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LECTIN-HISTOCHEMICAL REGULARITIES OF DIFFERENTIATION OF THE HUMAN PAROTID GLAND EPITHELIAL GERMS

ЛЕКТИНОГІСТОХІМІЧНІ ЗАКОНОМІРНОСТІ ДИФЕРЕНЦІЮВАННЯ ЕПІТЕЛІАЛЬНИХ ЗАЧАТКІВ ПРИВУШНОЇ ЗАЛОЗИ ЛЮДИНИ

Резюме. Глікополімерні сполуки складають структурну і функціональну основу клітин і тканин живого організму. Нами обґрунтовано потребу анатомо-лектиногістохімічного дослідження привушної залози у ранньому пренатальному періоді онтогенезу, оскільки відомості про становлення топографії фрагментарні та несистематизовані, а окремі аспекти їхнього морфогенезу дискусійні. Досліджено 35 зародків і передплодів людини віком від 21 доби до 12 тижнів внутрішньоутробного розвитку. Глікополімери виявляли шляхом обробки серійних зрізів лектинами, кон'югованими з пероксидазою хрону. У динаміці пренатального морфогенезу зародків та передплодів 4-го – 12-го тижнів ембріогенезу експресія глікополімерів – рецепторів лектинів на поверхні клітин, у цитоплазмі і на базальній мембрані епітеліальних зачатків привушної залози та ротової порожнини людини з її похідними за перерозподілом глікополімерів – схожі, що може слугувати лектиногістохімічним підтвердженням ектодермального джерела походження епітеліального зачатка привушної залози. Занурення клітин епітелію ділянок щічно-альвеолярних кишень у нижче прилеглу мезенхіму з формуванням у зародків 11,0-12,5 мм ТКД первинних зачатків привушної залози та перетворення їх в епітеліальні тяжі пов'язано з накопиченням сіалованих глікополімерів (N-ацетилнейрамінової кислоти), N-ацетил-D-глюкозаміну – специфічних до лектину зав'язі пшениці (WGA) і лектину бузини чорної (SNA); N-ацетил-2-дезокси-2-аміно-D-глюкопіранози, екранованої сіаловою кислотою ß-D-галактози та α-L-фукози – специфічних відповідно до лектинів виноградного слимака (HPA), кліщовини (RCA) та кори золотого дощу (LABA). Ці глікополімери присутні впродовж перших 12-ти тижнів як на цитолемі клітин епітеліальної закладки привушної залози, так і в їх цитоплазмі. Результати лектиногістохімічного дослідження раннього пренатального онтогенезу привушної залози можуть послужити основою у роботі лабораторій скринінгу морфологічного матеріалу для оцінки ступеня зрілості та прогнозування життєздатності плода і діагностики відхилень від нормального розвитку.

Ключові слова: лектини; привушна залоза; пренатальний онтогенез.

Lectins (Lc) are modern histochemical markers of cellular glucoconjugates and extracellular tissue structures [1]. Lectins are selectively bounded with final non-reducing mono- or oligosaccharide residues of glycopolymers (GPM). GPM compounds make up structural and functional basis of cells and tissues of a living organism [2]. Existence of identification and junction of such glycopolymers by endogenic Lc in the body, called lectin-receptor interactions, can trigger lectin-dependent regulations of cellular functions and cellular response in ontogenesis which stipulate differentiation of tissues and their structural components [3]. The necessity of anatomical-lectinohistochemical examination of the parotid gland (PG) in early prenatal period of ontogenesis is substantiated, as the evidences concerning its topography are fragmentary and unsystematized, and certain aspects of its ontogenesis are disputable [4-7].

The objective of the research: to study expression of Lc GPM-receptors on the surface of cells, cytoplasm and on the basal membrane of the human PG epithelial germs and oral cavity with its derivatives.

