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Реферати

МЕТОДОЛОГІЯ МАРКУВАННЯ ДЕНДРИТНИХ КЛІТИН В СЛИЗОВІЙ ТОВСТОЇ КИШКИ ПРИ ЕКСПЕРИМЕНТАЛЬНОМУ ВИРАЗКОВОМУ КОЛІТІ

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Існує проблема маркування імунних клітин моноклональними антитілами до CD-рецепторів, що постає в перехресній експресії клітинних детермінант відразу декількома видами лейкоцитів, а це вимагає задіяння двох-трьох маркерів, по асоціації яких і виявляються клітинні кластери. Не є винятком і дендритні клітини, які мають антигенні мітки ідентичні гістіоцитам, В-лімфоцитам. Мета дослідження - пошук специфічного маркера дендритних клітин у складі запаленої слизової оболонки товстої кишки при експериментальному виразковому коліті. Були вивчені фрагменти товстої кишки лабораторних щурів лінії Вістар, у яких моделювали виразковий коліт шляхом введення трінітробензолсульфонової кислоти в товсту кишку. Було встановлено, що з усіх вивчених маркерів дендритних клітин в складі слизової товстої кишки найбільш прийнятним є S-100, оскільки інтенсивно накопичується в цитоплазмі ДК, але не гістіоцитів та лейкоцитів, не вимагає диференціювання з нервовими елементами. Маркер дозволяє досліджувати деталі гетерогенності його накопичення в цитоплазмі ДК.

Ключові слова: дендритні клітини, виразковий коліт, імуногістохімічне маркування.

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METHODOLOGY OF LABELING DENDRITIC CELLS IN THE COLONIC MUCOSA IN EXPERIMENTAL ULCERATIVE COLITIS

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There is a problem - labeling of immune cells with monoclonal antibodies to CD-receptors because appears the cross expression of cell determinants by several types of leukocytes, that requires to use of two or three markers for identification some cell clusters. No exception the dendritic cells that have identical antigenic tags the same the histiocytes, B-lymphocytes. The aim - to find a specific marker of dendritic cells within the inflamed mucosa of the colon in experimental ulcerative colitis. We were studied colon laboratory Wistar rats, which modeled ulcerative colitis by administering trinitrobenzolsulfonic acid in the colon. It was found that the most important marker of dendritic cells in the mucosal part of the colon is S-100 because accumulates in the cytoplasm dendritic cells, but not leukocytes and histiocytes, not requires differentiation with neural elements. Marker allows to investigate the details of the heterogeneity of its accumulation in the cytoplasm of dendritic cells.

Key words: dendritic cells, ulcerative colitis, immunohistochemical markers.

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GLYCOSAMINOGLICANS DISTRIBUTION IN THE RATS' MAJOR SALIVARY GLANDS DURING EARLY POSTNATAL PERIOD AFTER ANTENATAL ANTIGEN ACTION

Purpose - to determine the features of glycoproteins' distribution in the structures of rats' major salivary glands in early postnatal period after intrauterine antigen action. In newborn animals receiving antigen in the antenatal period, in the cells' cytoplasm and extracellular matrix indicate the accumulation' increase of Alcian blue stain - positive compounds retained until the 11th and offset at the 45th day of postnatal life. The detected changes in the major salivary glands cells' are the basis for the development of inflammatory and dystrophic processes and can lead to the functional violations formation' hereinafter.

Key words: major salivary glands, intrauterine antigenic action, glycosaminoglycans, rats.

The major salivary glands synthetic and structural imbalance take one the main role in the general structure of oral pathology. Nowadays, one of the leading take pathological condition connecting with the salivary glands' inflammatory and dystrophic violations. The problem of etiology and pathogenesis not enough studied and demanded intent attention of researchers [5, 6, 7]. One of determinatives that result in violation of major salivary glands morphogenesis of and as a result the development of its pathology is the condition of pregnant health, more than half of them has chronic diseases and system functional disorders which is accompanied by the immune pathological condition, namely by antigen influence on a fetus [8]. Nowadays it is determined injection of the antigen in the antenatal period is the basis for the development of inflammatory processes in a postnatal period [9]. Afterbirth, the changes in connective tissue caused by antigen influence to fetus during the embryonic formation are the most striking manifestations of the undifferentiated connective tissue syndrome in newborn [9]. GAGs are the most susceptible component in dysplasia syndrome and perform the number of important functions in the salivary glands. Interconnection of glycoseaminoglicans (GAGs) distribution in the major salivary glands structures in a postnatal period injected intrauterine an antigen after birth is not enough studied.

