Transplantologiya - 2016. - № 4. – P. 39.-47.

Effect of the extract of Daigo lactic acid bacteria fermentation on the composition of the microflora and intestinal motor function in experimental dysbiosis

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Received: 17 August 2016

In experiments on rats, the efficacy of the fermentation extract of Daigo lactic acid bacteria was investigated as the means for the prophylaxis and correction of an impaired microflora composition, and small intestine motor activity changes in dysbiosis. Experimental dysbiosis induced by a 7-day oral administration of antimicrobials (Amoxycillinum and Metronidazolum) was manifested by considerable disturbances in qualitative and quantitative composition of the jejunum and cecum microflora. A preventive administration of Daigo prior to the exposure to antimicrobials eliminated the dysbiosis signs. Daygo administration after modeling the dysbiosis led to the recovery of intestinal motor function, normalized the numbers of conditionally-pathogenic microorganisms in the jejunum, and decreased the numbers of opportunistic microorganisms in the cecum.

Keywords: fermentation extract of lactic acid bacteria, microflora, intestinal motor function, experimental dysbiosis.

Introduction

Intestinal dysbiosis is known as a syndrome that accompanies many pathological conditions and is essentially a consequence of a pathological process [1]. Impaired microbiocenosis resulted from the intake of antimicrobial agents ranks high among many causes of dysbiosis. Modern antibiotics are the primary means to treat infectious diseases of bacterial origin. Their uncontrolled use often leads to killing not only pathogenic microorganisms in natural biotopes, but also to suppressing the normal human microflora species.

The concept of functional nutrition, first formulated in Japan in the late 1990s, has been widely recognized as the one addressing the task of microecological human system improvement, being a priority for the prevention and treatment of most chronic diseases in economically developed countries.

Functional nutrition combines products of natural or synthetic origin intended for regular daily use and having a regulating effect on physiological functions, biochemical reactions, and psychosocial human behavior through the normalization of its microecological status [2].

Recently, the issues of functional nutrition have been often discussed in the context of using pro-, pre- and synbiotics [3-5]. This is primarily due to the fact that the concept of functional nutrition has become more vigorously aimed at specific products that can positively influence the intestinal microflora composition. The first successful experience of small intestine transplantation in the N.V.Sklifosovsky Research Institute for Emergency Medicine demonstrated that the gastrointestinal tract microflora correction with a 2% solution of sugar beet pectin and probiotics resulted in the elimination of opportunistic pathogen microflora [6].

In April 2015, the Russian market of functional products was enriched with the Japanese Daigo product registered and approved for use for maintaining the gut microbiota, enhancing the immunity, and resolving gastroenterological problems. The active substance of Daigo contains a specially selected extract of lactic acid bacteria fermentation (16 strains).

Daigo has been developed as a new type of products prepared from the lactic acid bacteria that improve the gastrointestinal tract function. Unlike probiotics, Daigo does not contain live lactic acid bacteria. Daigo represents a mix of peptides, bioregulators extracted from the bacterial cells of physiological lactobacilli (16 strains) colonizing the intestine of healthy humans. The soy milk used as a medium for culturing the lactic acid bacteria is obtained from a special soybeans grown organically without pesticides and chemicals. The lactic acid bacteria extract is a mixture of lactic acid bacteria secretion products and cell substances obtained by fermentation. Recent studies have shown that the cell substances and fermentation products of metabolized lactic acid bacteria produce a more favourable effect on the gut regulation and vital functions than live lactic acid bacteria.

The purpose of the study was to investigate the efficacy of Daigo functional product in the prevention and correction of imbalanced microflora composition and small intestine motor disorders in experimental dysbiosis in rats.

Material and Methods

The study included 28 adult Wistar male rats (body weight about 300 g, aged 4 months). The rats were supplied by *NeoMarke Ltd.* breeding facility. All experimental animals were kept on a natural light:dark cycle under standard vivarium conditions of N.V.Sklifosovsky Research Institute and

provided ad libitum access to water and a balanced diet (pelleted feed custom-made by *Laboratorkorm Ltd.*).

Study design

All the animals were divided into 4 experimental groups of 7 animals each:

1. The Control Group included intact animals (n=7). In those rats, the intestinal evacuation activity was measured, and the samples of the jejunum and cecum contents were taken for a microbiology study.

