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LECTINOSPECIFIC CHARACTERISTICS OF CELL COMPONENTS IN THE ERYTHROBLASTIC ISLET IN RATS WHEN ADMINISTRATING CRYOPRESERVED PLACENTA DURING THE EXPERIMANTAL INFLAMMATION

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Summary. Having studied the lectinochemical reaction on cell surfaces of proerythroblasts, enhanced binding to lectin of edible snail, golden shower rind, and wheat germ was observed. A moderate binding reaction was observed on cell surfaces of proerythroblasts with peanut lectin, soybean lectin, European elder lectin and mistletoe lectin, basophilic erythroblasts gave enhanced binding only to the lectin of edible snail. It is found that there is a decrease in the frequency of binding to lectins of edible snail and mistletoe in polychromatophilic and orthochromatic erythroblasts. In the orthochromatic erythroblasts a weak reaction was observed with the lectins of edible snail, peanut, mistletoe and wheat germ, and a completely absent reaction was observed when linking cell surfaces in orthochromatic erythroblasts with lectins of soybean seeds and European elder.

Аннотация. Исследовав лектинохимичну реакцию на клеточных поверхностях проэритробластов, наблюдали усиленное связывание с лектином виноградной улитки, коры золотого дождя и лектином зародышей пшеницы. Умеренную реакцию связывания наблюдали на клеточных поверхностях проэритробластов с лектином арахиса, семенами сои, бузины черной и омелы белой, базофильные эритробласты давали усиленную реакцию связывания только с лектином виноградной улитки. Установлено, что в полихроматофильных и ортохромных эритробластах, наблюдается уменьшение частоты связывания с лектинами виноградной улитки и омелы белой. В ортохромных эритробластах обнаружена слабая реакция с лектинами виноградной улитки, арахиса, омелы белой и зародышей пшеницы, а полностью отсутствуе реакции наблюдалась при связывании клеточных поверхностей в ортохромных эритробластах с лектинами семян сои и бузины черной.

Key words: cryopreserved placenta, experimental inflammation, erythroblastic islet cells, carbohydrate residues.

Ключевые слова: криоконсервированная плацента, экспериментальное воспаление, клетки эритробластного островка, углеводные остатки.

Introduction. Prophylaxis of the blood system diseases allows to prevent hematological sicknesses, to detect them in the early stages, and to increase the life expectancy of the population. The complex of organs of the immune system perform hematopoietic function, provide immunity, which is a set of various reactions of the organism, which are aimed at preserving its genetic homeostasis [9].

A promising trend at the current stage of development of morphological studies is the use of monoclonal antibodies and lectins, but if using immunohistochemical methods revealed both polypeptide and carbohydrate chains of biopolymers, then using the lectin-chemical methods, it is possible to verify carbohydrate epitopes of biological macromolecules [2, 3, 4].

The phenomenon of reverse interaction of lectins with carbohydrates defines several types of biological reactions – it is the transport and accumulation of carbohydrates, which provide specificity of intermolecular interactions (processes of recognition of macromolecules and cells), intercellular interactions [1-4]. The introduction of biologically active substances into the body in the form of cryopreserved placenta preparations was studied on the structural components of the small intestine, which improves the expression of the protective function of cells (enterocytes) due to the intensity of synthesis of membrane glycoproteins with increased content of sialoglycan [7, 8].

The purpose of the study was to determine the receptors-glycopolymers of plant origin in the structural components of erythroblastic islet of the red bone marrow in rats with the introduction of cryopreserved placenta during experimental inflammation.

Material and methods of research. The study was performed on 45 mongrel white rats, which were administered with one-time subcutaneous transplantation of cryopreserved placenta during experimentally modeled acute aseptic inflammation caused by intraperitoneal injection of 5 mg of λ-carrageenan ("Sigma", USA) in 1 ml of isotonic sodium chloride solution per 1 animal.

The animals were withdrawn from the experiment by an overdose of ketamine anesthesia (administered

intraperitoneally at a rate of 75 mg / kg body of the animal weight) according to the prescribed terms on the 1st, 2nd, 3rd, 5th, 7th, 10th, 14th, 21st and 30th day. The study of red bone marrow was carried out in accordance with the established terms of the experiment.

A set of biological material for conducting the research was done under conditions of a small operating vivarium of the Ukrainian Medical Stomatological Academy in accordance with the "Rules for the Use of Laboratory Experimental Animals" (2006, Annex 4) and the Helsinki Declaration on the Humane Approach to Animals. For the study, preparations of red bone marrow in rats were used.

After gathering, the material was fixed in a 10% solution of neutral formalin with subsequent decalcification in a solution of ethylenediaminetetraacetic acid, with keeping to ph 7.4. For gaining observation preparations, sections with thickness up to 5 micrometer were stained with hematoxylin and eosin. Subsequently, the preparations were treated using standard sets of NPK "Lektinoest" (Lviv) in the development of lectins 1:50 by the method [5, 6].