Purpose. To determine the glycosaminoglycans distribution features in the rats' major salivary glands structures in early postnatal period after intrauterine antigen action.

Matirials and research methods. The object of the research was 224 salivary glands of white laboratory rats. Due to impossible quality materials' taking in the early periods of postnatal life parotid and sublingual salivary glands, the investigation done at the submaxillaris [5].

The rats were divided into three groups. The 1st group is intact rats. The 2d group is rats, which were introduced 0,05 ml solution of antigen in the amniotic fluid on the 18th day of pregnancy by the method of N. Voloshyn [10], the 3d group – control, the animals were introduced intrauterine 0,05 ml of physiological solution on the 18th day of pregnancy. The feeding of animals was twice a day at the same time.

For the study of peculiarities of GP distribution of the structures of major salivary glands of antigen's action on the foetus, chosen the model of transuterine, transmembrane introduction of antigen in amniotic waters by the method of N. Voloshyn [10]. The antigen was rare (killed) split - vaccine Vaxigrip 2009. Keeping the animals and experiments were carried out accordingly to regulations of European convention about the defense of spine animals which are used due to the experimental and other scientific aims (Strasbourg, 18.03.86), general ethic principles of the experiments on the animals taken by the first national congress of Bioethics (Kyiv, 2001). The animals' killing and taking of the material done from 13-00 till 14-00 on the 1st, 5th, 7th, 11th, 14th, 30th, 45th day of postnatal life. On every term in all groups of the animals were examined 5 - 6 animals from 2 - 3 afterbirth. For the investigation, the major salivary glands used during some minutes after killing. The samples fixed in 10% solution of formalin, dehydrated, filled in paraffin mixture and produced serial paraffin sections. The histochemical detecting of GAGs whole complex conducted by means of Alcian blue stain (pH 2,6; Critical Electrolyte Concentration (CEC) – MgCl2 0,2M) without and after previous testicular gyaluronidase' processing of sections. For sulfate GAGs differentiation stained by Alcian blue with CEC MgCl2 0,6M. The results of histochemical exposure of stain was done by semi - quantitative and determine as: +++ - turquoise, ++ - blue, + - pale blue, - - is absence of stain. Intermediate hues evaluated accordingly: ++/+++, +/++. The GAGs distribution detected in cells' cytoplasm and intracellular matrix of major salivary glands.

Results at its discussion. In intact and control group is the more intensive staining of cells' cytoplasm structures and presents - +. Staining of all above-stated structures for the control group of animals does not differ from the data got from the animals of intact group, that is why in the future control group will not be cited (Fig. 1). The results received from antigen injected animals showed the less intensive stain and is -/+. After testicular gyaluronidase sections processing the stain intensive gradually decrease in all observe structures. In GAGs differentiation via Alcian Blue staining with CEC MgCl2 0,6M observed the minor sulfated GAGs accumulation. This manifestation shows the existence in cells' cytoplasm a small quantity of low sulfated GAGs and determined as a pale blue stain in all animals groups.

The seventh day after birth characterized of GAGs accumulation in cells' cytoplasm in all animals group and determined as ++/+++. The fermentative control setting the stain intensity is increase in all observed structures.

At the 11th, 14th day of postnatal life, the GAGs accumulation increased for all animals groups to a pale blue. The sections have hardly noticeable coloration close to absence of stain after fermentative control. In Alcian blue staining with CEC MgCl2 0,6M in all observed structures the stain reaches a pale blue color. These data are unchanged for every animals group (Fig. 1).