2. The Dysbiosis Group (n=7) represented the group with experimental dysbiosis. The rats were administered an antimicrobial solution (amoxicillin + metronidazole) per os once daily for 7 days. On the 8-th day of the experiment, the intestinal evacuation activity in those rats was measured, and the samples of the jejunum and cecum contents were taken for a microbiology study.

3. The Dysbiosis + Daigo Group (n = 7) was the group with the experimentally induced dysbiosis and a remedial administration of Daigo investigational solution. The rats were administered the antimicrobial solution (amoxicillin + metronidazole) per os once daily for 7 days, and from day 8 to day 21, the animals received Daigo investigational solution. On the 22-nd day of the experiment, the intestinal evacuation activity in those rats was measured, and the samples of the jejunum and cecum contents were taken for a microbiology study.

4. The Daigo + Dysbiosis Group (n = 7) was the group with a prophylactic administration of Daigo investigational solution and a subsequent administration of antimicrobial agents (amoxicillin + metronidazole). The rats received an oral Daigo investigational solution once

daily for 14 days, and then they received the antimicrobial solution from day 15 to day 21. On the 22-nd day of the experiment, the intestinal evacuation activity in those rats was measured, and the samples of the jejunum and cecum contents were taken for a microbiology study.

Doses, regime, and route of drug administration

Daigo solution was given at a dose of 0.33 ml/kg; amoxicillin and metronidazole were administered at a dose of 28.5 mg/kg, and at a dose of 22.8 mg/kg, respectively.

Before each dosing, every animal was weighed, and a dose calculation was made basing on the rat individual body weight.

The study drugs (antimicrobials and Daigo investigational product) were dosed orally, from 9 a.m. to 11 a.m. daily.

Small intestine evacuation activity measurement

The small intestine evacuation activity in experimental animals was measured using Evans Blue inert marker. The Evans Blue marker was administered to the rats per os to measure the intestinal evacuation activity. After 20 minutes, the rats were euthanized. At autopsy, the small intestine (from pilorus to the cecum) was isolated, and the length of the entire intestine and the distance the marker traveled for 20 minutes were measured. The Transit Index (TI), expressed in per cent, was defined as the ratio of the distance traveled by the marker to the total intestine length, multiplied by 100.

Collection of samples for bacteriology study

A part of the jejunum, 5 cm long, at 15 cm from the Treitz ligament was isolated for sampling. The intestinal content was sampled into a sterile tube using a special spatula (to study the jejunal lumen microflora). Then, the isolated jejunal part was washed with sterile saline, the scrap of the wall layer was sampled (to study the jejunum parietal microflora) and the sample was placed in a sterile tube. Further, the cecum was isolated. The intestinal content was sampled into a sterile tube using a special spatula (to study the cecum lumen microflora).

Bacteriology studies

Microbiology studies of feces, the jejunum and cecum contents were performed in accordance with the Regulations adopted for the study of feces in humans: "Protocol of patient management. Intestinal dysbacteriosis", the Branch Standard 91500.11.00042003. The following 9 microorganism species isolated in the samples were under investigation: *Staphylococcus spp, Streptococcus spp, Enterococcus spp, Escherichia coli (E.coli), Proteus mirabilis, Klebsiella spp.,* non-fermentative Gram-negative bacteria *(*NFGNB), *Bifidobacterium spp.* and *Lactobacillus spp.*

Data are presented as medians and percentiles: Me (25; 75)%. The non-parametric Mann-Whitney U-test was used in the statistical analysis. The differences were considered statistically significant at $p < 0.05$.

Results and discussion

Studying the efficacy of Daigo functional product in the prevention and correction of the small intestine motor disorders in rat experimental dysbiosis

After 7 days of receiving the antimicrobial agents (amoxicillin + metronidazole), the Dysbiosis Group animals showed a statistically significant slowdown of the gut content transit compared to the Control Group from 30.34 (19.56; 44.55)% to 25.17 (23.41; 31.84)% ($p<0.05$).

