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AĞ SİÇOVULLARIN NAZİK BAĞIRSAQLARINDA LİMFOİD DÜYÜNLƏRİN NEOGENEZİNİN GENİŞ TƏSİR SPEKTRLİ ANTİBİOTİKLƏR VASİTƏSİLƏ İNDUKSİYASI

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Nazik bağırsağın limfoid düyünlərində geniş təsir spektrli antibiotiklərin induksiya etdiyi neogenez prosesini öyrənmək məqsədilə tədqiqat aparılmışdır.

Tədqiqata kütləsi 200±20 q olan 60 baş erkək cinsli ağ siçovul daxil edilmişdir. Heyvanlardan 30 baş birinci – kontrol qrupunu, 30 baş isə əsas qrupu təşkil etmişdir. Əsas qrupdakı heyvanlarda klaritromisin yeridildikdən sonra nazik bağırsaqların limfoid düyünlərində törənən morfoloji dəyişikliklər kontrol qrupu ilə müqayisədə tədqiq edilmişdir.

Tədqiqat göstərmişdir ki, nazik bağırsaqlarda qruplarla yerləşən və immun reaksiyaların inkişafı üçün zəmin yaradan limfoid törəmələr düyünlərinin sayına görə fərdi xüsusiyyət daşıyır və bu, bağırsaq möhtəviyyatının tərkibindəki antigenlərdən asılı olaraq, dəyişikliyə uğraya bilir. Bağırsaqların selikli qişasının və selikaltı bazal qatının limfoid törəmələri yeni yaranmış qrupşəkilli limfa düyünlərindən ibarət olur; onların yaranmasını geniş təsir spektrli antibiotik olan klaritromusinin bağırsaq mikrobiosenozunda törətdiyi pozuntularla izah etmək olar. Bundan əlavə, klaritromisin həm də immunotrop təsir xassəsinə malikdir. Antibiotikin tətbiqi zamanı immun sistemin ikincili (periferik) orqanları olan genetik determinasiyalı limfoid strukturların işçi kompensator hiperplaziyası müşahidə edilir.

Açar sözlər: *limfoneogenez, qrupşəkilli limfa düyünləri, nazik bağırsaq, selikli qişa, geniş təsir spektrli antibiotik*

Ключевые слова: *лимфонеогенез, групповые лимфоидные узелки, тонкая кишка, слизистая оболочка, антибиотик широкого спектра действия*

Keywords: *lymphoneogenesis, group lymphoid nodules, small intestine, mucous membrane, broad-spectrum antibiotic*

NEOGENESIS OF LYMPHOID NODULES OF SMALL INTESTINE OF WHITE RATS INDUCED BY A BROAD-SPECTRUM ANTIBIOTIC

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The article presents the results of a study conducted to investigate the neogenesis of lymphoid nodules in the small intestine of rats induced by a broad-spectrum antibiotic. The study was conducted on 60 white male rats of reproductive age, weighing about 200.0±20.0 grams, which were divided into two groups: control (n=30), and animals of the second group (n=30) in which studied the morphological state of the group lymphoid nodules of the small intestine after the clarithromycin administration. The genetically programmed total number of group lymphoid nodules in the small intestine of rats is a constant, while the number of lymphoid nodules in them is a variable value that depends on situational shifts in the microbiocenosis of the small intestine. Group lymphoid nodules are characterized by a large degree of individual variability in the number of lymphoid nodules involved in immune reactions, which depends on the variability of the antigenic composition of the contents of the small intestine.

Thus, lymphoid formations of the mucous membrane and submucosa of the small intestine of white rats are newly formed (initial) forms of group lymphoid nodules, the appearance of which can be explained by a violation of microbiocenosis in the small intestine under the influence of a broad-spectrum antibacterial drug - clarithromycin, which also has immunotropic properties. After taking an antibiotic, we observed the

Introduction. This article is consistent with the reports that have appeared in the literature in recent years about the possibility of re-formation of some secondary (peripheral) organs of the immune system, which have been called "tertiary lymphoid structures". These are mainly lymph nodes and lymphoid nodules associated with various organs [1-5]. Such ectopic formations may have different complexity of organization, ranging from simple accumulations of immunocompetent cells to the formation of highly ordered lymphoid nodules, which arise in the process of development of chronic inflammatory processes, oncological and autoimmune diseases, as well as in the process of allotransplant rejection [6-9].