At the 30th day in whole complex of GAGs detection the stain intensity of cells' cytoplasm is evenly increase in all animals group and determined as -/+. The fermentative control shows hardly noticeable coloration close to stain absence. In the sulfated GAG's detecting the stain intensity is increase to pale blue color.

At the 45th day of postnatal life in whole complex of GAGs detection and after testicular gyalyronidase' processing the all observed structures' stain is absence. The Alcian Blue stain with CEC MgCl2 0,6M is unchanged in major salivary glands cells' cytoplasm. In detecting GAGs whole complex in the major salivary glands intercellular matrix of a newborn experimental animals the stain intensity is +, in compared intact group – the stain intensity is +/++. Testicular gyaluronidase section processing results to moderate stain intensity increase to pale blue. In Alcian Blue staining with CEC MgCl2 0,6M detected the minor accumulation of low sulfated GAGs in the intracellular matrix structures' of intact animals group close to -/+. The 5th day after birth characterized intensive accumulation of GAGs in the intercellular matrix in all observed animals groups. In experimental animals group the observed structures is close to blue. The same structures in intact group, is turquoise. The fermentative control results

indicated the stain intensity increase in intercellular matrix for all groups. Sulfated GAGs accumulation is no different from the newborn animals' findings.

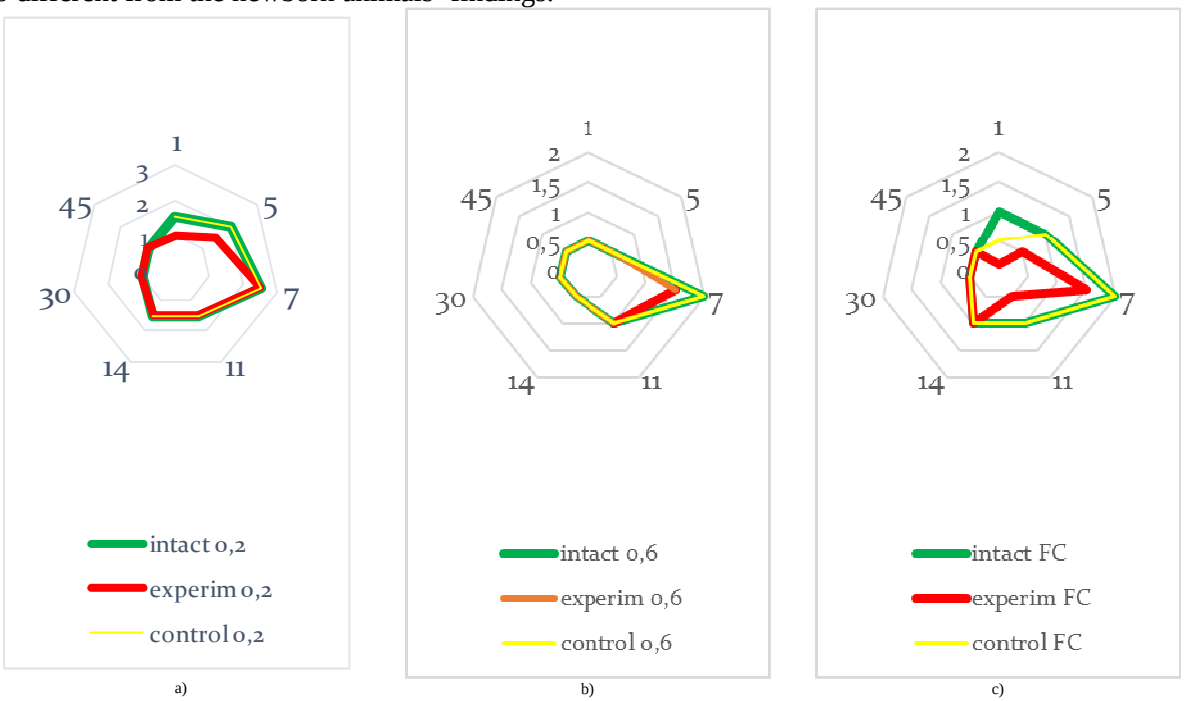

Figure 1. Stain intensity indices of the major salivary glands' epithelial structures. a) - Alcian blue stain with Critical Electrolyte Concentration – MgCl2 0,2M); b) - Alcian blue stain with Critical Electrolyte Concentration – MgCl2 0,6M; c) – Alcian blue stain after previous testicular gyaluronidase processing of sections.

