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*I.Y.Sydorchuk,**O.I.Holyar,**M.O.Sokolenko*Bukovyna state medical university,  
ChernivtsyTHE DEGREE OF TETRACYCLINE  
INFLUENCE ON THE INTESTINE  
MICROBIOCENOSIS AND ITS  
CORRECTION BY "BIFIFORM - 21"**Key words:** *microbiome, large and small intestine, normal flora, dysbacteriosis, tetracycline.***Abstract:** *The paper presents a theoretical synthesis and scientific solution of scientific problem, grounded on the experimental side effects of broad-spectrum antibiotic (tetracycline hydrochloride) on qualitative and quantitative composition of the microbiota in the biological layer on mucus membrane of colon and distal part of small intestine; substantiation of self-healing process within 10 days qualitative and quantitative composition of the microbiota of given biotype, depending on the dose of tetracycline (average and maximum therapeutic dose) orally administered white rats, and the influence of probiotic "Child powder Biform number 21" on the restoration of the qualitative and quantitative composition of the gut microbiome.*

Accumulated in our country and around the world a vast experience of antibiotics allowed review of the basic principles and concept of their purpose, to make the most efficient use of different groups of antibiotics, however, to prevent or minimize the level of side effects and complications of varying severity. Determination of the optimal dose of antibiotic in the causal treatment is extraordinary and crucial to the successful treatment of bacterial infectious diseases [1, 2]. The inefficiency of causal antibiotic therapy may be associated with uneffective selection of therapeutic doses. Small doses often provide therapeutic concentrations in the blood and tissues, but leading to a lack of success in treatment, or transition of diseases to a chronic form, and the formation of resistance of the pathogen to antibiotic [3]. A typical example of this error is the usage of tetracyclines in adult patients of a single dose of 10 mg / kg or less, while the average therapeutic dose is 20 mg / kg [4].

The use of antibiotics in medical practice, in some cases is accompanied by the development of side effects, reactions, life-threatening to patients. In recent years, this problem is given the utmost importance both in domestic and foreign publications. Years of experience in the use of antibiotics leads to the objective analysis and synthesis of their application and to the formulation of basic fundamental concepts of characteristics of adverse reactions and processes [5]. In most cases, causal treatment with antibiotics is accompanied by the formation of varying degrees of intestinal dysbiosis / dysbacteriosis. Formed under the influence of various factors, primarily on the use of antibiotics,

gastro - intestinal dysbiosis can be hardly corrected, which significantly complicates the course of disease treatment [6, 7].

However, the mechanism for violations microbiome with antibiotics, depending on the dose and the duration of use of broad-spectrum antibiotics is not known, and also the guidelines on use of probiotics after antibacterial agents depending on their doses is not developed [8, 9]. Experimental studies are aimed to establish violations species composition and population levels of microbiome under the influence of different doses of broad-spectrum antibiotics, duration of use, taking into account function of the immune system and the methods of application for pre- and probiotics to correct qualitative and quantitative composition of the microbiome habitat [10].

**Materials and methods**

The experimental study was conducted on 70 white inbred albino rats weighing 180 - 220 g. The animals were obtained from the vivarium of Bukovina state medical university, they were kept under standard conditions on a standard diet and taken in experiment after a 2 - week quarantine in the absence of any signs of disease. Experiments were conducted in accordance with the Law of Ukraine № 3447-IV and the European Convention for the Protection of Vertebrate Animals used for research and other purposes.

Effect of different doses of tetracycline on the microbiota of the large and distal small intestine was studied by daily oral administration with metallic probe directly into the rat's stomach within 5 days in a volume less than 1 ml tetracycline at avera-

therapeutic dose (20 mg / kg) and maximum (50 mg / kg). Control animals within 5 - days were injected daily with 1 ml isotonic sodium chloride solution into the stomach.

The process of self-healing was performed by accepted in standard vivarium conditions for 10 days after administration of different doses of tetracycline.

Species composition and population-level microbiome different habitats studied microbiological and mycological method I.Y.Sydorchuk, O.I.Holyar (2009 - 2010), [12 - 15].

#### Results and Discussion

The first stage of research was to study the species composition and population levels and the microbiota of biological layer in mucus membrane of the distal part of small intestine and the large intestine.

The effect of different therapeutic doses of tetracycline hydrochloride (medium - 20 mg / kg and maximum - 50 mg / kg) on the species composition (qualitative) and the population (quantitative) level of indigenous obligate anaerobic bacteria of the genus *Bifidobacterium*, *Lactobacillus*, *Peptostreptococcus*, *Eubacterium*, *Bacteroides* and facultative anaerobic bacteria of the genus *Enterococcus*, and the qualitative and quantitative composition of facultative anaerobic and aerobic *Enterobacteriaceae* (bacteria of the genus *Escherichia*, *Proteus*, *Klebsiella*, *Edwardsiella*, *Pantoteya*, *Staphylococcus*, etc.)

