

THE EFFECT OF MICROCRYSTALLINE CELLULOSE ON THE MICROFLORA OF THE COLON

D.B. Koval, *H.R. Malyarchuk, O.O. Levenets

I. HORBACHEVSKY TERNOPIL NATIONAL MEDICAL UNIVERSITY, TERNOPIL, UKRAINE

Background. Nowadays, much attention is paid to enterosorption methods that allow cleansing the internal organs and removing extraneous substances out of the body of a sick person.

Objective. The aim of the research was to study the effect of microcrystalline cellulose on the microflora of the large intestine.

Methods. The study was performed on 50 white laboratory Wistar rats weighing 180-270 g, which were divided into control and experimental groups. The experimental group was daily administered with microcrystalline cellulose at a dose of 500 mg/kg. The study followed ethical standards and recommendations for the humanization of work with laboratory animals according to the "European Convention for the protection of vertebrate animals used for experimental and other purposes" (Strasbourg, 1986, 2010), as well as the requirements of the Commission on Bioethics of I. Horbachevsky Ternopil National Medical University (Minutes No. 66, dated November 01, 2021). The first group involved the intact rats on standard diet, the second – the rats, which received normal feeding of microcrystalline cellulose.

Results. In the feces of the experimental white rats treated with microcrystalline cellulose, the level of *Escherichia coli* in the large intestine decreased by 22 and 25%. The number of these microorganisms increased by 20% in 7 days and by 14% in 14 days. The content of epidermal staphylococci in the stool decreased by 10% on the 7th day of administration. Microcrystalline cellulose increased the number of *Staphylococcus aureus* by 12%, but decreased the number of enterococci in the feces by 28%. In 7-14 days of the experiment, the content of these bacteria did not change significantly in the colon. In relation to anaerobic microorganisms – bacteroides and clostridia, this supplement caused a slight increase in the number of bacteroides – by 8.64% and the number of clostridia – by 11.54% on the 14th day. The content of fungi of the *Candida* genus on the 7th and 14th day increased by 8.3%.

Conclusions. In the 2nd period of the study, the microbiome of intestinal contents worsened: the process of dysbacteriosis increased, which was manifested by a significant increase in the number of *Proteus* spp., *Staphylococcus aureus*, anaerobes (bacteroides, clostridia) and *Candida* spp., as well as decreased *Escherichia coli* and Enterococci.

KEYWORDS: enterosorption; microcrystalline cellulose; colon microflora; enterosorbents.

Introduction

These days the incidence of the large intestine disorders due to various microorganisms is increasing. Therefore, it is important to study the mechanisms of its development, features of early diagnosis, prevention and treatment. The large intestine is the main reservoir of the human microbiota in general and the digestive tract in particular; it is always dominated by characteristic groups of microorganisms, the number of species of which is small, but in quantitative terms they form the basis of the biocenosis [1,4]. The main microflora of the

*Corresponding author: Hanna R. Malyarchuk, PhD., senior assistant professor, I. Horbachevsky Ternopil National Medical University, Ternopil, 46001, Ukraine
E-mail: malyarchuk@tdmu.edu.ua

colon contains obligate anaerobic bacteria of the *Bifidobacterium*, *Bacteroides*, *Lactobacillus*, *Propionibacterium*, *Peptostreptococcus* genera, as well as additional anaerobic and aerobic bacteria of the *Escherichia*, *Enterococcus* genera. Additional colon microflora includes anaerobic bacteria of the *Peptococcus*, *Clostridium*, *Eubacterium*, *Fusobacterium* genera and additional anaerobic and aerobic bacteria of the *Staphylococcus*, *Citrobacter*, *Proteus*, *Enterobacter*, *Edwardsiella*, *Klebsiella* and other genera [1,5]. Enterosorption is an important method based on the binding and excretion of toxic substances from the gastrointestinal tract for therapeutic and prophylactic purposes. Microcrystalline cellulose is of particular interest. The supplement

gently cleanses the intestinal wall, absorbs toxins, poisons and other dangerous accumulations. For active sorption of toxins, re-administration of enterosorbents should last at least two weeks, so an important factor for their effective and safe use is the preservation or restoration of physiological parameters of gastrointestinal function, in particular normal intestinal microbiocenosis [6,9].