Histopreparations were stained from light to dark brown and by two independent researchers they were exposed to protocol scores: 0 points - no reaction, 1 point - weak reaction, 2 points - moderate reaction, 3 points - strong reaction, 4 points - sharp reaction.

Control of the lectin binding reaction was performed by excluding diaminobenzidine from the scheme of preparation processing [1, 5, 6].

Results of the research and their discussion. As a result of the morphological and morphometric study it was found that the effect of placental tissue stimulates the reparative processes induced by the introduction of λ-carrageenan and thus confirming its anti-inflammatory effect. An increase in the number of pro-erythroblasts, basophilic, polychromatophilic and orthochromatic erythroblasts was detected at the early stages of observation, quantitative changes were of a phase nature. Histological examination revealed that the elements of the hemomicrocirculatory bed of the red bone marrow responded uniformly in the direction of the increase of the average diameters of the lumen compared with the intact group of animals.

It is proved that the restoration of the morphofunctional state of the structural components of the red bone marrow occurs in the late stages of the experiment, and the restoration of indicators to such as in the intact group of animals is observed from the 21st to the 30th day of the experiment, due to biologically active substances containing placental tissue.

Formulation of lectinochemical reactions on the histological preparations of the rat bone marrow, with the introduction of cryopreserved placenta during experimental inflammation, a strong reaction of binding on cell surfaces of macrophages with HPA, LABA, PNA, and WGA lectins, a moderate reaction with Con A and VA lectins were observed. A weak reaction was defined with SBA and SNA lectins (Table 1).

Table 1

Characteristics of the lectins specificity

№	Lectin	Abbreviated name	Source of production	Carbohydrate specificity
1		Con A		
2	Lectin of edible snail	HPA	Helix pomatia	αGalNAc
3	Lectin of golden shower rind	LABA	Laburnum anagyroides bark agglutinin	Fuc (Fucα1-2Gaβ1-4Glc)
4	Lectin of peanut	PNA	Arachis hypogaea	Т-антиген (Galβ1-3GalNAc-)
5	Soybean lectin	SBA	Glicine max	αGalNAc
6	European elder lectin	SNA	Sambucus nigra	NeuNAc(α2-6)DGal /DGalNAc
7	Mistletoe lectin	VAA	Viscum album	β-D-Gal
8	Lectin of wheat germ	WGA	Triticum vulgare	NAcDGlc, NANA

Note. Man - mannose; Glc - glycose; GlcNAc - N-acetyl- glycose substitute; Gal - galactose; GalNAc - N-acetyl-galactose substitute; Fuc - fucose; NeuNAc - N-acetylneuraminic (sialic) acid.

On cell surfaces of the proerythroblasts, a strong reaction was observed when binding to Con A, HPA, LABA, and WGA lectins; a moderate reaction was observed with PNA, SBA, SNA, and VAA lectins.

Table 2

Lectinochemical characteristics of the structural components in the erythroblastic islet when administrating crypreserved placenta during the experimental inflammation

Lectins	Macrophages	Erythroblastic islet			
		Proerythroblasts	Basophilic erythroblasts	Polychromatophilic erythroblasts	Orthochromaticerythroblasts
Con A	2	3	2	2	1
HPA	3	3	3	1	1
LABA	3	3	2	1	2
PNA	3	2	2	2	1
SBA	1	2	1	1	0
SNA	1	2	2	1	0
VAA	2	2	1	2	1
WGA	3	3	2	2	1

Discussion. During the study of lectinochemical reactions with carbohydrate residues of the basophilic erythroblasts in erythroblastic islet, a strong reaction was detected only with HPA lectin, a positive reaction was observed with Con A, LABA, PNA, SNA and WGA lectins, a weak reaction was noted with SBA and VAA lectins.

A study of the intensity of lectinochemical reactions with carbohydrate residues of polychromatophilic erythroblasts revealed a moderately positive reaction on cells, with Con A, PNA, VAA and WGA lectins, and a weak binding reaction was visualized with HPA, LABA, SBA, SNA lectins.

As a result of the study of histological preparations, a moderately positive reaction was observed in orthochromatic erythroblasts only with LABA lectin on the cell surfaces. A weak binding reaction was observed with Con A, HPA, PNA, VAA, and WGA lectins, no reaction was detected on carbohydrate residues with SBA, SNA lectins.

Conclusions

Increased expression of lectin receptors of edible snail, golden shower rind, and wheat germ on cell surfaces of macrophages and proerythroblasts was detected. It is found that there is a decrease in the frequency of binding to lectins of edible snail and mistletoe in polychromatophilic and orthochromatic erythroblasts. In orthochromatic erythroblasts a weak reaction was observed with the lectins of edible snail, peanut, mistletoe and wheat germ, and no reaction was observed, when binding carbohydrate residues in orthochromatic erythroblasts to lectins of soybean seeds and European elder.

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