of the offspring of the experimental group showed a slightly different exposure of the receptors of the lectins we used. Thus, on the 40th and 60th day of postnatal development, the cytotopography of the receptors DGlcNAc > NeuNAc of the WGA-specific lectin and LFuc of the LABA-specific lectin (Fig. 1B) did not change, compared to the control group. Still, the macrophages themselves, with these receptors on their surface, were identified to a lesser extent (Fig. 1D). Receptors of the fucose-specific LABA lectin were also exposed in the endothelium of vessels of the microcirculatory bed (Fig. 1B). Redistribution of αDMan-specific GNA lectin receptors was also characteristic. The absence of mannose glycans was ascertained in the composition of the lamina propria of the mucous membrane of the bronchi, as well as a decrease in the intensity of their exposure in the composition of the walls of the alveoli (Fig. 2B).

In the structural components of the lungs of the experimental group, the affinity to the αDGalNAc-specific SBA lectin was also slightly lower in elements of both the bronchial tree and alveoli (Fig. 2D).

The performed studies showed that the use of lectins of different carbohydrate specificity allows for obtaining valuable information about the redistribution of their receptors in postnatal development, which directly impacts various manifestations of functional activity both under normal conditions and during pathology.

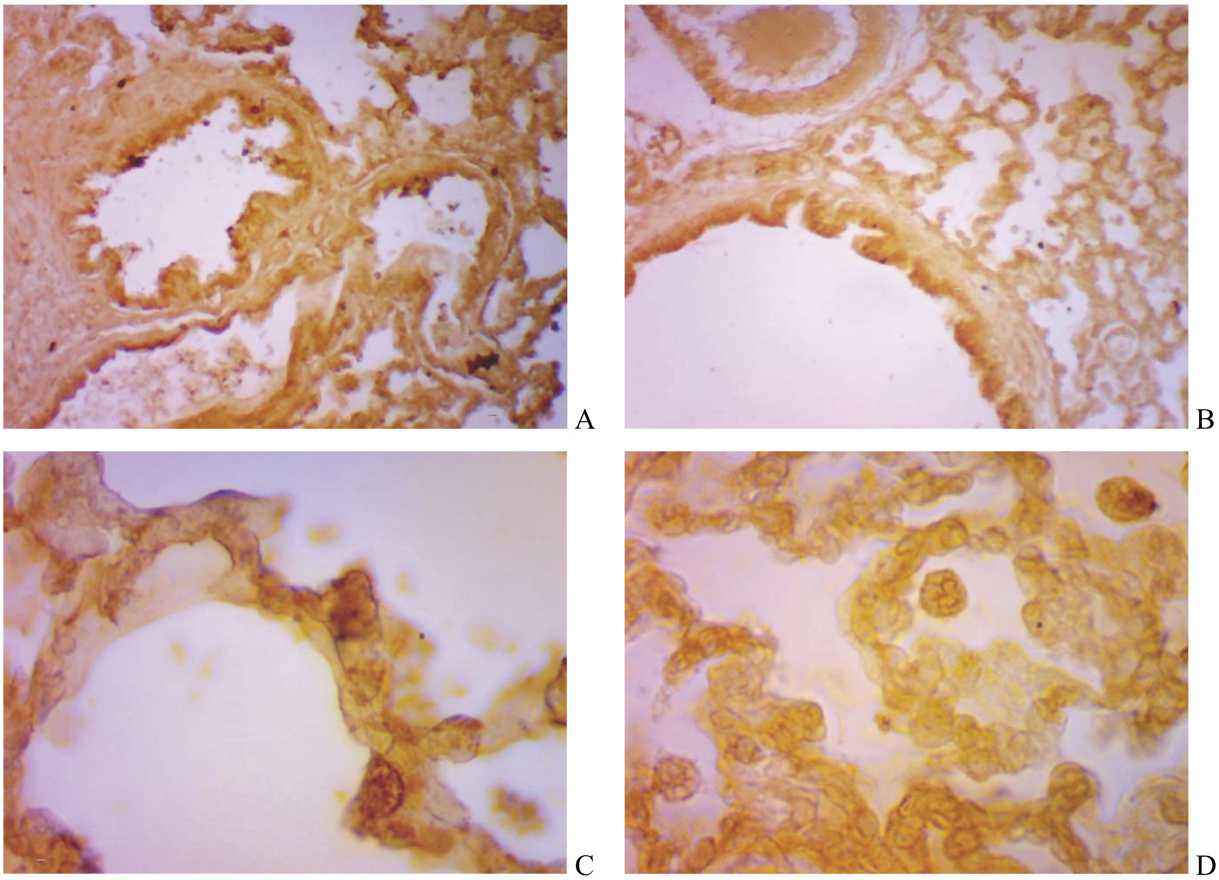

Fig.1. Exposure of LABA lectin receptors in the structural components of the offspring of females on the 40th day of postnatal development. A – intensive exposure of LABA lectin receptors in the bronchial epithelium; magn. x150. B – intensive exposure of fucose-specific receptors in the epithelium of the bronchi, in the wall of individual alveoli and endothelium of vessels; magn. x150. C – intensive exposure of LABA lectin receptors in the cell membrane of alveolar macrophages and moderate in alveolar epithelial cells; magn. x600. D – moderate exposure of LABA lectin receptors in alveolar cells and a decrease in the intensity of the reaction in macrophages; magn x600. A, B – control. C, D – experiment.