When studying the small intestine motor function changes we noted a tendency to recovery of the intestine content transit in Dysbiosis + Daigo Group to 32.17 (24.05; 37.76)% vs. the Dysbiosis Group showing 25.17 (23.41; 31.84)% ($p> 0.05$). No statistically significant difference was found when comparing the changes in the small intestine evacuation function between the Dysbiosis + Daigo Group and the Control Group: The TI was 32.17 (24.05; 37.76)% in the Dysbiosis + Daigo Group, and 30.34 (19.56; 44.55)% in the Control Group ($p> 0.05$), indicating the normalized intestinal transit after receiving Daigo.

When comparing the small intestine motor function changes in the animals of the Daigo + Dysbiosis Group to those in Dysbiosis Group, we identified a trend toward the recovery of intestinal content transit: TI was 30.16 (28.36; 36.15)% in the Daigo + Dysbiosis" Group, and 25.17 (23.41; 31.84)% in the Dysbiosis Group ($p>0.05$). The comparison of the small intestine evacuation function changes between the Daigo + Dysbiosis Group and the Control Group demonstrated no statistically significant differences. The TI was 30.16 (28.36; 36.15)% in the Daigo + Dysbiosis Group, and 30.34 (19.56; 44.55)% in the Control Group ($p>0.05$), indicating the normalization of the intestinal transit in the animals on Daigo therapy.

Thus, the experimentally induced dysbiosis in a rat causes the impairments of the small intestine motor function that are expressed in slowing down the rates of intestinal content transit. Daigo administration aimed at either improving or preventing dysbiosis leads to the normalization of the intestinal motor function.

Studying the efficacy of Daigo functional product in the prevention and correction of the altered microflora composition in rat experimental dysbiosis

Table 1 summarizes the results obtained while investigating the effect of a 7-day administration of antimicrobial agents (amoxicillin + metronidazole) on the jejunum and cecum microflora.

Table 1. The content of the various microorganisms in the jejunum and cecum in the Control Group and Dysbiosis Group CFU/ml, Me (25; 75)%

Microorganism	Jejunum, lumen microflora		Jejunum, parietal microflora		Cecum, lumen microflora	
	Control (Intact animals)	Dysbiosis	Control (Intact animals)	Dysbiosis	Control (Intact animals)	Dysbiosis
Staphylococcus spp.	0 (0;0)	—	—	0 (0;0)	10^4 (10^3;10^5)	0 (0;0)*
Enterococcus spp.	0 (0;10^2)	10^4 (10^4;10^4)*	—	10^3 (0;10^3)*	10^4 (0;10^5)	10^6 (10^5;10^8)*
E.coli	0 (0;10^2)	103 (10^3;10^4)*	0 (0;0)	10^4 (102;10^4)*	10^5 (10^5;10^6)	10^7 (10^6;10^7)*
Proteus mirabilis	—	10^3 (10^3;10^4)*	—	10^2 (0;10^2)*	—	10^4 (0;10^5)*
Enterobacter spp.	—	0 (0;10^6)	—	10^4 (0;10^5)*	—	10^6 (0;10^8)*
Kl.Pneumoniae	—	0 (0;10^5)	—	0 (0;10^2)	—	0 (0;10^8)

Moulds	—	0	—	—	—	—
Lactobacillus spp.	10^3 (10^3;10^4)	10^5 (10^5;10^6)*	10^3 (0;10^3)	10^5 (10^4;10^5)*	10^7 (10^7;10^8)	10^6 (10^5;10^6)*
Bifidobacterium spp.	10^3 (0;10^4)	10^5 (10^5;10^5)*	10^3 (0;10^4)	10^4 (10^4;10^5)	10^7 (10^7;10^7)	10^5 (10^5;10^6)*

Note: "-" absent in all the animals.

* P <0.05 - statistically significant differences when compared with the Control Group.

The data in the Table demonstrate the development of significant impairments in the qualitative and quantitative composition of jejunum and cecum microflora in experimentally induced dysbiosis in a rat. Thus, *Proteus mirabilis*, *Enterobacter spp.*, and *Kl.pneumoniae* in the jejunal lumen were found to appear in some animals. Meanwhile, *Enterococcus spp.*, *Proteus mirabilis*, *Enterobacter spp*., and *Kl. penumoniae* cultured on the wall layer of the jejunum. The presence of *Proteus mirabilis*, *Enterobacter* spp., and *Kl. penumoniae* was found in the cecum. It is worthwhile to note the absence of these organisms in all the animals of the Control group.