Objects and methods of the study. 35 human embryos and pre-fetuses aged from 21 days to 12 weeks of the intrauterine development, 2,5-70,0 mm of the parietal-coccygeal length (PCL) [according to G.A. Schmidt's periodization], were examined at the stages from the early period of the mature nerve groove and immature somites to the beginning of the fetal period (corresponding to X-XII levels of development and 9-23 stages approved at Carnegie Institute (Carnegie stages). Glycopolymers were found by means of treatment of serial sections with lectins (Table) conjugated with horseradish peroxidase

(HRP). The agents were treated with the sets produced by "Lectinotest" (Lviv). Visualization of points of lectin binding was performed in "diaminobenzidine- H_2O_2" system.

Table

Characteristics of lectin carbohydrate specificity used in the study of the early prenatal ontogenesis of the human parotid gland

Lectin name	Carbohydrate specificity
Wheat germ agglutinin (WGA)	N- acetylneuraminic acid (sialic) and less N-acetyl-D-glucosamine
Sambucus nigra agglutinin (SNA)	N- acetylneuraminic acid (sialic) and less ß-D-galactose
Helix pomatia (edible snail) agglutinin (HPA)	N-acetyl-2-desoxy-2-amino-D-glucopyranose
Rhizoctonia solani agglutinin (RCA)	ß-D-galactose screened by sialic acid
Solanum tuberosum (potato) agglutinin (STA)	N-acetyl-chitotriozamine
Laburnum anagyroides (Golden Rain shrub) agglutinin (LABA)	α-L-fructose
Peanut agglutinin (PNA)	ß-D-galactose
Lens culinaris agglutinin (LCA)	α-D-mannose

Intensity of the reaction developing from light to dark brown colour of sections by different lactins was assessed (points) by two researchers independently. The points 0,1,2,3,4 were respectively interpreted: absent reaction, mild positive, moderate positive, severe and very severe reaction. Specificity of reaction was controlled by means of diaminobenzidine inclusion from the scheme of agent treatment (carbohydrate specificity of lectin, *see the Table*).

The study is a fragment of the planned complex inter-department research issue of the Department of Anatomy, Topographic Anatomy and Operative Surgery (Chief – Professor O.M.Slobodian) "Peculiarities of Morphogenesis and Topography of the Organs and Systems in the Prenatal and Postnatal Periods of Ontogenesis ". State registration № 0115U002769.

Results and discussion. Distribution of GPM that are lectin receptors (LcR) during ontogenesis of the parotid gland is not investigated completely, irrespective of the fact that the rate of its accumulation and the character of distribution can be a criterion of normal or pathologic their development, and can help to solve the issue concerning confirmation of histogenetic source of its origin. Since the main form-building processes of human prenatal ontogenesis with isolation and differentiation of the epithelial and mesenchymal components occur during the first three months of the intrauterine development, we have chosen the age period from the 4^{th} to 12^{th} weeks of embryogenesis (3,2-70,0 mm of PCL) as the basis for the temporal duration (depth) of the study during examination of lectin-histochemical regularities in differentiation of the parotid gland and oral cavity with its derivatives.

Early histogenesis of PG, ralcavity with its derivatives are found to be accompanied by GPM synthesis with final non-reduced residues of N-acetyl-D-glucosamine, and to a lesser extent – N-acetylneuraminic acid, which appear to be Lc wheat germs (WGA). The epithelium lining theoral cavity, buccal-alveolar pockets, tongue, and forming PG germon the stages of its development or isolation from the previous germs, contains a number of GPM with final non-reduced residues of N-acetyl-D-glucosamine, and toal esserextent – N-acetylneuraminic acid. Development and growth of these organs result in a complete reduction of LcR of wheat germs on the basal membrane (BM) of the epithelium. To the extent of growth and branching of the epitheliumtaenia into smaller ducts of PG these substances are deposited on the epithelial apical surface (AS) of the major efferent ducts, and the epithelium of newly formed small ducts does not contain them.