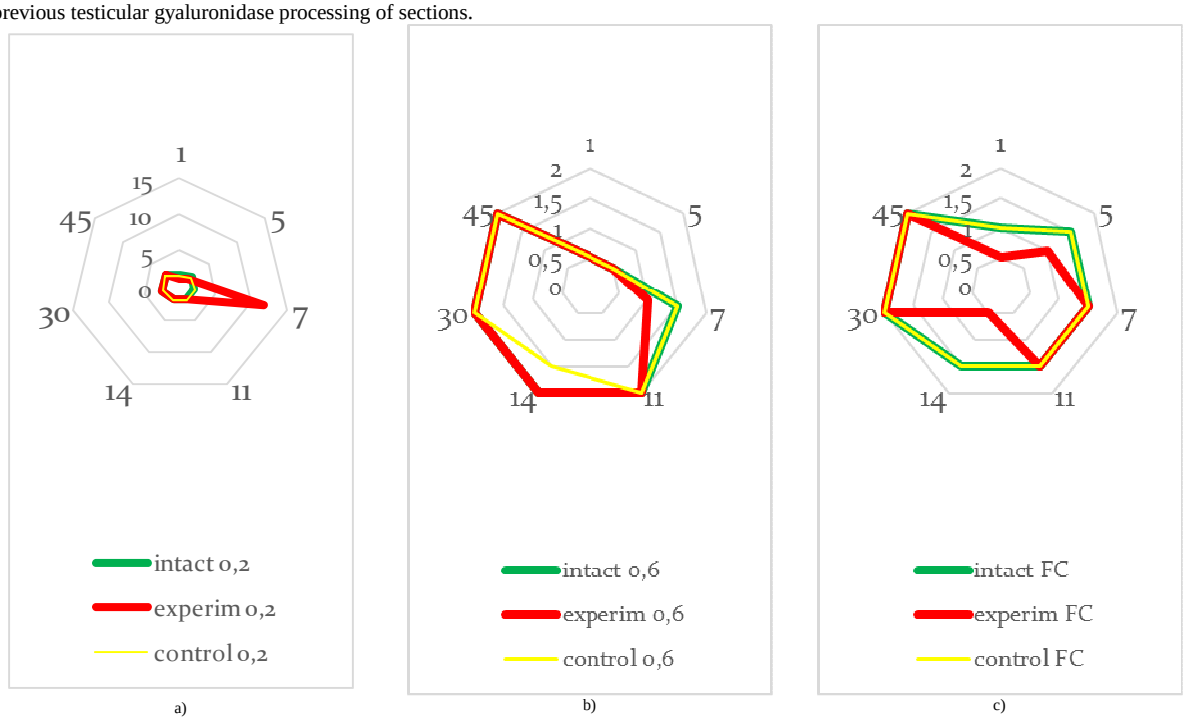

Figure 2. Stain intensity indices of the major salivary glands' stromal structures. a)- Alcian blue stain with Critical Electrolyte Concentration – MgCl2 0,2M); b) - Alcian blue stain with Critical Electrolyte Concentration – MgCl2 0,6M; c) – Alcian blue stain after previous testicular gyaluronidase processing of sections.

In all animals' groups at the 7th day of postnatal life noted the stain intensity increase in the extracellular matrix to ++. After fermentative processing the stain intensity of all above-stated structures in experimental group is some decreased - +/++. The results received in intact group showed that stain intensity is practically not changing and remain at the level of blue stain.

At the 11th, 14th the GAGs contain in the extracellular matrix increased almost to +/++ and remain unchanged in all animals group. Since 11th day after birth the sulfated GAGs contain is increased compared 7th day. The extracellular matrix' stain reaches the pale blue color. The period from 7th day to 30th day of postnatal life characterized of GAGs considerable accumulation in major salivary glands'

extracellular matrix. The observed structures' stain intensity is ++/+++ and remains the same to 45th day after birth (Fig. 2).