The aim of the research was to study the effect of microcrystalline cellulose on the microflora of the large intestine.

Methods

The study was performed on 50 white laboratory Wistar rats weighing 180-270 g, which were divided into control (20 rats) and experimental (30 rats) groups. The experimental group was daily administrated with microcrystalline cellulose at a dose of 500 mg/kg.

The study was carried out following ethical standards and recommendations for the humanization of work with laboratory animals according to the "European Convention for the protection of vertebrate animals used for experimental and other purposes" (Strasbourg, 1986, 2010), as well as requirements of the Committee on Bioethics of I. Horbachevsky Ternopil National Medical University (Minutes No. 66, dated November 1, 2021). The study protocol was approved by the university review boards and ethics committees of the participating centers.

Sampling was performed from the rectum with a disposable sterile cotton swab to remove feces. Disposable cotton swabs by Copan (Italy) were used. Before and after material collection, the tampon was weighed on torsion scales. The difference in weight was taken as the mass of stool. Then the swab was placed in 1 ml of 0.1% solution of Triton X-100 in 0.075 M phosphate buffer pH 7.9, and shaken thoroughly for 10-15 minutes. The prepared ten-fold dilutions of the material, inoculated on nutrient media of MPA, YSA, Endo, Sabouraud, Blaurock, MRS, agar-EDDS, was incubated at the optimum temperature of 37 °C.

In 24-96 hours of incubation the number of colonies was counted and the result was expressed by the number of colony-forming units per 1 gram of feces according to the formula: $X1 = 20 \cdot M \cdot N / t$, where X1 is the number of CFU/ml; 20 is the constant coefficient, when 0.1 ml of the sample is inoculated; M is the number of colonies that grew; N is the dilution (in 10, 100, 1000 times etc.); t is the

mass of feces. Since the number of microbes per unit can reach tens of thousands, we used the decimal logarithm of this indicator – log CFU/m.

The arithmetic mean and standard error ($M \pm m$) were used to describe the data in the normal distribution. Since the data obtained as a result of the clinical study had deviations from the normal distribution of the variation series, nonparametric statistical methods to compare groups – the Mann – Whitney U-test (for independent groups) was used. The software and mathematical complex for the personal computer Microsoft Excel 2016 was used for processing of the results of the study.

Results

The groups were statistically comparable at the beginning of the study. During the experiment, no significant changes of the microbiome of feces of the control rats on a fiber-free diet were evidenced, when comparing the two terms of the study (Tables 1 and 2). However, in the control group of animals, the composition of feces showed degree 1 dysbacteriosis (the concentration of normal microflora was below standard levels).

Microcrystalline cellulose reduced the level of *Escherichia coli* in the large intestine of the experimental rats up to 5.3 ± 0.2 and 5.2 ± 0.2 log CFU/g (by 22 and 25%, respectively), by 20% in 7 days, and by 14% in 14 days. On the 7th day of microcrystalline cellulose administration, the content of epidermal staphylococci in feces decreased by 10%. However, the microcrystalline cellulose increased the number of *Staphylococcus aureus* by 12%.

Microcrystalline cellulose reduced the number of enterococci in the feces by 28%. In contrast, after taking microcrystalline cellulose in 7-14 days of the experiment the content of these bacteria did not change significantly in the colon. In relation to anaerobic microorganisms: bacteroides and clostridia, this supplement caused a slight increase in the number of bacteroides – up to 8.8 ± 0.3 log CFU/g (8,64%) and a more significant increase in the number of clostridia – 8.7 ± 0.2 log CFU/g (11,54%) on the 14th day. After taking microcrystalline cellulose, the content of fungi of the *Candida* genus on the 7th and 14th day slightly increased by 8,3%.

