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GENERAL HISTOLOGICAL CHARACTERISTICS OF LYMPHOID NODULES OF PEYER'S PATCHES OF THE SMALL INTESTINE IN ALBINO RATS AFTER ADMINISTRATION OF A BROAD-SPECTRUM ANTIBIOTIC*

Hryn V. H., Kostylenko Y. P., Hryn K. V.

Ukrainian Medical Stomatological Academy.

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Дисбактеріоз може бути викликаний введенням антибіотика в організм будь-яким способом, але найбільш високий його ризик при пероральному прийомі, так як препарат потрапляє прямо в кишечник, безпосередньо впливаючи на мікрофлору. Після курсового прийому антибіотика широкого спектру дії пейєрові бляшки тонкої кишки білих щурів залишаються незмінними як по топографії, так і за загальним кількісним складом. Але при цьому більш ніж в два рази відбувається збільшення їх загальної площі за рахунок появи в них нової генерації лімфоїдних вузликів. Мета: вивчення гістологічної характеристики лімфоїдних вузликів пейєрових бляшок тонкої кишки білих щурів після курсового прийому кларитроміцину. В експерименті задіяно 30 білих щурів-самців репродуктивного віку, масою 200,0±20,0 грам. Прийом антибіотика тваринами з їжею проводився в режимі дворазового їх годування на добу. Матеріалом для вивчення служили ділянки тонкої кишки з пейєровими бляшками. Вивчали серійні парафінові зрізи під світловим мікроскопом «Konus». Морфометричні характеристики тканинних структур отримували за допомогою об'єкт-мікрометра Sigeta X 1 мм/100 Div.x0.01мм. Встановлено, що після курсового прийому антибіотика широкого спектру дії (кларитроміцину) в тонкій кишці білих щурів топографія і загальна кількість пейєрових бляшок залишаються незмінними, тоді як їх загальна площа зростає більш ніж в два рази. Дана гіперплазія структурованої лімфоїдної тканини в слизовій оболонці тонкої кишки тварин при впливі антибактеріального препарату відбувається за рахунок появи в пейєрових бляшках нових генерацій лімфоїдних вузликів, серед яких виділяються малі, середні та великі форми. Кожна з них відрізняється своїми морфологічними особливостями, які полягають в основному в перетворенні лімфоїдно-асоційованого епітелію. Отже, генетично запрограмована загальна кількість пейєрових бляшок в тонкій кишці статевозрілих білих щурів є константою, тоді як кількість в них лімфоїдних вузликів – величиною змінною, яка залежить від стану мікробіоценозу кишечника.

Ключові слова: пейєрові бляшки, лімфоїдно-асоційований епітелій, крипти, кларитроміцин.

Administration of an antibiotic by any route can cause dysbacteriosis, but its risk is the highest when taken orally, since the drug gets directly into the intestine, affecting microflora. After administration of a broad-spectrum antibiotic, Peyer's patches of the small intestine of albino rats remained unchanged both topographically and in their total amount. But at the same time, their total area is more than doubled, which, according to our data, becomes possible due to the appearance of a new generation of lymphoid nodules in them. The aim of the research was to study the histological characteristics of lymphoid nodules of Peyer's patches of the small intestine in albino rats after administration of clarithromycin. 30 mature albino male rats weighing 200.0±20.0 g were involved in the experiment. The antibiotic was administered to the rodents as a supplement to food during their two-meals-a-day feeding. Areas of the small intestine with Peyer's patches have been studied. Serial paraffin sections have been analyzed using the "Konus" light microscope. Morphometric characteristics of the tissue structures were obtained using the Sigeta X 1 mm / 100 Div.x0.01mm stage micrometer. It has been established that after the course of administration of a broad-spectrum antibiotic (clarithromycin) in the small intestine of albino rats, the topography and total number of Peyer's patches remain unchanged, while their total area increased by more than twice. This hyperplasia of structured lymphoid tissue in the mucous membrane of

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the small intestine of animals under the influence of the antibacterial drug occurs due to the appearance of new generations of lymphoid nodules in the Peyer's patches, among which small, medium and large forms are distinguished, similar to the stages of their development. Each of them is distinguished by its morphological features, primarily, the conversion of the lymphoid-associated epithelium. Therefore, the genetically programmed total number of Peyer's patches in the small intestine of mature albino rats is constant, whereas the number of lymphoid nodules in them is a variable that depends on the state of the intestinal microbiocenosis.