On early stages of human embryogenesis the cells of the PG epithelial germ synthesize a considerable amount of GPM with final non-reduced residues of N-acetylneuraminic acid. Cell migration in the process of dichotomic branching of the epithelialtaenia of PG duct germs is connected with accumulation of sialic GPM on BM and AS, as well as in the cytoplasm of epithelial cells.

In the major excretory ducts differentiating epithelial cells retain these compounds only on the apical surface. At the end of principal branching (dichotomic divisions of PG germ) – to the 12^{th} week of embryogenesis, LR of wallwart (Sambucusnigra) undergo reduction and are contained only in the cellular cytoplasm. The dynamics of expression and reduction of sialic-containing glucoconjugates which appear to be Lc of wallwart (Sambucusnigra), in the epithelial germs of the PG and oral acidy with its derivatives is

similar and consists of biosynthesis and accumulation of a noticeable amount of these biopolymers on the earliest stages of the intrauterine development on the AC of the epithelial layer and in the cytoplasm inclusions. During the second and at the beginning of the third month of embryogenesis the concentration of these compounds remains on a high level in the same areas of localization. At the end of the third month of the intrauterine development the cytoplasm of epitheliocytes gets free from LR at the expense of their reduced amount on AS. BM of the PG epithelial germ and the oral epithelium with its derivatives during the whole period of the study on the action of Lc of wallwart (Sambucusnigra) remainSNA-negative.

In the epithelial germs of the oral cavity with its derivatives lectin receptors of Helix pomatia (edible snail) agglutinin (HPA) interacting with biopolymer molecules and final non-reducing residues of N-acetyl-D-galactosamine are first determined on the apical surface of the epithelial cells in the germs aged 39 days (11,0 mm of PCL). Beginning of biosynthesis on the apical surface of the parotid gland epithelial germ cells of HPA-binding compounds, which practically immediately manifest strong intensive reaction with Helix pomatia agglutinin, is determined by the beginning of formation of the parotid gland epithelial germ due to immersion of the epithelial cells of the buccal-alveolar pockets of the primary oral cavity into the adjacent mesenchyme (embryos of 39-40 days; 11,0-12,5 mm of PCL). During further examination of the embryogenesis (embryos and pre-fetuses of 12,0-45,0 mm of PCL) accumulation of these glycoconjugates increases. Progressive decrease of staining intensity (similar to decrease of HPA-positive compounds) was found in the following 11th and 12th weeks of the intrauterine development which is the result of N-acetyl-D-galactosamine conjugates reduction. At the end of the 12th week Helix pomatia agglutinins are found only on the apical surface of the epithelial cells of the oral cavity mandibular processes, and they are very mildly expressed on the apical surface and in the basal membrane of the epithelial germs of the parotid gland excretion ducts.

Examination by the series histological sections of GPM cytotopography with final non-reducing residues of N-acetylneuraminic acid that screens ß-D-galactose and is bound with RCA, determined the occurrence of these macromolecules in the series histological sections containing the parotid gland epithelial germs (embryos of 39 days; 11,0 mm of PCL). And RCA-positive macromolecules are mostly localized (moderate degree of deposition) on the apical surface and less (weekly positive degree of deposition) – on the basal membrane and in the cytoplasm of cells of the parotid gland epithelial germ. It is significant that epithelial germs of the oral cavity in embryos and pre-fetuses of 24-43 days (3,2-14,0 mm of PCL) are rich in receptors to RCA. Glycopolymers with terminal residues of ß-D-galactose, screened by sialic acid, are concentrated on the apical and basal surfaces of the epithelial layer cells lining the maxillary processes of the oral cavity. Considerably less RCA-positive compounds are located in the cytoplasm of epithelial cells, although certain inclusions with GPM are found in the cells. During the second month of the intrauterine development all the mentioned epithelial germs become rich in RCA-positive glycoconjugates mostly concentrated on the apical and basal surfaces of the epithelial cells, and less – in the cytoplasm of the cells. The third month of the prenatal development in pre-fetuses with 30,0-70,0 mm of PCL is characterized by reduction of RCA receptors in the cellular cytoplasm and on the basal membrane, while there are a number of them on the apical surface of the epithelial cell germs.