According to the existing ideas, these formations appear as a result of different pathological factors, which can be considered as a process of adaptive multiplication (as a result of additional generation) in the corresponding affected organs of lymphoid structures which conform in form to the secondary, genetically determined, organs of the immune system [10].

Purpose. To study neogenesis of the lymphoid nodules in the small intestine of rats, induced by a broad-spectrum antibiotic.

Material and Methods. The study was conducted on 60 white male rats of reproductive age, weighing about 200.0±20.0 grams, which were divided into two groups equal in number and physiological state, one of which served for obtaining initial control data (n=30), and the animals of the second group (n=30) were used in the experiment to study the morphological state of group lymphoid nodules (GLN) of the small intestine after a course administration of a broad-spectrum antibacterial medicine, which was clarithromycin. Before the experiment, all animals were kept in standard conditions of the experimental-biological clinic (vivarium) of Poltava State Medical University, according to the rules of keeping experimental animals, established by the Directive of the European Parliament and the Council (2010/63/EU), the order of the Ministry of Education and Science, Youth and Sports of Ukraine from 01.03.2012 №249 "On approval of the order of scientific institutions to conduct experiments, experiments on animals" and "General ethical principles of experiments on animals", adopted by the Fifth National Congress on Bioethics (Kyiv, 2013) [11-12].

In order to avoid stressful condition of animals from clarithromycin administration by means of a probe, which may adversely affect the functional state of the digestive system, we resorted to a natural, physiological method, which consisted in administering the antibiotic to animals with food in the mode of two times a day (morning and evening) for 10 days. However, the coarse fodder products usually used in the diet of animals were replaced by stale bread, which allows absorption of clarithromycin solution into itself [13].

After completion of the experiment and alternate vivisection of all animals, this was accomplished by an overdose of ether anesthesia [14]. The material for the study was the small intestine, starting from the pyloric part of the stomach to the border with the cecum. This allowed to determine its length (which was about 1 meter) without much difficulty and to count along its entire length the GLN, which are clearly visible under the serous membrane (from the side opposite to the place of mesentery attachment) in the form of different in shape and size whitish spots. At the same time, in each individual case intragroup differentiation by size was carried out among them, as a result of which their small, medium and large specimens were isolated in separate variants, which were further subjected to planimetric analysis. Considering that they have a round or oval shape, their area was estimated using known formulas used in mathematics to calculate the area of the corresponding figures. The Digital Caliper 150 mm Miol served as a measuring instrument. The obtained quantitative and planimetric data were subjected to statistical analysis using EXCEL 2010 program (Microsoft Excel Corp., USA). The mean value - M, error of mean value - m were calculated. Reliability of differences between mean values was determined on the basis of Student's t-criterion. The result was considered reliable at $p < 0.05$ [15].

The next stage was the preparation of small intestine GLN preparations for histologic studies, for which we selectively excised small sections of the small intestine containing GLN of different sizes. The latter were individually enclosed in paraffin blocks so that serial sections (4 μm thick) in the cross section of the small intestine could be obtained, which were stained with hematoxylin-eosin.

The obtained histological preparations were studied and documented using the Konus light microscope equipped with the Sigeta DCM-9009.0MP digital microphotographic attachment with the Biorex 3 software adapted for these studies (serial number 5604). Sigeta X 1 mm/100 Div.x0.01mm micrometer object served as a metric scale, the scale bar of which (equal to 1 mm, where the smallest division corresponds to 10 μm) was applied to the corresponding micro-photograph obtained at equal magnification.