Key words: Peyer's patches, lymphoid-associated epithelium, crypts, clarithromycin.

It is obvious that the choice of the topic of the present paper has been determined by the urgent issue of contemporary medicine about the pathogenesis of dysbacterioses associated with antibiotic therapy, which, especially when using broad-spectrum antibiotics, causes partial or complete suppression of not only pathogenic, but also normal microflora, which is the reason for functional disorders of the intestinal tract [1, 11]. Notably, administration of antibiotics by any route can cause dysbacteriosis, but its risk is the highest when taken orally, since the drug gets directly into the intestine, affecting microflora. It has been reported that in most patients, signs of dysbacteriosis in the form of diarrhea appeared within 1-2 weeks after the beginning of treatment [13, 15].

Many publications pay enough attention to the clinical and bacteriological aspects of this problem [4, 9]. At the same time, the issues related to the morphofunctional states of the most representative formations of the immune system of the intestinal mucous membranes, which are Peyer's patches, are underestimated, which, consequently, prompted the present experimental study, involving albino rats. The findings of morphometric analysis showed that after administration of a broad-spectrum antibiotic, Peyer's patches of the small intestine of albino rats remained unchanged both topographically and in their total amount, which contradicts the findings of some authors [8]. But at the same time, their total area is more than doubled, which, according to our data, becomes possible due to the appearance of a new generation of lymphoid nodules in them, the study of which was the aim of the present research.

The aim of present research was to study the histological characteristics of lymphoid nodules of Peyer's patches of the small intestine in albino rats after administration of clarithromycin.

Material and methods

30 mature albino male rats weighing 200.0±20.0 g were involved into the experiment. Before the experiment, all animals were kept in standard conditions of the experimental biological clinic (vivarium) at Ukrainian Medical Stomatological Academy in compliance with the regulations on keeping experimental animals, adopted by the European Parliament and Council Directive (2010/63 / EU), the Order of the Ministry of Education and Science, Youth and Sports of Ukraine as of 01.03.2012, No. 249 "On approval of the procedure for conducting tests, experiments on animals by research institutions" and "General ethical principles of experiments on animals", adopted by the Vth National Congress on Bioethics (Kiev, 2013), (Minutes No. 155 as of 26.04.2017 of the meeting of the Committee on Biomedical Ethics at Ukrainian Medical Stomatological Academy) [2, 14].

First of all, we faced the task of choosing a method for oral administration of a broad-spectrum antibiotic, namely, clarithromycin, (500 mg tablets) to animals [13, 15]. One of the most targeted methods used in the practice of such experiments is the use of a flexible cannula inserted through the mouth into the esophagus [10]. However, taking into account that this procedure inevitably causes a stress in animals, which can adversely affect the functional state of the digestive system, we were forced to abandon it. Instead, it was decided to apply a natural, physiological method, when antibiotic is administered to the rodents as a supplement to their food during their two-meals-a-day feeding (morning and evening), which corresponds to the housing conditions in the vivarium. However, harsh food nutrients, commonly used in animal diet, were replaced by more high-calorie foods, which include stale bread, absorbing clarithromycin solution. The dose of the latter was calculated based on the data from the veterinary directory, according to which the dosage of clarithromycin for rats is 10 mg/kg [3].

The rodents were restricted to such a diet for 10 days. It should be noted that during and at the end of administration of the antibiotic as a supplement to high-calorie foods for experimental animals, no signs indicating the development of intestinal disorder in the form of diarrhea were noted.