In the epithelial germs of the oral cavity with its derivatives STA-positive material is registered in embryos in the term of 43 days (14,0 mm of PCL). The place of its greatest localization is the apical surface of the epithelial layer cells, and less – on the basal membrane and intra-cytoplasmatic inclusions. The content of these biopolymers during the second and third months of the prenatal ontogenesis changes inconsiderably. On the 12th week of the intrauterine development the difference between organs is determined in the concentration and histotopography of STA between the proximal and distal portions of the parotid gland excretory ducts.

Summing up the determined results of histotopography and dynamics of distribution of hydrogen containing molecules – glycoconjugates with final non-reducing residues of α-L-fructose in the series histological sections of the parotid gland epithelial germ and the oral cavity with its derivatives stained with LABA, it should be noted that at different stages of the prenatal development (embryos in the term of 24-37 days; 3,2-9,0 mm of PCL) LABA receptors are present in considerable amount in the epithelial germ of the primary oral cavity lining. A clear concentration of LABA-positive glycopolymers are found on the basal surface of the epithelium, apical surface of the cells and less – in the intracellular inclusions and cytoplasm of the epithelial layer. At the early stages of development (embryos in the term of 24-38 days; 3,2-10,0 mm of PCL) the process of the parotid gland germ has not started yet. Therefore, only between the

5th and 6th weeks of embryogenesis (beginning of the second month of the intrauterine development; embryos with 11,0-12,5 mm of PCL) intensification of production and accumulation to valuable measures of LABA-positive material occurs on the apical surface, basal membrane and cellular cytoplasm of the parotid gland epithelial germs that correlates with the time of beginning of its forming processes. During the second month of the embryonic development (embryos and pre-fetuses with 10,0-30,0 mm of PCL) a tendency to enrichment of the parotid gland epithelial germs and oral cavity with its derivatives α-L-glucose conjugates is found, especially on the basal membrane of the oral cavity epithelial layer. On the third month of the intrauterine development (10th-12th weeks; pre-fetuses with 32,0-70,0 mm of PCL) LABA-binding sites in the basal membrane and the cytoplasm of cells in the oral cavity epithelial layer with its derivatives are gradually decreasing. On the apical surfaces of all the examined germs and in the basal membrane and cytoplasm of the parotid gland epithelial germ the concentration of places of lectin conjugation remains practically unchanged.

Glycopolylmers with final non-reducing residues of β-D-galactose conjugated with PNA were first determined in the cells of the oral cavity epithelial germs with its derivatives in pre-fetuses in the term of 45 days (16,0 mm of PCL). The first weak conjugation of PNA with appropriate glycoconjugates for it of the parotid gland epithelial germ marking is found in the embryo of 38-40 days (12,0 mm of PCL) that leaves behind the development of the oral cavity epithelial germ by the time of the embryonic development. PNA-positive compounds are found in very small amounts in the basal membrane and the cytoplasm of epithelial germ cells. β-D-galactoconjugates in the epithelial germs of the parotid gland and oral cavity with its derivatives are concentrated on the apical surface and internal cytoplasmatic inclusions. With increase of the prenatal age of the embryos and pre-fetuses (from the 6th to 12th days of the intrauterine development; 12,0-70,0 mm of PCL) the concentration of β-D-galactoconjugates in the cells of the epithelial germs increases. The minimal PNA concentration first found in embryos and pre-fetuses (12,0 and 16,0 mm of PCL) on the basal membrane of the parotid gland epithelial germ and oral cavity with its derivatives remained unchanged in the course of the prenatal development, and it gradually disappears when pre-fetuses become 11-12 weeks of the intrauterine development (56,0-70,0 mm of PCL).