Results and Discussion. According to the obtained planimetric indices (Table), the total area of small intestine GLN increased more than twice, namely, if according to control data it was equal to 220.9±14.4 mm^2, after a course administration of clarithromycin for 10 days it expanded to 476.8±10.1 mm^2.

Based on the results of our studies, we can draw a general conclusion that the genetically programmed total number of GLN in the small intestine is a constant, whereas the number of lymphoid nodules in them is a variable value depending on situational shifts in the microbiocenosis of the small intestine. During the study of serial histologic slices, the mucous membrane formations of the small intestine of experimental animals, which attracted attention by their atypical form, were found quite unexpectedly. At low magnifications of a light microscope, they look like a close row of several alternating, different in size, dome-shaped elevations of the mucosa, which are surrounded by typical intestinal villi (Fig. 1).

It can be clearly seen that each such elevation is a protrusion of the intrinsic lamina of the mucosa, covered with a layer of intestinal epithelium and crypts embedded in it (from the muscular side). At high magnification, it can be seen that these crypts are short tubular branches of a common cisterna embedded in the basal part of these elevations (Fig. 2).

In this case, some of these crypts open with an orifice on their luminal surface, while others communicate with slit-like gaps between adjacent elevations, which is typical for the general structure of lymphoid nodules [16], which in this case are in their rudimentary state.

Table. **Results of quantitative and planimetric analysis of group lymphoid nodules of small intestine of white rats in normal and after clarithromycin introduction (n=60), M±m**

Groups of animals	Indicators	Total number of GLN	Number and area (S) by size of GLN						Total GLN area value (mm^2)
			Small		Medium		Large		
			Quantity	S ($мм^2$)	Quantity	S ($мм^2$)	Quantity	S ($мм^2$)	
Control group n=30	**M±m**	19,9±0,7	12,6±0,4	64,9±2,9	5,8±0,5	97,6±8,0	1,5±0,3	58,4±10,3	220,9±14,4
	Min	12	8	1,57	2	10,6	0	31,4	87,3
	Max	28	17	9,8	11	27,5	5	60,4	406,7
After clarithro-mycin administ-ration n=30	**M±m**	19,4±0,5	3,3±0,4	17,7±1,7	10,4±0,4	201,5±9,0*	5,7±0,2	258,2±10,7*	476,8±10,1*
	Min	13	0	3,1	5	12,6	3	31,4	406,4
	Max	25	8	9,4	16	28,3	8	100,5	593,7

Note: GLN – group lymphoid nodules; S – square, M – mean value, m – mean value error, Min – minimal value, Max – maximal value; «*» – reliability of differences with control ($p<0,05$).

Figure 1. Rudimentary forms of lymphoid nodules of the small intestine mucosa of the white rat after a course clarithromycin administration. Paraffin section; hematoxylin-eosin staining; magnification: x100. The smallest division of the scale bar is 10 μm.
1 – muscular layer; 2 – intestinal crypts; 3 – rudiments of lymphoid nodules; 4 – intermediate form between intestinal villi and rudimentary lymphoid nodules; 5 – intestinal villi. Arrows indicate the orifices of intestinal crypts.

As an additional confirmation of this conclusion, the structure of the connective tissue located between the crypt formations and the covering intestinal epithelium of these formations (Fig. 2) can serve as a peculiarity of the connective tissue structure. It is characterized by blood vessels and various cellular elements, among which lymphocytes predominate. It is noteworthy that the peripheral (lateral) zones of the dome sections of rudimentary lymphoid nodules are the predominant zone of concentration of the latter.