The numbers of *Enterococcus spp.*, *E.coli*, *Proteus mirabilis*, *Lactobacillus spp.*, and *Bifidobacterium spp.* significantly increased in the jejunal lumen; and a significant increase in the numbers of *Enterococcus spp.*, *E.coli*, *Proteus mirabilis*, *Enterobacter spp.*, and *Lactobacillus spp.* was seen in the wall layer of the jejunum. The changes in the numbers of microorganisms in the cecum vs. the Control Group were statistically significant for all species studied, except *Kl. Pnumoniae.* The numbers of *Enterococcus spp.* and *E.coli* were found to have increased 10-fold, and the numbers of *Staphylococcus spp.*, *Lactobacillus spp.*, and *Bifidobacterium spp.* decreased.

Thus, our data have demonstrated that a 7-day intake of antimicrobials (amoxicillin + metronidazole) is associated with significant qualitative and quantitative impairments of miscroflora composition both in the jejunum and in the cecum. The changes manifest themselves in the increased numbers of *E.coli* in the small intestine and the cecum, in the bifidobacteria and lactobacilli redistribution between the upper and lower intestine (their number reduction in the cecum and increase in the small intestine), and in the appearance of the opportunistic microorganism species not typical for the small intestine and the cecum of healthy animals; all these may indicate the occurrence of the third grade dysbiosis (Classification by V.M. Bondarenko).

Having studied the changes in the jejunum and cecum microflora after the remedial administration of Daigo in experimentally induced dysbiosis (the Dysbiosis + Daigo Group), we obtained the following results (Table. 2).

Table 2. The content of various microorganisms in the jejunum and cecum in the group of animals with Daigo remedial administration in presence of experimental dysbiosis, and in the Dysbiosis Group, CFU/ml, Me (25; 75)%

Microorganism	Jejunum, lumen microflora		Jejunum, parietal microflora		Cecum, lumen microflora	
	Dysbiosis	Dysbiosis + Daigo	Dysbiosis	Dysbiosis + Daigo	Dysbiosis	Dysbiosis + Daigo
Staphylococcus spp.	—	—	—	—	—	10^3 (0;10^4)*
Enterococcus spp.	10^4 (10^4;10^4)	0 (0;10^3)*	10^3 (0;10^3)	—	10^6 (10^5;10^8)	10^5 (10^5;10^6)

E.coli	10^3 (10^3;10^4)	0 (0;10^2)*	10^4 (10^2;10^4)	—	10^7 (10^6;10^7)	10^4 (10^4;10^7)
Proteus mirabilis	10^3 (10^3;10^4)	0 (0;0)*	10^2 (0;10^2)	—	10^4 (0;10^5)	—
Enterobacter spp.	0 (0;10^6)	—	10^4 (0;10^5)	—	10^6 (0;10^8)	0 (0;10^5)
Kl.Pneumoniae	0 (0;10^5)	—	0 (0;10^2)	—	0 (0;10^8)	—
Moulds	—	0(0;0)	—	—	—	—
Lactobacillus spp.	10^5 (10^5;10^6)	10^5 (10^5;10^5)	10^5 (10^4;10^5)	10^4 (10^4;10^4)	10^6 (10^5;10^6)	10^6 (10^5;10^6)
Bifidobacterium spp.	10^5 (10^5;10^5)	10^5 (10^5;10^5)	10^4 (10^4;10^5)	0 (0;10^4)*	10^5 (10^5;10^6)	10^5 (10^5;10^6)

Note: "-" absent in all the animals.

* P <0.05 - statistically significant differences between the Dysbiosis + Daigo Group and the Dysbiosis Group.

As seen in the table, the animals that received Daigo after the experimentally induced dysbiosis had significant qualitative and quantitative changes in jejunal and cecal microflora composition compared to the animals of the Dysbiosis Group. Thus, *Enterobacter spp.,* and *Kl.Pneumoniae* disappeared from the jejunal lumen of all the animals taking Daigo, and the jejunal wall layer became free from *Enterococcus spp.*, E.coli, *Proteus mirabilis, Enterobacter spp.*, and *Kl.Pneumoniae. Proteus mirabilis*, and *Kl.Pneumoniae* disappeared from cecum.