Hydrochloride tetracycline in dose of 20 mg / kg (therapeutic dose) administered for 5 days directly into the stomach of intact white rats leads to minor changes in species composition and population levels but deep violations of oral microbiota of the colon by reducing the quantitative composition of autochthonous obligate anaerobic bifidobacteria, lactobacilli, eubacteria ( $p < 0.001$ ) and significant ( $p < 0.001 - < 0.05$ ) increase in population-level electives *Bacteroides*, peptokokki, clostridia, enterobacteria (*edwardsiyelamy*, *pantoteyamy*, *Klebsiella*, *Proteus*).

That dose of tetracycline hydrochloride weakens the colonization resistance of the mucous membrane of the colon due to profound changes in species composition of the microbiota biological layer on mucus membrane of habitat. That leads to the elimination of bifidobacteria, eubacteria, enterococci, peptostreptokokki and colonization of the colon mucosa conditionally pathogenic enterobacteria (*edwardsiyelamy*, *Klebsiella*, *pantoteyamy*, *Proteus*), staphylococci, peptokokki, clostridium and pseudomonas.

Average dose of tetracycline hydrochloride in intact animals after 5 days of administration leads to a significant ( $p < 0.01$ ) reduction in the number of lactobacilli and increase population levels of *Enterobacteriaceae* and *Bacteroides* and *E. coli*.

*bacteriaceae* and *Bacteroides* and *E. coli*.

Of particular importance in the formation of infection in the distal small intestine, which is characterized by significant vascularization and localization of lymphoid clusters associated with mucous membrane, the surface of which, is the most capable for resorption of antigens (microorganisms) comparatively other parts of the intestine. This gives it a propensity to contact with microorganisms, antibiotics and various chemicals. The treatment of acute infectious intestinal diseases using causal antibiotics, effects the intestines endogenous microbiota overgrowth (dysbiosis).

Colonization resistance of the mucosa of the distal small intestine under the influence of medium doses of tetracycline is undergoing profound changes, characterized by changes in qualitative and quantitative interrelations, thus reveals a pronounced deficit of obligate indigenous bifidobacteria, lactobacilli, enterococci, and peptostreptokokkipryepitelanoyi biofilm contamination of the mucous membrane of pathogenic and conditionally pathogenic enterobacteria, staphylococcus, clostridium and peptokokki that reach this biotope high population level, quantitative dominance coefficient and significance.

Thus, average therapeutic dose of tetracycline hydrochloride (20 mg / kg) per oral administration in intact white rats for 5 days leading to the elimination of the distal small intestine cavity enterococci, hay bacillus, peptostreptokokki, in some animals - bifidobacteria and lactobacilli. Under the impact of this dose it comes a powerful oral contamination of the distal small bowel pathogens (*Escherichia enterotoksyhenny*) and opportunistic (*Proteus*, *Klebsiella*, *pantoteyamy*, *edwardsiyelamy*) enterobacteria, staphylococci and bacteria of the genus *Clostridium*. As a result, in the cavity of the small intestine it creates a pronounced shortage of indigenous obligate anaerobic bifidobacteria, lactobacilli, eubacteria and enterococci, peptostreptokokki. Against this background, disrobed increases the level of pathogenic and conditionally pathogenic enterobacteria, staphylococci and clostridia.

Using a medium therapeutic doses of tetracycline hydrochloride does not affect the quality and quantity of *Bacteroides*, eubacteria, but a growing number of *E. coli*.

Despite the frequent use of tetracycline at present, the effect of these drugs on the normal intestinal flora has not been studied. In previous studies we examined the influence of the features on abdominal mukoznu microbiota and the colon and the distal small intestine average therapeutic dose (20 mg / kg) of tetracycline hydrochloride. It is necessary to study side effects of antibiotic use at the maximum

therapeutic dose - 50 mg / kg.

The maximum therapeutic dose (50.0 mg / kg) of tetracycline hydrochloride introduced directly into the stomach for 5 days leads to a pronounced imbalance of microflorapreepithelial biofilm of mucosa membrane of the colon, characterized by sterilizing effect of antibiotic concerning endogenous obligate anaerobic, facultative anaerobic and aerobic bacteria. In preepithelial biofilm of mucosa membrane of the colon bacteroides are only in small amounts and conditionally pathogenic enterobacteria (*Klebsiella*, *pantoteyi*) peptokokki and clostridia, which contaminate mucosa membrane of the colon and reach the minimum population level.