The presented microphotographs (Fig. 2) show that such populations of lymphocytic elements are located subepithelially, where there are individual cells containing several lymphocytes together with macrophages. It is noticeable that these immunocompetent cells have a close connection with the polarized monolayer of intestinal epithelium of rudimentary lymphoid nodules, which acquires

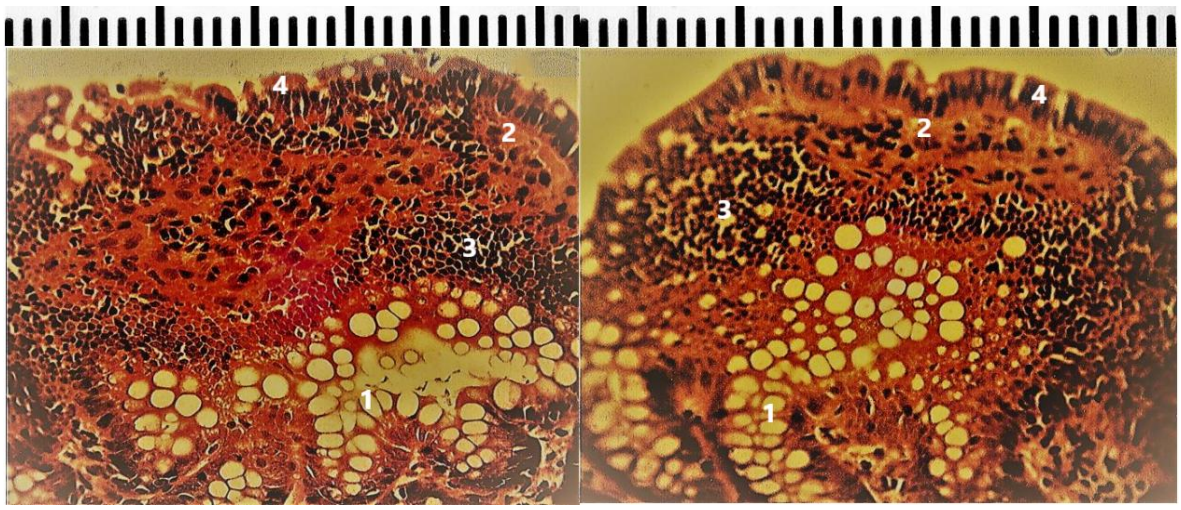

Figure 2. Microscopic structure of two rudiments of lymphoid nodules. Paraffin section; hematoxylin-eosin staining; magnification: x400. The smallest division of the scale bar is 10 μm.
1 – goblet cells in the epithelial wall of intestinal crypts; 2 – connective tissue base; 3 – lymphocytic infiltration; 4 – intestinal covering epithelium (future follicle-associated epithelium).

an uneven, torn surface characteristic of the so-called follicle-associated epithelium of mature GLN [17].

Obviously, the question arises about the morphological sources of formation of these rudimentary forms of GLN in the mucosa of the small intestine. To explain this phenomenon, we pay attention to the fact that they are always found in the continuum of typical intestinal villi, with transitional forms on the border zone between them, which is quite well seen on the overview microphotograph (Fig. 1). This suggests that the newly formed lymphoid nodules arise as a result of morphogenetic transformation of intestinal villi on the preformed base of intestinal crypts, the epithelium of which contains stem cells that are a source of renewal and proliferation of all types of enterocytes of follicle-associated GLN epithelium. It is quite possible that this refers to the process of their formation in the embryonic period of development.

First of all, it should be noted that, according to our data, the character of topographic distribution of GLN in the wall of small intestine of experimental animals does not differ from that of the control group. The same can be said about their total number. Consequently, the topography and total number of GLN in the small intestine of white rats after exposure of the small intestine microflora to an antibacterial preparation remain unchanged, which contradicts the data of some authors [18-19].

It is noteworthy that the main share of the increase in the area of GLN is provided by medium and, especially, large specimens, while the number and area of small GLN are subjected to a noticeable decrease. This phenomenon can be explained by the fact that small-sized GLN are transformed into larger group clusters. Consequently, the result of the action of clarithromycin used in the experiment is hyperplasia of the organized lymphoid tissue of the small intestine.