Enterococcus spp., *E.coli, Proteus mirabilis* significantly decreased in numbers in the jejunal lumen, and the numbers of *Bifidobacterium spp.* significantly decreased in the jejunal wall layer. The number of *Staphylococcus spp.* significantly increased in the cecum.

Thus, the most significant remedial effect of Daigo therapy in the experimentally induced dysbiosis includes the disappearance of

opportunistic pathogen microflora in the small intestine wall layer, and the significant reduction in the small intestine lumen, as compared to the animals in the Dysbiosis Group.

To answer the question whether the remedial administration of Daigo in experimental dysbiosis can normalize microflora, we made a comparison of bacteriology test results between the animals of Dysbiosis + Daigo Group and the Control Group (Table. 3).

Table 3. The content of various microorganisms in the jejunum and cecum in the group of animals with Daigo remedial administration in presence of experimental dysbiosis and in the Control Group (intact animals) CFU/ml, Me (25; 75)%

Microorganism	Jejunum, lumen microflora		Jejunum, parietal microflora		Cecum, lumen microflora	
	Control (Intact animals)	Dysbiosis + Daigo	Control (Intact animals)	Dysbiosis + Daigo	Control (Intact animals)	Dysbiosis + Daigo
Staphylococcus spp.	0 (0;0)	—	—	—	10^4 (10^3;10^5)	10^3 (0;10^4)*
Enterococcus spp.	0 (0;10^2)	0 (0;10^3)	—	—	10^4 (0;10^5)	10^5 (10^5;10^6)
E.coli	0 (0;10^2)	0 (0;10^2)	0 (0;0)	—	10^5 (10^5;10^6)	10^4 (10^4;10^7)
Proteus mirabilis	—	0 (0;0)	—	—	—	—
Enterobacter spp.	—	—	—	—	—	0 (0;10^5)
Kl.Pneumoniae	—	—	—	—	—	—
Moulds	—	0(0;0)	—	—	—	—
Lactobacillus spp.	10^3 (10^3;10^4)	10^5 (10^5;10^5)*	10^3 (0;10^3)	10^4 (10^4;10^4)*	10^7 (10^7;10^8)	10^6 (10^5;10^6)*
Bifidobacterium spp.	10^3 (0;10^4)	10^5 (10^5;10^5)*	10^3 (0;10^4)	0 (0;10^4)	10^7 (10^7;10^7)	10^5 (10^5;10^6)*

Note: "-" absent in all the animalsl.

* P <0.05 - statistically significant differences between the Dysbiosis + Daigo Group and the Control Group (intact animals).

As seen in the Table, there are no statistically significant differences between the animals that received Daigo after the experimentally induced dysbiosis and the animals of the Control Group in the numbers of opportunistic microorganisms in the jejunal lumen and parietal microflora; that suggests a quantity normalization of these microorganism species when Daigo has been administered after experimentally induced dysbiosis. Moreover, the Daigo administration resulted in a 100-fold increase in the numbers of *Lactobacillus spp.,* and *Bifidobacterium spp.* in the jejunal lumen compared to those of intact animals, and in a 10-fold increase in the number of *Lactobacillus spp.* in the jejunal wall layer. In the cecum (as compared to the Control Group) we observed significantly decreased numbers of *Staphylococcus* spp. (10-fold*), Lactobacillus spp.* (10-fold), and *Bifidobacterium spp*. (100-fold).

Thus, the remedial administration Daigo after experimentally induced dysbiosis led to normalized numbers of opportunistic microorganisms in the jejunum. The numbers of opportunistic microorganisms in the cecum decreased, as well as lactobacilli and bifidobacteria did, when compared to healthy animals, which may suggest an effective correction of experimental dysbiosis. It was interesting to note that Daigo remedial administration after the experimentally induced dysbiosis led to an increased number of *Lactobacillus spp.* in the lumen and the wall layer of the small intestine. In addition, there was a redistribution of bifidobacteria between the upper and lower intestine: the increase of their numbers in the small intestine lumen and the reduction in the cecum.

The study of jejunum and cecum microflora changes after the prophylactic administration of Daigo in experimental dysbiosis (the Daigo + Dysbiosis Group) gave the following results (Table. 4).