In the epithelial germs of the parotid glands GPM with final non-reducing residues of α-D-mannose interacting with LCA and first appearing on the apical surface of the epithelial cells in embryos in the term of 39-40 days (11,0-12,5 mm of PCL) till the end of the second month of embryogenesis remain practically on the same level. A relative constant amount and histotopography of LCA receptors found on the apical surface of the oral cavity epithelial germs in the embryos in the term of 24-37 days (3,2-9,0 mm of PCL) till the end of the second month of embryogenesis (pre-fetuses with 23,0-27,0 mm of PCL) remain on the same level as well. At the same time, in the epithelial germs of the salivary glands and oral cavity with its derivatives the end of the second month of the prenatal development is associated with appearance of a small amount of LCA-positive macromolecules on the basal membrane, and for the germ of the parotid gland – in the cytoplasm of cells. At the same time, the cellular cytoplasm of the oral cavity epithelial layer remains LCA-areactive. During the third month of the prenatal ontogenesis (pre-fetuses with 30,0-70,0 mm of PCL) α-D-mannose conjugates that increased at the beginning of the month, remain stable till the end of the examined period of the intrauterine life.

Conclusions. The dynamics of prenatal morphogenesis of the embryos and pre-fetuses of the 4th-12th week so fembryogenesis expression of glycopolymers – lectinreceptors on the surface of cells, in the cytoplasm and on the basal membrane of the parotid gland epithelial germs and the human oral cavity with its derivatives by re-distribution of glycopolymers are similar, which can be the evidence of ectodermal source of the parotid gland epithelial germ.

Immersion of the epithelial cells of the buccal-alveolar pockets into the lower adjacent mesenchyme with the formation of primary parotid gland germs in embryos with 11,0-12,5 mm of PCL and their transformation into the epithelial taeniae is associated with accumulation of sialic glycopolymers (N-acetyl neuraminic acid), N-acetyl-D-glucosamine – specific to Wheat germ agglutinin (WGA) and Sambucus nigra agglutinin (SNA); N-acetyl-2-desoxy-2-amino-D-glucopyranose, screened by sialic acid of ß-D-galactose and α-L-fucose – specific to Helix pomatia (edible snail) agglutinin (HPA), Rhizoctonia solani agglutinin (RCA) and Laburnum anagyroides (Golden Rain shrub) agglutinin (LABA) respectively. These glycopolymers are present during the first 12 weeks both on the cytolemma of the cells of the parotid gland epithelial germ and in their cytoplasm.

Outlooks of the scientific inquiry. The results of lectin-histochemical examination of the early pre-

natal ontogenesis of the parotid gland can form the basis for the work of laboratories dealing with screening of morphological material in order to assess the degree of maturation and prognosis of fetus viability and diagnostics of deviations from normal development.

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ЛЕКТИНОГИСТОХИМИЧЕСКИЕ ЗАКОНОМЕРНОСТИ ДИФФЕРЕНЦИРОВКИ ЭПИТЕЛИАЛЬНЫХ ЗАЧАТКОВ ОКОЛОУШНОЙ ЖЕЛЕЗЫ ЧЕЛОВЕКА

