

THE STUDY OF THE ANTAGONISTIC EFFECT OF PROBIOTICS IN DYSBIOTIC DISORDERS IN RAT INFECTIOUS COLITIS MODEL

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Background. *Clostridium difficile* (*C. difficile*) is the most common etiological factor for antibiotic-associated diarrhea. The development of this type of infection can lead to serious complications such as pseudomembranous colitis. This condition is difficult to treat and can lead to the persistent disability of working-age patients. Probio re and Probio re plus are modern high-dose probiotics.

Objective – to determine the effect of the use of Probio re and Probio re plus for the treatment of dysbiotic disorders that occurred in the modelling of infectious colitis in rats.

Material and methods. Clinically healthy white outbred rats weighing 200.0-250.0 grams were taken for the experiment. To create experimental dysbiosis, animals were immunosuppressed by intramuscular injection of cyclophosphamide: the daily dose was 0.6 mg/kg for 7 days. Thereafter, exogenous microbial loading was performed by introducing into the stomach 1 ml of a suspension of *St. aureus*, *C. albicans* and *Cl. perfringens* for 3 days. The animals were treated for 5 days starting from the 11th day of the experiment. Rats were divided into the groups of 6 animals: group 1 - Probio re; group 2 - Probio re plus; group 3 - placebo (water); group 4 - intact animals.

Results. The antagonistic effects of Probio re and, to a greater extent, Probio re plus against pathogenic microorganisms were detected. These probiotics restored the indices of normal intestinal microflora and suppressed pathogenic and conditionally pathogenic microflora.

Conclusios. Probio re and Probio re plus can be used for the treatment of the dysbiotic disorders associated with infectious colitis.

Keywords: probiotics, diarrhea, infectious colitis, *Saccharomyces boulardii*, *Lactobacillus rhamnosus* GG.

ИССЛЕДОВАНИЕ АНТАГОНИСТИЧЕСКОГО ЭФФЕКТА ПРОБИОТИКОВ ПРИ ДИСБИОТИЧЕСКИХ НАРУШЕНИЯХ НА МОДЕЛИ ИНФЕКЦИОННОГО КОЛИТА У КРЫС

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Введение. *Clostridium difficile* (*C. difficile*) чаще всего является этиологическим фактором антибиотик ассоциированной диареи. Развитие данного вида инфекции может привести к таким тяжелым осложнениям, как псевдомембранозный колит. Это состояние трудно поддается лечению и может завершиться стойкой инвалидизацией пациентов трудоспособного возраста. Пробио-ре и пробио-ре плюс – современные высокодозные пробиотики.

Цель исследования – определение эффекта применения пробио-ре и пробио-ре плюс для лечения дисбиотических нарушений, которые возникали при моделировании инфекционного колита у крыс.

Материал и методы. Для эксперимента брали беспородных клинически здоровых белых крыс массой 200,0-250,0 граммов. С целью создания экспериментального дисбиоза животным проводили иммуносупрессию путем внутримышечного введения циклофосфамида: суточная доза составляла 0,6 мг/кг в течение 7 дней. После этого экзогенную микробную нагрузку осуществляли введением в желудок 1 мл суспензии *St. aureus*, *C. albicans* и *Cl. perfringens* в течение трех дней. Лечение животных начинали с 11 суток эксперимента и проводили в течение пяти дней. Крысы были разделены на группы по 6 особей: группа 1 – пробио-ре; группа 2 – пробио-ре плюс; группа 3 – плацебо (вода) группа 4 – интактные животные.

Результаты. Выявлены антагонистические эффекты пробио-ре и в большей степени – пробио-ре плюс в отношении патогенных микроорганизмов. Эти пробиотики восстановили показатели нормальной микрофлоры кишечника и подавляли патогенную и условно патогенную микрофлору.

Выводы. Для лечения дисбиотических нарушений, ассоциированных с инфекционным колитом, целесообразно назначать пробио-ре плюс и пробио-ре.

Ключевые слова: пробиотики, диарея, инфекционный колит, *S. boulardii*, *L. rhamnosus* GG.

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Introduction

Antibiotic associated diarrhea (AAD) is an urgent problem in modern medicine. It develops as a result of the use of broad-spectrum antibiotics and is treated by the use of antibiotics (vancomycin, metronidazole) which do not always lead to complete recovery and have side effects. *Clostridium difficile* (*C. difficile*) is a gram-negative spore-forming bacterium that is the most common etiological factor of the antibiotic-associated diarrhea [1]. The development of this type of infection can lead to serious complications such as pseudomembranous colitis. This condition is difficult to treat and can lead to persistent disability of working-age patients. *C. difficile* infection occurs mostly in hospitalized patients. Current scientific evidence suggests that probiotics, namely *Saccharomyces boulardii* (*S. boulardii*) and *Lactobacillus rhamnosus*, may be used as prophylactic agents [2, 3]. Probio re and Probio re plus are modern high-dose probiotics containing *S. boulardii*, *Lactobacillus helveticus*, *Lactobacillus rhamnosus* GG and *Bifidobacterium longum*. Considering that dysbiotic phenomena occur in AAD, the rational scientific direction is to study the antagonistic effect of probiotics on the model of infectious colitis in laboratory rats.