According to [2, 6], the dynamics of changes in biologically important macromolecules (including glycoconjugates, which are lectin receptors) in the composition of cytological membranes, intracellular compartments, and non-cellular structures is most pronounced in the early stages of ontogenesis. The lumens of the alveoli look somewhat narrower on the 10th-20th day of postnatal ontogenesis, as shown in our previous studies [4].

Some authors [9] noted a significant increase in sialic acids in epithelial cells in autoimmune thyroiditis, especially noticeable on the lumenal surface of follicular cells, which probably changed the functional activity of the latter. Such changes have a corresponding effect on the synthesis of thyroid hormones and their effect on other organs, which is confirmed by the results of our research. Scientific works [3] show that the effect of thyroid hormones on the respiratory system is multifaceted: it consists of directly ensuring the act of breathing by influencing the respiratory muscles and increasing the synthesis of surfactant, which contains lipophosphoglycoprotein complexes. Studying the lungs under the conditions of their obstructive disease [1] showed a change in the exposure of lectin receptors in individual structural components. Our previous studies of the lungs of rats with experimental hypothyroidism also showed a change, like glycoprotein complexes on the surface of the epithelium of the bronchial tree and the air-blood barrier [15].

Data from preclinical and clinical studies suggest that the thyroid may be a target organ for SARS-CoV-2 [11]. In the context of the COVID-19 pandemic, patients who did not control the level of thyroid hormones in their blood showed a severe course of the disease and an increased risk of post-COVID complications.