Резюме. Соединения гликополимеров составляют структурную и функциональную основу клеток и тканей живого организма. Нами обоснована необходимость анатомо-лектиногистохимического исследования околоушной железы в раннем пренатальном периоде онтогенеза, поскольку сведения о становлении топографии фрагментарные и несистематизированы, а отдельные аспекты их морфогенеза дискуссионные. Исследовано 35 зародышей и передплодов человека в возрасте от 21 суток до 12 недель внутриутробного развития. Гликополимеры определяли путем обработки серийных срезов лектинами, конъюгированными с пероксидазой хрена. В динамике пренатального морфогенеза зародышей и передплодов 4-й - 12-й недели эмбриогенеза экспрессия гликополимеров - рецепторов лектинов на поверхности клеток, в цитоплазме и на базальной мембране эпителиальных зачатков околоушной железы и ротовой полости человека с его производными по перераспределению гликополимеров - похожи, что может служить лектиногистохимическим подтверждением эктодермального источника происхождения эпителиального зачатка околоушной железы. Погружение клеток эпителия участков щечно-альвеолярных карманов в ниже прилегающую мезенхиму с формированием у зародышей 11,0-12,5 мм ТКД первичных зачатков околоушной железы и превращение их в эпителиальные тяжи связано с накоплением сиалованых гликополимеров (N-ацетилнейраминовой кислоты), N -ацетил D-глюкоза-

мина - специфических к лектинам завязи пшеницы (WGA) и лектина бузины черной (SNA) N-ацетил-2-дезокси-2-амино-D-глюкопираноз, экранированной сиаловой кислоты ß-D-галактозы и α-L-фукозы - специфических к лектинам виноградной улитки (HPA), клещевины (RCA) и коры золотого дождя (LABA). Эти гликополимеры присутствуют в течение первых 12-ти недель как на цитолеме клеток эпителиальной закладки околоушной железы, так и в их цитоплазме. Результаты лектиногистохимических исследований раннего пренатального онтогенеза околоушной железы могут послужить основой в работе лабораторий скрининга морфологического материала для оценки степени зрелости и прогнозирования жизнеспособности плода и диагностики отклонений от нормального развития.
Ключевые слова: лектины; околоушная железа; пренатальный онтогенез.

LECTIN-HISTOCHEMICAL REGULARITIES OF DIFFERENTIATION OF THE HUMAN PAROTID GLAND EPITHELIAL GERMS

Abstract. Glycopolymer compounds make up structural and functional basis of cells and tissues of a living organism. The necessity of anatomical-lectinohistochemical examination of the parotid gland in early prenatal period of ontogenesis is substantiated, as the evidences concerning its topography are fragmentary and not systematized, and certain aspects of its ontogenesis are disputable. 35 human embryos and pre-fetuses aged from 21 days to 12 weeks of intrauterine development were examined. Glycopolymers were found by means of treatment of serial sections with lectins conjugated with horseradish peroxidase. The dynamics of prenatal morphogenesis of the embryos and pre-fetuses of the 4th-12th week of embryogenesis expression of glycopolymers – lectinreceptors on the surface of cells, in the cytoplasm and on the basal membrane of the parotid gland epithelial germs and the human oral cavity with its derivatives by re-distribution of glycopolymers are similar, which can be the evidence of ectodermal source of the parotid gland epithelial germ. Immersion of the epithelial cells of the buccal-alveolar pockets into the lower adjacent mesenchyme with the formation of primary parotid gland germs in embryos with 11,0-12,5 mm of PCL and their transformation into the epithelial taeniae is associated with accumulation of sialic glycopolymers (N-acetyl neuraminic acid), N-acetyl-D-glucosamine – specific to Wheat germ agglutinin (WGA) and Sambucus nigra agglutinin (SNA); N-acetyl-2-desoxy-2-amino-D-glucopyranose, screened by sialic acid of ß-D-galactose and α-L-fucose – specific to Helix pomatia (edible snail) agglutinin (HPA), Rhizoctonia solani agglutinin (RCA) and Laburnum anagyroides (Golden Rain shrub) agglutinin (LABA) respectively. These glycopolymers are present during the first 12 weeks both on the cytolemma of the cells of the parotid gland epithelial germ and in their cytoplasm. The results of lectin-histochemical examination of the early prenatal ontogenesis of the parotid gland can form the basis for the work of laboratories dealing with screening of morphological material in order to assess the degree of maturation and prognosis of fetus viability and diagnostics of deviations from normal development.
Key words: lectins; parotid gland; prenatal ontogenesis.

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