Objective was to investigate the effect of the use of probiotics probio re and probio re plus for the treatment of dysbiotic disorders that occurred in the modelling of infectious colitis in rats.

Material and methods

Clinically healthy white outbred rats weighing 200.0-250.0 grams, previously quarantined, were taken for the experiment. All animals were kept under the same conditions (temperature, humidity, lighting, diet). Induction of dysbacteriosis, infection, treatment and removal of rats from the experiment were carried out in accordance with the provisions of the Decree of the First National Congress on Bioethics "General Ethical Principles of Experiments on Animals" (2001), the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes of 18.03.1986, EEC Directive No. 609 of 24.11.1986, the provisions of the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty". All animals were kept under the same conditions (temperature, humidity, lighting, and diet).

To prove the experimentally created infectious colitis, the animals were examined for the

compliance with the normal intestinal microbiota of the rat flora [4].

To create experimental dysbiosis, animals were immunosuppressed by intramuscular injection of cyclophosphamide: the daily dose was 0.6 mg / kg for 7 days.

Subsequently, exogenous microbial loading was performed by introducing into the stomach using a special curved metal cannula 1 ml of a suspension of *Staphylococcus aureus* (10⁹ cells/ml), *Candida albicans* (10¹⁰ cells/ml) and *Clostridium perfringens* (10⁸ cells/ml) for 3 days [5]. *Clostridium perfringens* culture was grown on solid nutrient medium under anaerobic conditions (anaerostat).

Suspensions with the required number of microorganisms were prepared using an electronic device Densi-La-Meter (manufactured by PLIVA-Lachema, Czech Republic; wavelength 540 nm) according to the instrument instruction and information sheet on innovations in the healthcare system № 163-2006 "Standardization for the preparation of microbial suspensions", Kyiv.

The animals were treated for 5 days from the 11th day of the experiment. Rats were divided into groups of 6 animals: group 1 – the treatment was with Probio re at a dose of 40 mg/kg; group 2 – the treatment was with Probio re plus at a dose of 40 mg/kg; group 3 - placebo group (water); group 4 - intact animals.

Microbiological study of the faeces was performed on the 11th and 15th days of the experiment in the laboratory of biochemistry and biotechnology, I.I. Mechnikov Institute of Microbiology and Immunology.

Isolation of pathogens from the faeces was performed by conventional methods of bacteriological examination of dysbiosis [6]. Identification of the extracted cultures of microorganisms was carried out by morphological, tinctorial, cultural, biochemical properties by conventional methods in accordance with the determinant of bacteria Bergie [7]. The obtained results allow to characterize the quantitative and qualitative composition of the tested samples.

All results were summarized in the table and analysed mathematically using non-parametric Mann-Whitney criteria.

Results and discussion

In the study of the samples of infected animals on the 11th day of the experiment the signs of

Table 1. – The results of microbiological study of the faeces samples on the 11th day of the experiment

Number of Animals	Animal group	Total amount of <i>E. coli</i> , CFU/g	<i>S. aureus</i>	<i>Bifidobacteria</i> , CFU/g	<i>Lactobacilli</i> , CFU/g	<i>Candida</i> , CFU/g	<i>Clostridia</i>
	Normal indicators	10 ⁴ -10 ⁷	10 ³ -10 ⁵	10 ⁶ -10 ⁹	10 ⁶ -10 ⁸	0-10 ³	–
6	Intact animals	10 ⁵ – 10 ⁶	10 ² – 10 ³	10 ⁸ – 10 ⁹	10 ⁶ – 10 ⁷	10 ² – 10 ³	–
6	Infected animals	10 ²	10 ⁶	10 ⁵	10 ⁵	10 ⁴	+
7		10 ³	10 ⁶	10 ⁵	10 ⁵	10 ⁴	+
4		10 ³	10 ⁷	10 ⁵	10 ⁵	10 ⁴	+
1		10 ³	10 ⁵	10 ⁵	10 ⁵	10 ⁴	+

dysbiotic disorders development were revealed in all rats except group 4. In comparison with the intact and control groups, the normal intestinal microflora significantly decreased - the total number of *E. coli*

in 100% of cases ($p = 0.02$) by 2-3 ranks. *Bifidobacteria* and *Lactobacilli* decreased in 100% of animal samples ($p = 0.05$).

The number of *S. aureus* and *C. albicans* exceeded normal levels by 1-2 ranks in 100% of cases. *Clostridia* was isolated from all animals.

During the whole experiment, the representatives of the control group 4 (intact animals) revealed normal intestinal microbiota (Table 1).

The obtained results indicate that dysbiotic phenomena associated with infectious colitis have developed in laboratory rats.