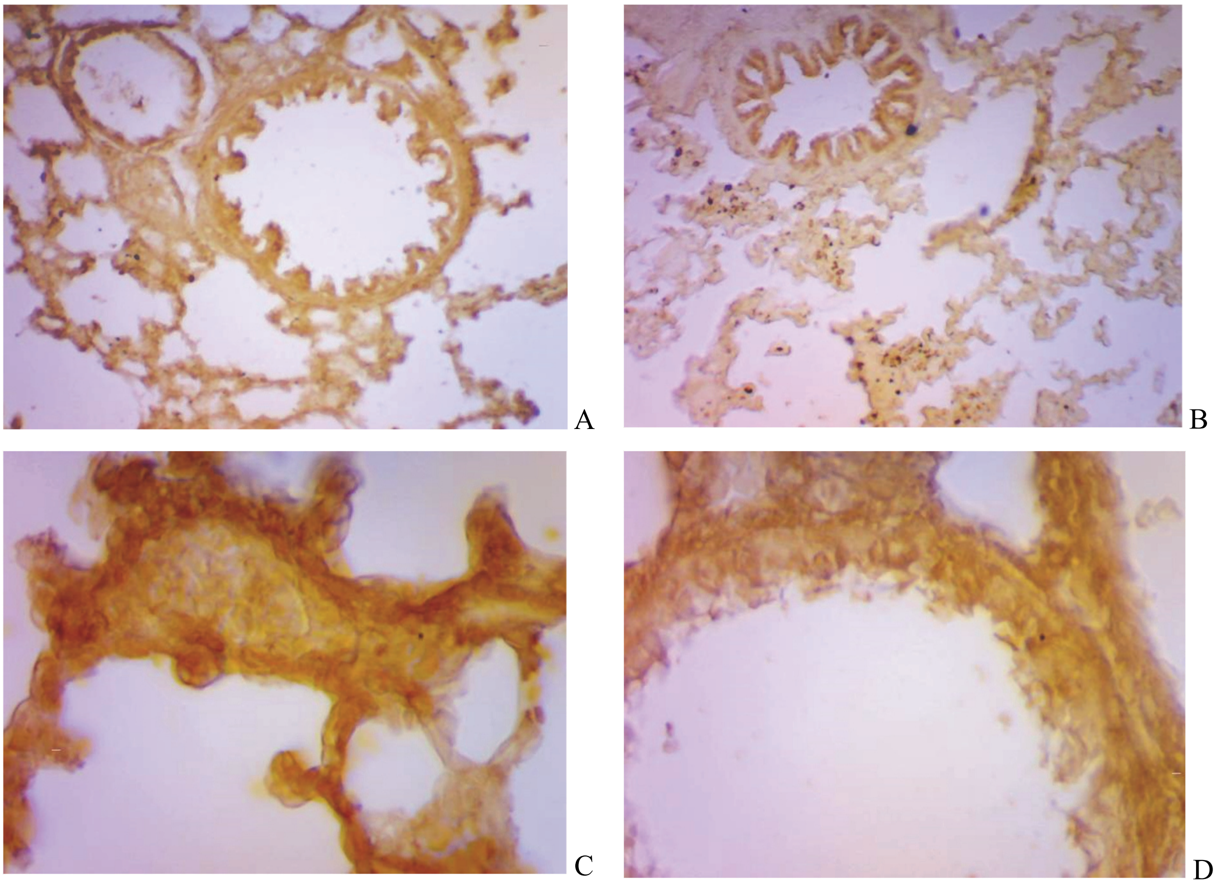

Fig. 2. Exposure of GNA and SBA lectin receptors in the structural components of the offspring of females on the 40th day of postnatal development. A – exposure of GNA lectin receptors in the epithelium of small bronchi, in the wall of alveoli and the endothelium of vessels; magn. x150. B – reduction of exposure in the lamina propria of the bronchi and the alveoli's wall; magn. x150. C – intensive exposure of SBA lectin receptors in type II pneumocytes; magn. x600. D – a moderate reaction with SBA lectin in the epithelium of the bronchi and alveoli; magn. x600. A, B – control. C, D – experiment.

In the course of the study, we have shown the modification of carbohydrate determinants, both in the airways and in the structural components of the aerogematic barrier, in the composition of which receptors for the lectins WGA, LABA, GNA, and SBA were identified with varying intensity. The reaction of alveolar macrophages, which showed a more significant affinity to the fucose-specific LABA lectin than other lectins, is noteworthy, which makes it possible to consider it a marker of the latter. It should be noted that previous studies of the lungs of sexually mature rats with experimental hypothyroidism induced by mercazolyl administration showed that αDGalNAc receptors – a specific SBA lectin – predominated in alveolar macrophages [15]. Presumably, the thyroid gland's hormonal activity affects the glycome's structure in different ways.

Taking into account the identified changes in the cytotopography of lectin receptors of the air-blood barrier and structural components of the bronchial tree, which obviously affect the functional activity of the above-mentioned lung structures, can be informative in diagnosing and treating pathological processes.

Conclusion

In experimental hyperthyroidism of the mother's organism induced by the L-thyroxine administration, a modification of the glycome is observed in the lungs of the offspring on the 40th and 60th day of postnatal development. It is an essential functional component of the glycocalyx and intracellular compartments. It can change the functional features of the structural elements of the bronchial tree and the air-blood barrier, modify immune reactions, influence gas exchange processes, and trigger pathological lung processes.

Prospects for further research. It is planned to study the structural components of the lungs of the offspring of female rats using a wider panel of lectins of different carbohydrate specificity.

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