Upon the experiment and after vivisection, which was carried out by an overdose of thiopental anesthesia (75 mg/kg of animal body weight intramuscularly in the upper third of the thigh of the hind paw) [10, 12] in compliance with the procedure and requirements for conduct of experiments on animals, the abdominal cavity was dissected with subsequent washing of its entire content with saline and embedment of whole carcasses into 10% formalin solution. After washing in the running water, the rodents' gastrointestinal tract was examined and sections of the small intestine, containing Peyer's patches, were selectively excised in its proximal, medial and distal parts. After their embedment into paraffin, serial histological sections of 4 μm thick (Microm HM 325) were obtained and stained with hematoxylin-eosin. Their study and documentation was carried out using the "Konus" light microscope equipped with the Sigeta DCM-900 9.0MP digital microphoto attachment and the Biorex 3 program (serial number 5604) adapted for these studies. The morphometric characteristics of the tissue structures of the corresponding specimens were obtained using a system of visual analysis of histological specimens, as well as using the Sigeta X 1 mm / 100 Div.x0.01mm stage micrometer, the scale of which (equal to 1 mm, where a small step corresponds to 10 μm) was applied to the corresponding microimage obtained in the same magnification.

Results and discussion

The findings of our previous study have established that that in animals, after 10-day administration of clarithromycin, Peyer's patches of the small intestine (compared to their regular state) on the average were larger in area due to a quantitative increase of the amount of lymphoid nodules in them. Moreover, histological sections showed no significant difference of the latter in appearance and relationship with adjacent structures (intestinal villi and crypts) from their regular form, which was described in detail in the previous publications

[5, 6, 7]. Among them, with regular constancy, large-, medium- and small-sized lymphoid nodules were located. And only a more careful and detailed study of their structure and a sequential serial analysis of histological sections enabled detecting some specific features that prompted to examine them starting from the small-sized forms.

Notably, in some cases, the apical portions of the small lymphoid nodules were covered with adjacent intestinal villi, connected to each other by their apices (Fig. 1A). The corresponding intestinal crypts were opened into the narrow space, located between the apical surface of the lymphoid nodule and the overhanging villi. In other cases, the lymphoid nodules of the same size but with more pronounced domed evaginations, were fully opened into the inner lumen of the small intestine (Fig. 1B). When compared with the previous species, it seemed that such an open form resulted from the dome-shaped evagination of the apical portion of the lymphoid nodule and disconnection of intestinal villi. Apparently, such an assumption is completely consistent with the publications on the formation of Peyer's patches embryogenesis. But the most notable for them is the form of their most apical portions, which is expressed in the formation of villous proliferation of the covering, lymphoid-associated epithelium in this site. It is quite obvious that such formations are an example of its fractalization, due to which the surface of its contact with the intestinal contents significantly increases. Furthermore, let us point out that such formations are not peculiar to medium- and large-sized lymphoid nodules.