Testicular gyaluronidase sections' processing showed the increase stain intensity in the observed structures to ++ in the experimental and intact groups. In Alcian Blue staining with CEC MgCl2 0,6M detected the blue color in all animals groups from 30th to 45th day after birth (Fig. 2).

Discussion. Immunohistochemically, several GAG fractions such as chondroitin-6-sulfate, unsulfated chondroitin sulfate, and keratan sulfate were seen mainly at the plasma membrane of both pleomorphic adenoma and adenoid cystic carcinoma cells and of myoepithelial cells of normal salivary glands [7]. Sialic acid concentrations were strong in retrolingual and moderate in submaxillary glands. Uronic acid concentrations were high in retrolingual, intermediate in parotid, and low in submaxillary glands. The major fraction in the submaxillary and parotid glands was hyaluronic acid, whereas in the retrolingual gland, the major fractions were glycoproteins and dermatan-sulfate [5]. Sialic acid was assayed in hypertrophic submaxillary and retrolingual glands after periodic incisor amputations. In the submaxillary glands, an increased sialic acid concentration and total content found. In the retrolingual glands, the sialic acid concentration did not change, but total content of the acid increased [4]. Secretory units synthesized a GAG mixture which was 20–25 percent hyaluronic acid, 70–75 percent heparan sulphate, and only 3–5 per cent chondroitin or dermatan sulphates, similar to that synthesized in vivo. No GAG was present in the secretory material, suggesting that all GAG synthesized was destined for the basement membrane or cell surface [2]. In animals group after antigen action in the fetal period, was detected synthesis increase of the low sulfated GAG in the major salivary glands' epithelial structures compared intact animals and highly sulfated GAG appearance. The testicular gyaluronidase sections' processing leaded to stain intensity increase of acinar cells' cytoplasm [3]. That is a mapping of chondroitin and hyaluronic acid increase content in experimental group major salivary glands' epithelial structures. The obtained results partially according to the changes of the GAG' accumulation, coincided with the data received early by several authors in the throat' mucous membrane, gums [1,11] and show the major salivary glands' epithelial structures reactivity to the antenatal antigen influence. In newborn experimental animals observed the chondroitin, hyaluronic acid and low sulfated GAG increase content in the major salivary glands' stromal structures. These data indicated the microenvironment changes in the major salivary glands. The similar data received early by several authors in the study of periodontium, knee joint [1,9,11].

Conclusion

The results received in animals' experimental group at the 1st, 5th, 7th day of postnatal life showed increase intensity of not sulfated and low sulfated GAGs accumulation in cells' cytoplasm and intracellular matrix of major salivary glands. At the background of the salivary glands weight increase in experimental group observed the quantity, synthesis and accumulation' increasing of not sulfated GAGs namely hyaluronic acid. The indicated imbalance between sulfated and not sulfated GAGs contain showed the changes in microenvironment of salivary glands cells' structures, secretion and excretion function disorders and can be the basis of inflammatory processes. The indicated synthesis and accumulation changes of GAGs in major salivary glands structures' are offset at the 11th day after birth.

Further researches prospects. *In our further researches, we will analyze the lectin receptors distribution and the distribution of PNA - positive lymphocytes define state of the microenvironments of the major salivary glands' structures.*

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Summary

РОЗПОДІЛ ГЛІКОЗАМІНОГЛІКАНІВ У ВЕЛИКИХ СЛИННИХ ЗАЛОЗАХ ЩУРІВ В РАННЬОМУ ПОСТНАТАЛЬНОМУ ПЕРІОДІ ПІСЛЯ АНТЕНАТАЛЬНОЇ АНТИГЕННОЇ ДІЇ

Сирцов В. К., Маслова І. М.

Мета роботи - встановити особливості розподілу глікозаміногліканів в клітинах великих слинних залоз в ранньому постнатальному періоді після внутрішньоутробної антигенної дії. У новонароджених тварин, що отримали антиген в антенатальному періоді, в цитоплазмі клітин та в міжклітинні речовині виявлено зниження накопичення альціанофільних сполук, яке зберігається до 11-ї та нівелюється на 45-ту добу. Виявлені зміни є підгрунтям для розвитку запальних та дистрофічних процесів в слинних залозах, що, в подальшому, може призвести до виникнення різних функціональних порушень.