Therefore, the question arises: what causes this overgrowth of lymphoid tissue in the mucosa of the small intestine, which leads to a doubling of the area of its contact with the wall microflora? In our opinion, it becomes possible either due to the formation of new additional lymphoid nodules in the GLN, or as a result of hyperplasia of the existing ones. To discuss this question it is necessary to involve our data, where it is clearly shown that separately taken GLN is an association of several, different in size and shape lymphoid nodules, among which small, medium and large are distinguished. At the same time, very small lymphoid nodules are found among them. This gives grounds to believe that every GLN, whatever their size, consists of a certain number of lymphoid nodules of different generation times, among which there are rudimentary forms, as well as those in the stage of development (small and medium nodules) and definitive lymphoid nodules (the largest). According to our data, the large lymphoid nodules are the same size in all cases, and only they have germinal (germinative) centers. It follows from this that in each GLN the necessary conditions are preserved for the emergence of new lymphoid nodule rudiments, which during their development will lead to the expansion of their area. In other words, GLN are characterized by a large degree of individual variability in the number of lymphoid nodules involved in immune reactions, which depends on the variability of the antigenic composition of the contents of the small intestine.

In rudimentary forms of lymphoid nodules, the predominant zone of lymphocyte concentration is the peripheral zones of their dome sections. As it is known, these zones in definitive GLN are the place of settled concentration of T-lymphocytes (T-dependent zones). This morphological fact can be seen as the beginning of the process of settlement (first wave) of new, developing GLN by lymphocytic elements, after which, according to literature data, lymphocytes migrate to follicle-associated epithelium of GLN.

Conclusion. Thus, all the facts presented above leave no doubt that the described formations of the mucosa and submucosa of the small intestine of sexually mature white rats involved in the experiment are newly formed (rudimentary) forms of group lymphoid nodules, the appearance of which can be explained by the disturbance of microbiocenosis in the small intestine under the influence of a broad-spectrum antibacterial medicine - clarithromycin, which also has immunotropic properties. So, at its course administration within 10 days there is not only a significant

increase (more than twice) of the area of the former (stationary in localization and quantity) GLN, but also an additional appearance of similar structured lymphoid formations in their rudimentary form outside them.

It follows from this that neogenesis of lymphoid structures can in principle be induced not only by some pronounced pathological processes, but also by other factors that are not related to pathology as such. But the problem under consideration is how to interpret these processes of neogenesis in morphogenetic aspect. We believe that in this case we observe the phenomena of working, compensatory hyperplasia of genetically determined lymphoid structures belonging to secondary (peripheral) organs of the immune system. In other words, there is no question of any fundamentally new formations. Therefore, such a name as "tertiary lymphoid structures" seems to us devoid of logic.

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НЕОГЕНЕЗ ЛИМФОИДНЫХ УЗЕЛКОВ ТОНКОЙ КИШКИ БЕЛЫХ КРЫС, ИНДУЦИРОВАННЫЙ АНТИБИОТИКОМ ШИРОКОГО СПЕКТРА ДЕЙСТВИЯ

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Резюме. В статье представлены результаты исследования, проведенного с целью изучить неогенез лимфоидных узелков тонкой кишки крыс, индуцированного антибиотиком широкого спектра действия. Исследование проведено на 60 белых крысах-самцах репродуктивного возраста, массой около 200,0±20,0 грамм, которые были разделены на две группы: животные первой группы (n=30) служили контролем, а животные второй группы (n=30) использованы в эксперименте в целях изучения морфологического состояния групповых лимфоидных узелков тонкой кишки после введения кларитромицина. Исследование показало, что групповые лимфоидные узелки отличаются большой степенью индивидуальной изменчивости по количеству лимфоидных узелков, задействованных в иммунных реакциях, что зависит от переменчивости антигенного состава содержимого тонкой кишки.

Лимфоидные образования слизистой оболочки и подслизистой основы тонкой кишки белых крыс являются вновь образующимися (зачаточными) формами групповых лимфоидных узелков, появление которых можно объяснить нарушением микробиоценоза в тонкой кишке под влиянием антибактериального препарата широкого спектра действия – кларитромицина, обладающего, еще и иммунотропными свойствами. При приеме антибиотика наблюдаются явления рабочей, компенсаторной гиперплазии генетически детерминированных лимфоидных структур, относящихся к вторичным (периферическим) органам иммунной системы.

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