УДК 615.246.8:616.34-002.191]-092.9 doi: 10.25298/2616-5546-2020-4-1-86-89

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

Yu. V. Verkhovodova, I. V. Kireev
National University of Pharmacy, Kharkiv, Ukraine

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

Ю. В. Верховодова, И. В. Киреев
Национальный фармацевтический университет, Харьков, Украина

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.*

Автор, ответственный за переписку:
Ю. В. Верховодова, Национальный фармацевтический университет,
e-mail: juliaverh73@gmail.com

Corresponding author
Vekhovodova Yulia; National university of pharmacy, Ukraine, Kharkiv,
e-mail: juliaverh73@gmail.com

Для цитирования:
Verkhovodova, Yu. V. Study of the antagonistic effect of probiotics in dysbiotic disorders in rat infectious colitis model / Yu. V. Verkhovodova, I. V. Kireev // Гепатология и гастроэнтерология. 2020. Т. 4, № 1. С. 86-89. https://doi.org/10.25298/2616-5546-2020-4-1-86-89

For citation:
Verkhovodova YuV, Kireev IV. Study of the antagonistic effect of probiotics in dysbiotic disorders in rat infectious colitis model. Hepatology and Gastroenterology. 2020;4(1):86-89. https://doi.org/10.25298/2616-5546-2020-4-1-86-89

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 11^{th} and 15^{th} 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 *E. coli*, CFU/g	*S. aureus*	*Bifidobacteria*, CFU/g	*Lactobacilli*, CFU/g	*Candida*, CFU/g	*Clostridia*
	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 (pu = 0.02) by 2-3 ranks. Bifidobacteria and Lactobacilli decreased in 100% of animal samples (pu = 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 (pu = 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 E.coli, CFU/g	S. aureus, CFU/g	*Bifidobacteria*, CFU/g	*Lactobacilli*, CFU/g	*Candida*, CFU/g	*Clostridia*
	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 мл)	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	+
		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 (pu = 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 (pu = 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 (pu = 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.

References

1. Arjaev NL, Gudz VA, Kozhevin RV. Profilaktika antibiotik-associirovannoj diarei, vyzvannoj toksigennym shtammom Clostridium difficile u detej (obzor). *Ditjachij likar*. 2017;4(55):28-34. (Russian).
2. Zyrjanov SK, Galeeva ZhA, Belousov JuB. Probiotiki i prebiotiki pri antibiotik-associirovannoj diaree: chto dokazano? *Lekarstvennyj vestnik*. 2016;2(62):16-19. (Russian).
3. Wanke M, Szajewska H. Probiotics for preventing healthcare-associated diarrhea in children: A meta-analysis of randomized controlled trials. *Pediatria Polska*. 2014;89(1):8-16. doi: 10.1016/j.pepo.2013.12.003.
4. Putnikov AV, Golota JuV, Sergijchuk TM. Kilkisni ta funkcionalni pokazniki kishkovoї normobioti shhuriv. *Mikrobiologija ta biotehnologija*. 2015;2:89-100. (Ukrainian).
5. Verhovodova JuV, Kireyev IV, Koshovij OM, Miga MM, Potapov SM, Osolodchenko TP. Sposib modeljuvannja kolitu z disbiotichnimi porushennjami. UK patent 201907465. 2019 April 04. (Ukrainian).
6. Vandepitte J, Engbaek K, Rohner P, Piot P, Heuck C, Claus C. Basic laboratory procedures in clinical bacteriology. Geneva; 1994. p. 30-32.
7. Brenner DJ, Krieg NR, Staley JT, editors. The proteobacteria. Part C, The alpha-, beta-, delta-, andepsilonproteobacteria. 2nd ed. New York: Springer; c2005. 1388 p. (Garrity GM, editor. Bergey's manual of systematic bacteriology; vol. 2, pt. C).

Conflict of interest. The authors declare no conflict of interest.

Financing. The study was performed without external funding.

Conformity with the principles of ethics. The study was approved by the local ethics committee.

Information about authors:

Vekhovodova Yulia; National university of pharmacy, Ukraine, Kharkiv, e-mail: juliaverh73@gmail.com; ORCID: 0000-0002-5047-375X

Kireyev Igor, PhD, MD (Medicine), Professor; National university of pharmacy; e-mail: ivkireev@ukr.net, ORCID: 0000-0002-5413-9273

Поступила: 04.03.2020
Принята к печати: 13.03.2020

Конфликт интересов. Авторы заявляют об отсутствии конфликта интересов.

Финансирование. Исследование проведено без спонсорской поддержки.

Соответствие принципам этики. Исследование одобрено локальным этическим комитетом.

Сведения об авторах:

Верховодова Юлия Владимировна; Национальный фармацевтический университет, Харьков, Украина; e-mail: juliaverh73@gmail.com; ORCID: 0000-0002-5047-375X

Киреев Игорь Владимирович, д.м.н., профессор; Национальный фармацевтический университет, Харьков, Украина; e-mail: ivkireev@ukr.net, ORCID: 0000-0002-5413-9273

Received: 04.03.2020
Accepted: 13.03.2020