Discussion

The effect of crystalline microcellulose on the intestinal microbiota has not been studied comprehensively. According to the literature,

Table 1. Effect of microcrystalline cellulose on the microflora of the large intestine of white rats, log CFU/g (on the 7th day of the study)

Types of microorganisms	Control (n=20)	Microcrystalline cellulose (n=30)
Escherichia coli	6.79±0.2	5.3±0.2 p<0.01
Enterobacteriaceae spp.	5.34±0.49	6.4±0.2 p<0.01
Staphylococcus epidermidis	5.44±0.2	4.9±0.3 p<0.05
Staphylococcus aureus	5.0±0.0	5.5±0.34 p<0.05
Streptococcus faecalis	5.53±0.2	4.0±0.2 p<0.002
Lactobacillus spp.	6.10±0.4	6.2±0.3 p<0.1
Bifidobacterium spp.	6.3±0.23	6.4±0.3 p<0.1
Bacteroides spp.	7.3±0.22	8.4±0.3 p<0.02
Clostridium spp.	7.3±0.12	7.4±0.3 p<0.1
Candida spp.	3.61±0.2	3.91±0.26 p<0.05
Proteus vulgaris	2.5±0.1	3.0±0.1 p<0.01

Table 2. Effect of microcrystalline cellulose on the microflora of the large intestine of white rats, log CFU/g (on the 14th day of the study)

Types of microorganisms	Control (n=20)	Microcrystalline cellulose (n=30)
Escherichia coli	6.9±0.2	5.2±0.2 p<0.01
Enterobacteriaceae spp.	5.6±0.3	6.4±0.2 p<0.02
Staphylococcus epidermidis	5.4±0.2	4.9±0.3 p<0.05
Staphylococcus aureus	5.0±0.2	5.6±0.34 p<0.05
Streptococcus faecalis	5.5±0.2	4.2±0.2 p<0.01
Lactobacillus spp.	6.1±0.4	6.2±0.3 p<0.1
Bifidobacterium spp.	6.3±0.2	6.4±0.3 p<0.1
Bacteroides spp.	8.1±0.2	8.8±0.3 p<0.05
Clostridium spp.	7.8±0.2	8.7±0.2 p<0.05
Candida spp.	3.6±0.2	3.9±0.26 p<0.05
Proteus vulgaris	2.5±0.1	3.5±0.1 p<0.001

concomitant therapy in patients with various pathologies shows significant effectiveness [10, 11]. The results of various studies prove a positive effect of enterosorbents on the manifestations of oxidative stress; it normalizes prooxidant-antioxidant balance [12,13]. However, no studies have been presented on the effects of enterosorbents on the microflora of the colon, especially in its prolonged administration.

Enterosorbents are biomaterials that "work" in the lumen of the gastrointestinal tract and have only local pharmacokinetics. However, in cases of sorption detoxification of the body, they act not only locally, but also have remote long-term effects [14]. The sorption of toxic metabolites and compounds relieves the main organs of metabolism and excretion. This explains the positive effect of enterosorbent in many comorbid pathologies. The effect of enterosorption on the intestinal microflora is also positive [15].

Conclusions

Microcrystalline cellulose entering the stomach has the ability to swell when absorbing fluid and is used to mechanically achieve a feeling of satiety and suppress appetite.

Mechanical cleaning of the intestinal mucosa and effective absorption of harmful substances when passing through the gastrointestinal tract allows using it in poisoning by chemicals, toxins and salts of heavy metals. However, in the 2nd period of the study, the microbiome of intestinal contents worsened: dysbacteriosis developed, which was manifested by a significant increase in the number of *Proteus vulgaris*, *Staphylococcus aureus*, anaerobes (*Bacteroides spp.*, *Clostridia spp.*) and *Candida spp.*, as well as decreased intestinal and intestinal bacteria.