Thus, the maximum therapeutic dose (50.0 mg / kg) of tetracycline hydrochloride, applied directly into the stomach of white rats for 5 days, caused the elimination of oral colon autochthonous obligate anaerobic bacteria of the genus *Bifidobacterium*, *Lactobacillus*, *Peptostreptococcus* and facultative anaerobic and aerobic - *Enterococcus*, *B. subtilis* (transient microorganisms) and contamination by pathogenic oral colon (enterotoksyhennymy *Escherichia*) and opportunistic pathogens (*Klebsiella*) enterobacteria, peptokokki and clostridium. Last microorganisms reach high population levels. This dose results in a sterilizing effect on the content of oral colon of experimental animals.

The main source of oral microflora in the distal small intestine is mucous microflora (microbiota preepithelial biological film mucosa) and to a lesser extent allochthonous microflora that falls into this habitat with food and drinks. Microflorapreepithelial biological film is the most stable and plays a significant role in colonization resistance and maintaining the health of the host. Therefore, the next step on the impact of the maximum therapeutic dose (50.0 mg / kg) of tetracycline on the microbiota of the distal small intestine microflorapreepithelial was to study the biological film of this habitat.

The process of self-healing within 10 days of the oral microbiota in the colon in experimental animals that received within 5 days maximum therapeutic dose of tetracycline hydrochloride (50 mg / kg), is characterized by the appearance of moderate population level in all animals indigenous obligate anaerobic bacteria of the genus *Lactobacillus*, *Bifidobacterium*, *Bacteroides*, *Peptostreptococcus* and facultative anaerobic and aerobic bacteria of the genus *Escherichia*, *Enterococcus*, *Bacillus*, decrease opportunistic enterobacteria sowing and emergence of yeasts of the genus *Candida* and other opportunistic microorganisms.

After the cessation of oral administration of tetracycline in doses of 50 mg / kg in the self-healing

within 10 days of emerging tend of normalization of oral microflora in the distal small intestine due to the appearance and achieving moderation in oral ulcers obligate indigenous bacteria of the genus *Bifidobacterium*, *Lactobacillus*, *Peptostreptococcus*, *Enterococcus* and elimination of oral ulcers some genera of *Enterobacteriaceae* (*Klebsiella*).

Preepithelial biofilm microbiota on mucosa of the distal small intestine tends to normalize due to the elimination of this habitat specific *Enterobacteriaceae* (*Pantotea*) and the emergence of indigenous obligate anaerobic bacteria of the genus *Bifidobacterium*, *Lactobacillus*, *Eubacterium*, *Peptostreptococcus*, facultative anaerobic and aerobic bacteria of the genus *Enterococcus*, *Escherichia*, that achieve moderate population level and become dominant, forming the main habitat microbiota.

After creating a model of intestinal dysbiosis administration orally daily for 10 days of tetracycline in maximum therapeutic dose on the sixth day spent decontamination measures aimed to correcting qualitative and quantitative composition of the microbiota of oral and preepithelial biological film mucosa of colon and distal small intestine, with "Bififormu children powder 21."

Thus, oral use in animals of *Bififormu* at dysbiosis of III - IV degree for 10 days significantly improves the qualitative and quantitative composition of the microbiota of oral colon due to the appearance and achieving moderate (normal) population-level quantitative dominance coefficient and coefficient significance bacteria of the genus *Bifidobacterium*, *Lactobacillus*, *Bacteroides*, *Peptostreptococcus*, *Enterococcus*, *Escherichia*.

Above listed proved that the use of *Bififormu* within 10 days actively improves the qualitative and quantitative composition of microflora content cavity distal small intestine. However, full recovery during this period does not occur, requiring continuation of probiotics administration.

#### Conclusions

1. The average therapeutic dose of tetracycline hydrochloride (20 mg / kg), introduced directly into the stomach by metallic probe leads to minor changes in species composition and population levels deep violated of oral microbiota and biological preepithelial film mucosa of the colon and distal small intestine - reducing the quantitative composition of indigenous obligate anaerobic bacteria of the genus *Bifidobacterium*, *Lactobacillus*, *Eubacterium* ( $p < 0,001$ ) and significant ( $p < 0,001 - < 0,05$ ) increase in population levels of opportunistic bacteria of the genus *Bacteroides*, *Peptococcus*, *Clostridium* and *Enterobacteriaceae* (bacteria of the genus *Klebsiella*, *Edwardsiella*, *Pantotea*, *Proteus*). The indicated dose

of antibiotics leads to the weakening of colonization resistance of the mucous membrane of the colon due to the profound changes of the quality of the microbiota preepithelial biological film that manifests elimination of biofilm preepithelialmucosa of the colon by representatives of indigenous obligate anaerobic microflora.

2. Maximum therapeutic dose of tetracycline (50 mg / kg) daily orally for 5 days shows a sterilizing effect on the content and in the biofilm preepithelialny mucosa of the colon and distal small intestine, contributing significantly reduced colonization resistance of the colon mucosa and distal small intestine and it can become a gateway to the penetration of pathogenic and conditionally pathogenic enterobacteria and others. through the wall of the colon into the blood and internal organs.