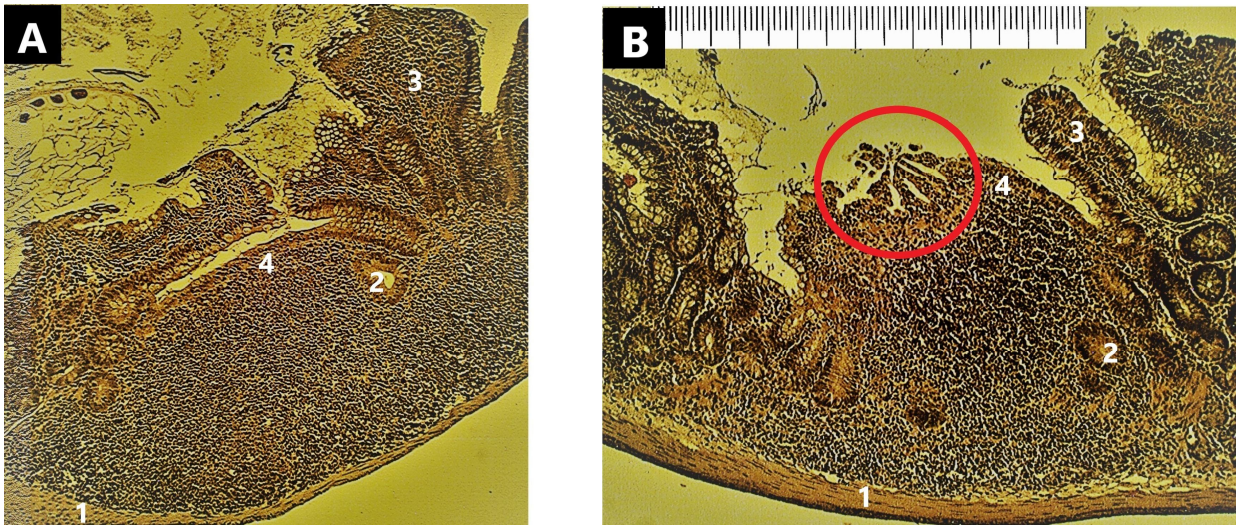

Fig. 1 (A, B). Small forms of lymphoid nodules of Peyer's patches of the small intestine in albino rats after administration of the antibiotic (clarithromycin). A - lymphoid nodule covered with interconnected adjacent intestinal villi; B - "open" form of a small lymphoid nodule. Paraffin sections; H&E stain; lens 10 × magnification. The smallest step of the scale is equal 10 μm.
1 – muscular tunic; 2 – crypts; 3 – intestinal villi; 4 – apical surface of the lymphoid nodule. The red circle outlines the villous (proliferative) proliferation of lymphoid-associated epithelium, which is shown in the next microimage at a higher magnification.

Eventually, some features of the structure of the apical surface of the medium-sized lymphoid nodules of Peyer's patches are of great interest (Fig. 2 A, B).

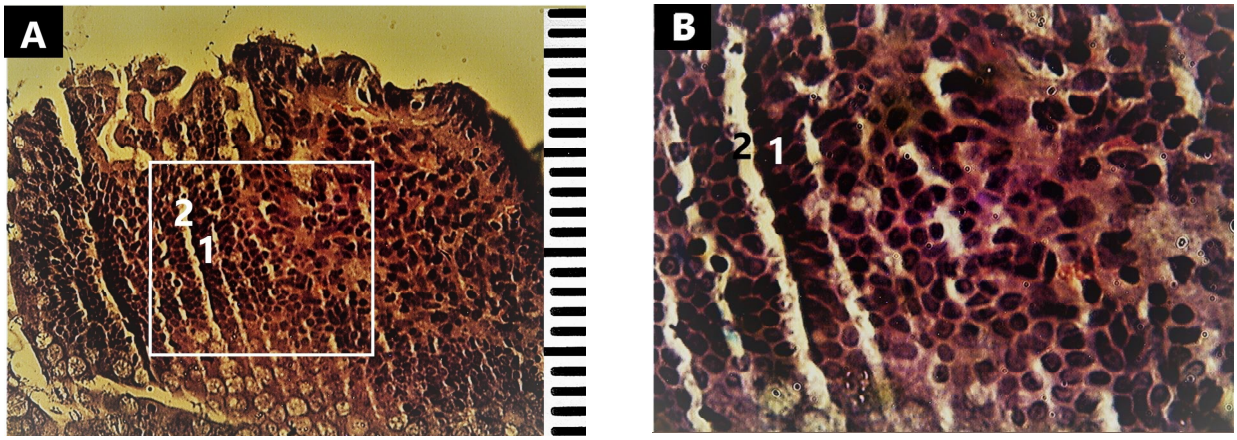

Fig. 2 (A, B). Apical part of the medium-sized lymphoid nodule of the Peyer's patch of the small intestine in albino rats after administration of the antibiotic (clarithromycin). Paraffin section; H&E stain; A - lens 40 × magnification; B - lens 100 × magnification. The smallest step of the scale is equal 10 μm.
1 – lymphoepithelial columns and 2 – interstitial fissures separating them.

In this case the unique formations, which we first described as columnar lymphoepithelial portions, are considered, which have an apparent fractal nature, clearly demonstrating a close relationship (symbiosis) between

the intestinal epithelium and lymphoid structures of Peyer's patches. Noteworthy, isolated lymphoepithelial columns of these formations have the same structure as the villous strands of the bushy proliferations of the apical portions of small lymphoid nodules. We hypothesize that in the course of development (within isolated Peyer's patches) of the small-sized lymphoid nodules, there is initially (in response to a certain antigenic stimulus) disordered proliferation of the lymphoid-associated epithelium, subsequently converted (with the enlargement of lymphoid nodule) into regular, strictly ordered ones in the form of columnar-lymphoepithelial portions.

Notably, in large lymphoid nodules such formations have not been detected. The findings of the study showed that they were not significantly different from their regular form and shape (after the course of antibiotic administration). However, in the vast majority of observations, their domes were flattened (Fig. 3 A).