Limitations of the study are the restricted number of laboratory animals and the limited range of methods for studying the large intestine microflora.

Conflict of Interests

Authors declare no conflict of interest.

Authors Contributions

Dmytro B. Koval, Hanna R. Malyarchuk – conceptualization, methodology, formal analysis, writing – original draft, writing – reviewing and editing; *Dmytro B. Koval, Hanna R. Malyarchuk* – data curation, writing – reviewing and editing; *Dmytro B. Koval, Hanna R. Malyarchuk, Olexandr O. Levenets* – investigation, formal analysis.

ВПЛИВ МІКРОКРИСТАЛІЧНОЇ ЦЕЛЮЛОЗИ НА МІКРОФЛОРУ ТОВСТОЇ КИШКИ

Д.Б. Коваль, *Г.Р. Малярчук, О.О. Левенець

ТЕРНОПІЛЬСЬКИЙ НАЦІОНАЛЬНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ І. Я. ГОРБАЧЕВСЬКОГО МОЗ УКРАЇНИ,
ТЕРНОПІЛЬ, УКРАЇНА

Вступ. У наш час велика увага надається методам ентеросорбції, які дозволяють очищати внутрішнє середовище та виводити з організму хворої людини чужорідні речовини.

Метою дослідження було вивчення впливу мікрокристалічної целюлози на мікрофлору товстого кишечника.

Методи. Дослідження проведено на 50 білих лабораторних щурах лінії Вістар масою 180-270 г, які були розділені на контрольну і експериментальну групи. В експериментальній групі шляхом щоденного введення мікрокристалічної целюлози з розрахунку 500 мг/кг. Роботу із піддослідними тваринами виконували згідно з правилами Європейської конвенції про гуманне ставлення до лабораторних тварин (Страсбург, 1985). Першу групу склали інтактні щури, годування яких було стандартним, другу – щури, які при звичайному годуванні отримували мікрокристалічну целюлозу.

Результати. У випорожненнях дослідних білих щурів, які отримували мікрокристалічну целюлозу знижувався рівень кишкової палички в товстому відділі кишечника на 22 і на 25%. Збільшилася кількість цих мікроорганізмів на 20% через 7 днів і на 14% – через 14 днів. Зменшувався вміст епідермальних стафілококів у випорожненнях на 10% після 7-го дня прийому. Прийом мікрокристалічної целюлози

збільшував кількість золотистих стафілококів на 12%, проте зменшував кількість ентерококів у фекаліях на 28%. В товстій кишці через 7-14 днів експерименту зміст цих бактерій не зазнав помітних змін. По відношенню до анаеробних мікроорганізмів – бактероїдів і клостридій цей препарат викликав незначне збільшення кількості бактероїдів – на 8,64% і кількості клостридій – на 11,54% після 14-го дня. Вміст грибів роду *Candida* після 7-го та 14-го дня збільшувалася на 8,3%.

Висновки. В 2-й термін дослідження мікробіологічний краєвид кишкового вмісту погіршувався: збільшувався процес дисбактеріозу, що виражалося в істотному збільшенні кількості протея, золотистих стафілококів, анаеробів (бактероїдів, клостридій) і грибів роду *Candida*, а також зниженням змісту кишкової палички та ентерококів.

КЛЮЧОВІ СЛОВА: ентеросорбція; мікрокристалічна целюлоза; мікрофлора товстого кишечника; ентеросорбенти.

Information about the authors

Dmytro B. Koval – student, I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine.

ORCID 0000-0002-8958-1731, e-mail: koval_dmybog@tdmu.edu.ua

Hanna R. Malyarchuk – Assistant Professor, I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine.

ORCID 0000-0003-2647-2703, e-mail: malyarchuk@tdmu.edu.ua

Olexandr O. Levenets – student, I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine.

ORCID 0000-0002-1155-5525, e-mail: levenets_oleole@tdmu.edu.ua

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