3. When using the average therapeutic dose of tetracycline (20 mg / kg) after 10 days oral microbiota and mucosa of the colon and distal small intestine is characterized by gradual slow (at 3.33 - 35.1%) growth of obligate indigenous bacteria of the genus Bifidobacterium, Lactobacillus, Eubacterium, but their population level remains unchanged. The state of mucosamicroflora (preepithelial biofilm) is characterized by increasing population levels, constancy index and quantitative dominance factor in bacteria Lactobacillus, Bacteroides, Peptococcus, Clostridium, Staphylococcus and opportunistic enterobacteria. This preepithelialbiofilm mucosa of the colon and distal small intestinearecontaminated with yeast fungi of Candida.

4. The process of self-healing within 10 days of the oral microbiota of the colon and distal small intestine of white rats for 5 days receive maximum therapeutic dose (50 mg / kg), tetracycline, characterized by the appearance of moderate population level in all animals indigenous obligate bacterial genus Bifidobacterium, Lactobacillus, Bacteroides, Peptostreptococcus, Enterococcus, Escherichia; decline in a sowing of opportunistic enterobacteria and yeast-like fungi contamination habitat genus Candida, other opportunistic microorganisms. The broken qualitative and quantitative composition of the microbiota preepithelial biofilm mucosa of the colon and distal small bowel restored by colonization of mucosal endogenous obligate autochthonous bacteria of the genus Bifidobacterium, Lactobacillus, Bacteroides, etc.

5. Comparative analysis of the natural self-healing oral microbiota and preepithelial biofilm intestinal mucosa (colon and distal small intestine) after using the average and maximum doses of broad-spectrum antibiotics, confirming that after using the maximum therapeutic dose that causes a sterilizing effect, the

process of the natural self-healing occurs more actively and intensively, which makes it possible to recommend the use of broad-spectrum antibiotics in maximum therapeutic doses for 5 days.

6 "Biform baby powder number 21" is an effective means of restoring the qualitative and quantitative composition of microflora and oral preepithelial biological film mucosa of the colon and distal small intestine of white rats in which intestinal dysbiosis was formed by a five-day administration of tetracycline in the maximum therapeutic dose (50 mg / kg).

Usage of Biform leads to decontamination and elimination of pathogens (*Escherichia enterotoksyhennyh*) and opportunistic enterobacteria, peptococci, clostridia, staphylococci and other microorganisms.

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**СТЕПЕНЬ ВЛИЯНИЯ ТЕТРАЦИКЛИНА НА  
МИКРОБИОЦЕНОЗ КИШЕЧНИКА И ЕГО  
КОРРЕКЦИЯ ПРЕПАРАТОМ "БИФИФОРМ-21"**

*И.И. Сидорчук, О.И.Голяр, М.О.Соколенко*

**Резюме.** В работе приведено теоретическое обобщение и научное решение научной задачи, которая проявляется в экспериментальном обосновании побочного действия

антибіотика широкого спектра діяння (тетрацикліна гідрохлориду) на якісний та кількісний склад мікробіоти порожнини і приєпітеліальної біологічної плівки слизової оболонки товстої та дистального відділу тонкої кишки; обґрунтуванні процесів самовідновлення протягом 10 днів якісного і кількісного складу мікробіоти цього біотопу у залежності від дози (середньої і максимальної терапевтичної дози) при пероральному введенні білим крысам, а також вплив пробіотика "Біфіформ дитячий порошок № 21" на відновлення порушеного якісного і кількісного складу мікробіоти кишечника.

**Ключевые слова:** мікробіома, товста і тонка кишка, нормофлора, дисбактеріоз, тетрациклін.

**СТУПІНЬ ВПЛИВУ ТЕТРАЦИКЛІНУ НА  
МІКРОБІОЦЕНОЗ КИШЕЧНИКУ ТА ЙОГО  
КОРЕКЦІЯ ПРЕПАРАТОМ "БІФІФОРМ-21"**

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**Резюме.** У роботі наведено теоретичне узагальнення і наукове вирішення наукової задачі, що виявляється в експериментальному обґрунтуванні побічної дії антибіотику

широкого спектру дії (тетрацикліну гідрохлориду) на якісний та кількісний склад мікробіоти порожнини і приєпітеліальної біологічної плівки слизової оболонки товстої та дистального відділу тонкої кишки; обґрунтуванні процесів самовідновлення протягом 10 днів якісного і кількісного складу мікробіоти цього біотопу у залежності від дози (середньої і максимальної терапевтичної дози) при пероральному введенні білим крысам, а також вплив пробіотика "Біфіформ дитячий порошок № 21" на відновлення порушеного якісного і кількісного складу мікробіоти кишечника.

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