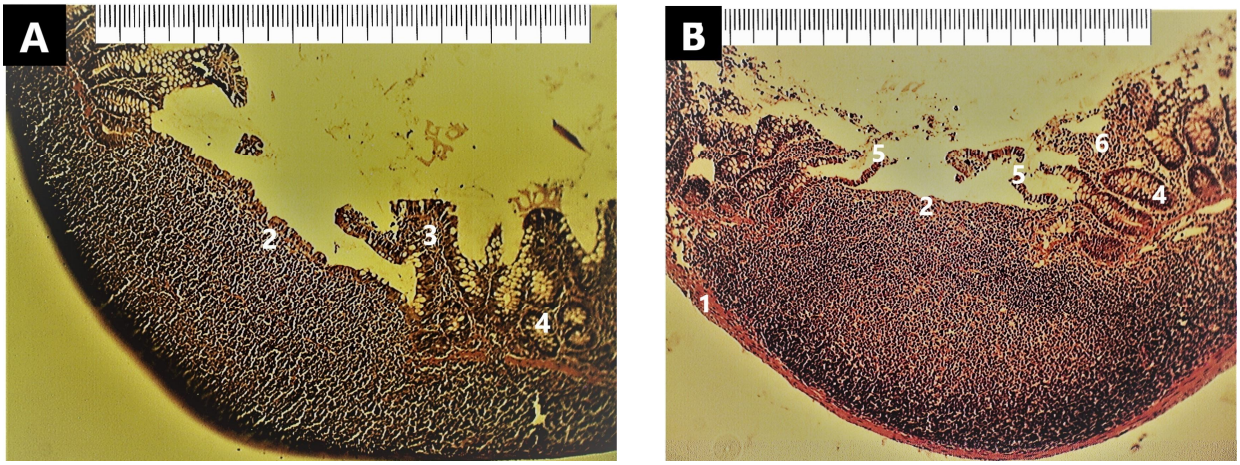

Fig. 3 (A, B). Large lymphoid nodules of Peyer's patches of the small intestine in albino rats after administration of the antibiotic (clarithromycin). A – intact lymphoid-associated epithelium and adjacent structures (intestinal villi and crypts); B – the phenomena of destructive changes. Paraffin sections; H&E stain; lens 10 × magnification. The smallest step of the scale is equal 10 μm. 1 – muscular tunic; 2 – the apical surface of the lymphoid nodule; 3 – intestinal villi; 4 – intestinal crypts; 5 – excoriation of lymphoid-associated epithelium; 6 – destruction of intestinal villi and crypts.

At the same time it has been noted that in this type of nodules partial or complete excoriation of lymphoid-associated epithelium occurred; as a result, underlying lymphoid tissue was in direct contact with the contents of the small intestine (fig. 3 B). This clearly pronounced destructive phenomenon can also apply to adjacent intestinal villi and crypts. It is noteworthy that such alternative processes were not detected in the small- and medium-sized lymphoid nodules.

Consequently, we hypothesize that the destructive changes in lymphoid nodules of the Peyer's patches, caused by administration of the antibiotic refer only to their "old" forms, which were replaced by new generations. However, due to the fact that the "old" lymphoid nodules persist for a certain time (the time period is unknown), the area of Peyer's patches might increase due to the increase of newly formed nodules in them.

Conclusion

1. It has been established that after the course of administration of a broad-spectrum antibiotic (clarithromycin) in the small intestine of albino rats, the topography and total number of Peyer's patches remain unchanged, while their total area increased by more than twice.

2. We hypothesize, that this hyperplasia of structured lymphoid tissue in the mucous membrane of the small intestine of animals under the influence of antibacterial drug occurs due to the appearance of new generations of lymphoid nodules in the Peyer's patches, among which small, medium and large forms are distinguished, similar to the stages of their development. Each of them is distinguished by its morphological features, primarily, the conversion of the lymphoid-associated epithelium.

3. Therefore, the genetically programmed total number of Peyer's patches in the small intestine of mature albino rats is constant, whereas the number of lymphoid nodules in them is a variable that depends on the state of the intestinal microbiocenosis.

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