The table data on Daigo prophylactic administration to the animals in modeling the experimental dysbiosis indicate significant qualitative and quantitative changes observed in the jejunal and cecal microflora composition when compared with the animals of the Dysbiosis Group. Thus, *Kl.Pneumoniae* disappeared from jejunal lumen of all the animals taking Daigo; and *Proteus mirabilis*, *Enterobacter spp.*, and *Kl.Pneumoniae* disappeared from the jejunal wall layer. *Kl.Pneumoniae* disappeared from the cecum.

Table 4. The content of various microorganisms in the jejunum and cecum in a group of animals with Daigo prophylactic administration before inducing the experimental dysbiosis, and in the animals of he Dysbiosis Group, CFU/ml, Me (25; 75)%

Microorganism	Jejunum, lumen microflora		Jejunum, parietal microflora		Cecum, lumen microflora	
	Dysbiosis	Daigo + Dysbiosis	Dysbiosis	Daigo + Dysbiosis	Dysbiosis	Daigo + Dysbiosis
Staphylococcus spp.	—	0 (0;0)	—	—	—	—
Enterococcus spp.	10^4 (10^4;10^4)	10^3 (0;10^5)	10^3 (0;10^3)	0 (0;10^2)	10^6 (10^5;10^8)	10^7 (10^6;10^9)
E.coli	10^3 (10^3;10^4)	10^2 (10^2;10^4)	10^4 (10^2;10^4)	0 (0;10^2)*	10^7 (10^6;10^7)	10^8 (108;10^8)*
Proteus mirabilis	10^3 (10^3;10^4)	0 (0;10^2)*	10^2 (0;10^2)	—*	10^4 (0;10^5)	10^3 (0;10^3)

Enterobacter spp.	0 (0;10^6)	0 (0;10^4)	10^4 (0;10^5)	—*	10^6 (0;10^8)	10^7 (105;10^8)
Kl.Pneumoniae	0 (0;10^5)	—	0 (0;10^2)	—	0 (0;10^8)	—
Moulds	—	—	—	—	—	—
Lactobacillus spp.	10^5 (10^5;10^6)	10^5 (10^5;10^6)	10^5 (10^4;10^5)	10^4 (10^4;10^4)	10^6 (10^5;10^6)	10^6 (10^6;10^7)
Bifidobacterium spp.	10^5 (10^5;10^5)	10^5 (10^5;10^6)	104 (10^4;10^5)	10^4 (10^3;104)	10^5 (10^5;10^6)	10^6 (10^5;10^6)

Note: "-" absent in all the animals.

* P <0.05 - statistically significant differences between the Daigo + Dysbiosis Group and the Dysbiosis Group.

Moreover, the number of *Proteus mirabilis* in the jejunal lumen significantly decreased (1000-fold), the number of *E.coli* significantly decreased (10,000-fold) in the jejunal wall layer. The numbers of *E.coli* in the cecum increased significantly (10-fold).

Thus, the most significant effect of Daigo prophylactic administration in modeling the experimental dysbiosis has been the disappearance and(or) a significant reduction of opportunistic pathogen microflora in the small intestine lumen and wall layer when compared to the animals of the Dysbiosis Group.

In order to answer the question whether the prophylactic administration of Daigo in modeling experimental dysbiosis can normalize microflora, we made a comparison of bacteriology test results between the animals of Daigo + Dysbiosis Group and the Control Group (Table. 5).

Table 5. The content of various microorganisms in the jejunum and cecum in the group of animals with Daigo prophylactic administration before inducing the experimental dysbiosis and in the Control Group, CFU/ml, Me (25; 75)%

Microorganism	Jejunum, lumen microflora		Jejunum, parietal microflora		Cecum, lumen microflora	
	Control (intact animals)	Daigo + Dysbiosis	Control (intact animals)	Daigo + Dysbiosis	Control (intact animals)	Daigo + Dysbiosis
Staphylococcus spp.	0 (0;0)	0 (0;0)	—	—	10^4 (10^3;10^5)	—*
Enterococcus spp.	0 (0;10^2)	10^3 (0;10^5)	—	0 (0;10^2)	10^4 (0;10^5)	10^7 (10^6;10^9)*
E.coli	0 (0;10^2)	10^2 (10^2;10^4)*	0 (0;0)	0 (0;10^2)	10^5 (10^5;10^6)	10^8 (10^8;10^8)*
Proteus mirabilis	—	0 (0;10^2)	—	—	—	10^3 (0;10^3)*
Enterobacter spp.	—	0 (0;10^4)	—	—	—	10^7 (10^5;10^8)*
Kl.Pneumoniae	—	—	—	—	—	—
Moulds	—	—	—	—	—	—
Lactobacillus spp.	10^3 (10^3;10^4)	10^5 (10^5;10^6)*	10^3 (0;10^3)	10^4 (10^4;10^4)*	10^7 (10^7;10^8)	10^6 (10^6;10^7)*
Bifidobacterium spp.	10^3 (0;10^4)	10^5 (10^5;10^6)*	10^3 (0;10^4)	10^4 (10^3;10^4)	10^7 (10^7;10^7)	10^6 (10^5;10^6)*