Ключові слова: великі слинні залози, глюкозаміноглікани, внутрішньоутробна антигенна дія, щурі.

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РАСПРЕДЕЛЕНИЕ ГЛИКОЗАМИНОГЛИКАНОВ В БОЛЬШИХ СЛЮННЫХ ЖЕЛЕЗАХ КРЫС В РАННЕМ ПОСТНАТАЛЬНОМ ПЕРИОДЕ ПОСЛЕ АНТЕНАТАЛЬНОГО АНТИГЕННОГО ДЕЙСТВИЯ

Сырцов В. К., Маслова И. Н.

Цель работы - установить особенности распределения гликопротеинов в клетках больших слюнных желез крыс в раннем постнатальном периоде после внутриутробного антигенного действия. У новорожденных животных, получивших антиген в антенатальном периоде в цитоплазме клеток и в межклеточном веществе больших слюнных желез выявлено снижение накопления альцианофильных соединений, которое сохраняется до 11-х и нивелируется на 45-е сутки. Выявленные изменения могут быть основой для развития воспалительных и дистрофических процессов в слюнных железах, что в последующем, может привести к возникновению различных функциональных нарушений.

Ключевые слова: большие слюнные железы, глюкозаминогликаны, внутриутробное антигенное действие, крысы.

Рецензент Єрошенко Г.А.

УДК 611.817.1

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СТРОЕНИЕ НЕКОТОРЫХ ГЛУБОКИХ УЧАСТКОВ БЕЛОГО ВЕЩЕСТВА ЧЕРВЯ МОЗЖЕЧКА ЧЕЛОВЕКА

Восемь ветвей белого вещества образуют десять долек червя мозжечка. Иногда две или три ветви начинаются общим участком белого вещества. **Цель** работы – установить строение участков белого тела мозжечка, общих для нескольких его ветвей. 230 мозжечков – объект исследования, возраст 20–99 лет. Проводили анализ оцифрованных изображений сагиттальных сечений мозжечка. Общее начало третьей и четвертой ветвей не имеет собственных листков серого вещества. Общий ствол имеет один или два листка серого вещества. Общее начало пятой и шестой ветвей не имеет собственных листков серого вещества. Их общий ствол имеет от одного до семи листков. Ствол, общий для шестой и седьмой ветвей, имеет собственный листок серого вещества на нижней поверхности. Ствол, общий для пятой, шестой и седьмой ветвей, в 84 % имеет два собственных листка, лежащих зеркально: один – сверху, один – снизу. Стволы белого вещества, общие для пятой и шестой ветвей и шестой и седьмой ветвей, в половине случаев начинаются от ствола, общего для пятой, шестой и седьмой ветвей.

Предлагаются их названия. Так, ствол, общий для третьей и четвертой ветвей – truncus communis r. paleocerebellaris superioris III–IV, или truncus paleocerebellaris superior. Так как отходящие от него ветви образуют дольки III и IV-V, его можно обозначить и как truncus communis ll. III–V. Ствол, от которого вместе начинаются пятая и шестая ветви - truncus communis r. V–VI, или truncus communis ll.VI–VIII. Ствол общий для шестой и седьмой ветвей – truncus communis paleocerebellaris inferior, или truncus communis *r.* VI–VII, или truncus communis lobules VIII–IX. Ствол, от которого вместе начинаются три ветви, пятая и шестая и седьмая, - truncus communis r. V–VII, или truncus communis lobules VI–IX.

Ключевые слова: мозжечок, белое вещество,червь, кора.

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Мозжечок имеет сложную пространственную конфигурацию, связанную с организацией белого вещества, являющегося основой его коры [3, 6]. В его составе различают центральную часть – червь, и полушария. Червь и полушария делятся на десять долек главными бороздами, переходящими с одного полушария через червь на другое. Долькам червя соответствуют определенные дольки полушарий [3, 6].