After a five-day administration of Probio re at a dose of 40 mg/kg for 15 days in group 1, 100% of cases increased the total number of *E. coli* ($p = 0.05$) – equally by distribution for each rank (33.3%), 10⁴ CFU/g, 10⁵ CFU/g, 10⁶ CFU/g. After the treatment with Probio re plus for five days at a dose of 40 mg/kg, the total number of *E. coli* also recovered in 100% of cases. In 67% of cases, the indicators increased to 10⁶ CFU/g, in the remaining 33% to 10⁵ CFU/g (Table 2).

Table 2. – The results of microbiological study of the samples of faeces of experimental animals in groups on the 15th day of the experiment

Animal group	The substance used and dose, mg/kg	Total number of <i>E. coli</i> , CFU/g	<i>S. aureus</i> , CFU/g	<i>Bifidobacteria</i> , CFU/g	<i>Lactobacilli</i> , CFU/g	<i>Candida</i> , CFU/g	<i>Clostridia</i>
	Normal indicators of microbiota	10 ⁴ -10 ⁷	10 ³ -10 ⁵	10 ⁶ -10 ⁹	10 ⁶ -10 ⁸	0-10 ³	–
1	Probio re Dose 40 mg/kg	10 ⁴	10 ⁵	10 ⁶	10 ⁶	10 ³	–
		10 ⁴	10 ⁵	10 ⁶	10 ⁶	10 ³	–
		10 ⁵	10 ⁵	10 ⁶	10 ⁶	10 ³	–
		10 ⁵	10 ⁴	10 ⁶	10 ⁶	10 ³	–
		10 ⁶	10 ⁴	10 ⁶	10 ⁶	10 ³	–
		10 ⁶	10 ⁴	10 ⁶	10 ⁶	10 ³	–
2	Probio re plus Dose 40 mg/kg	10 ⁶	10 ⁴	10 ⁹	10 ⁸	10 ²	–
		10 ⁶	10 ⁴	10 ⁹	10 ⁸	10 ²	–
		10 ⁶	10 ⁴	10 ⁹	10 ⁸	10 ²	–
		10 ⁶	10 ⁴	10 ⁸	10 ⁸	10 ²	–
		10 ⁵	10 ³	10 ⁸	10 ⁷	10 ³	–
		10 ⁵	10 ³	10 ⁸	10 ⁷	10 ³	–
3	Placebo (Sterile water 3 ml)	10 ²	10 ⁶	10 ⁵	10 ⁵	10 ⁴	+
		10 ²	10 ⁶	10 ⁵	10 ⁵	10 ⁴	+
		10 ²	10 ⁶	10 ⁵	10 ⁵	10 ⁴	+
		10 ²	10 ⁷	10 ⁴	10 ⁵	10 ⁴	+
		10 ²	10 ⁷	10 ⁴	10 ⁴	10 ⁴	+
4	Intact animals	10 ⁵ -10 ⁶	10 ² -10 ³	10 ⁸ -10 ⁹	10 ⁶ -10 ⁷	10 ² -10 ³	–

In group 1 *S. aureus* decreased to normal in 100% of cases, specifically 50% of cases to 104 CFU/g, the rest to 105 CFU/g, respectively. In group 2, the indicators of *S. aureus* also decreased to normal in all animals. In 67% of cases up to 104 CFU/g, in 33% of cases up to 103 CFU/g ($p = 0,02$).

The number of *C. albicans* in groups 1 and 2 decreased to normal in all animals, but differed in distribution and rank – in group 1 in all cases the number reached 103 CFU/g, in group 2 in 67% of cases - 102 CFU/g, in 37% of animals 1 * 103 CFU/g ($p = 0,02$).

In the study of the representatives of the normal intestine flora and *Lactobacillus*, it was found out that in all tested samples there was a recovery of these parameters ($p = 0,05$). In group 1, the number of *B.* reached 106 CFU/g in 100% of cases, in group 2 the number of *Bifidobacteria* in 50% of animals increased to 108 CFU/g and in 50% of animals to 109 CFU/g. The number of *Lactobacillus* in group 1 increased to 106 CFU/g in all animals: in group 2 to

107 CFU/g in 33% of cases and in 67% of cases to 108 CFU/g. This can be explained by the presence in *Probio re plus L. helveticus*, *L. rhamnosus GG* and *B. longum*.

Summarizing all of the above, *Probio re plus* in the experiment showed a higher efficiency than *Probio re* for the correction of dysbiotic disorders - restoration of normal microflora and inhibition of pathogenic one.

Conclusions

The study revealed the antagonistic effect of *Probio re* and, to a greater extent, *Probio re plus*. These probiotics significantly restored the indicators of normal intestinal microflora and suppressed pathogenic and conditionally pathogenic microflora. Thus, considering the composition of these probiotics, it can be concluded that their use is rational for the treatment of dysbiotic disorders associated with infectious colitis.

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