Note: "-" absent in all the animals.

* P <0.05 - statistically significant differences between the experimental and control groups.

According to the data given in the table, there are no statistically significant differences in the animals that received prophylactic Daigo in modeling the experimental dysbiosis compared to the Control Group in the quantities of opportunistic microorganisms in jejunal wall microflora, indicating normalized numbers of these microorganisms with Daigo prophylactic administration. Moreover, there was a 10-fold increase in the number of *Lactobacillus spp.* in the jejunal wall layer compared to that in

intact animals. The quantities of opportunistic microorganisms in the jejunal lumen microflora were not significantly different from those in the Control Group, except for *E.coli* that increased in number 100-fold compared to the Control Group. Furthermore, the numbers of *Lactobacillus spp.,* and *Bifidobacterium spp.* in the jejunal lumen increased 100-fold in jejunal contents *Lactobacillus spp.* and *Bifidobacterium spp.* compared to the intact animals. In the cecum, we observed the appearance of *Proteus mirabilis,* and *Enterobacter spp.,* the growth of *Enterococcus spp.,* and *E.coli,* reduced numbers of *Lactobacillus spp.* and *Bifidobacterium spp.*

Thus, the prophylactic Daigo administration in modeling the experimental dysbiosis has led to the disappearance of opportunistic microorganisms in the jejunal wall layer and to normalized numbers of opportunistic microorganisms (except *E.coli)* in the jejunal lumen. As in the series with Daigo remedial administration, its prophylactic administration in modeling the experimental dysbiosis has led to increased numbers of *Lactobacillus spp.* both in the small intestine lumen, and wall layer. Besides, there occurs a redistribution of bifidobacteria content between the upper and lower intestine towards their numbers getting increased in the small intestine and reduced in the cecum.

Conclusion

In this study, the experimental dysbiosis associated with the use of antimicrobial agents in rats was caused by a 7-day oral administration of the two broad spectrum antimicrobials: amoxicillin and metronidazole. It was shown that a 7-day intake of antimicrobials led to significant qualitative and quantitative impairments of the microflora composition in the jejunum and cecum. Those changes manifested themselves in the increased numbers of

E.coli both in the jejunum and cecum; in the bifidobacteria and lactobacilli redistribution between the upper and lower intestine (their numbers decreased in the cecum and increased in the small intestine); and the appearance of opportunistic microorganism species not typical for the small intestine and the cecum of healthy animals. And those changes might indicate the occurrence of severe grade III dysbiosis (Classification by V.M. Bondarenko). Severe dysbiotic changes in the rat intestine were accompanied by an impaired intestinal motor function manifested as a slowdown of the intestinal content passage.

Daigo remedial administration after the experimentally induced dysbiosis resulted in normalized numbers of opportunistic microorganisms in the jejunum. In the cecum, the numbers of opportunistic microorganisms decreased compared to those of healthy animals that might indicate an effective control of experimental dysbiosis. Those changes in microflora were accompanied with the recovery of intestinal motor function.

Daigo prophylactic administration before the experimentally induced dysbiosis led to the disappearance of opportunistic microorganisms from the jejunal wall layer and to the normalization of their number (except *E.coli)* in the jejunal lumen. Those changes in microflora were accompanied by the normalization of intestinal motor function.

Thus, the demonstrated experiments in rats have shown a significant efficacy of the fermentation extract of Daigo lactic acid bacteria for the prevention and improvement of the impaired motor activity of the small intestine and the gut microbiocenosis in experimental dysbiosis.

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