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Our Experience in Studying the Effect of a Genetically Modified Products on the Colon Microflora Laboratory Animals

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Abstract The purpose of the experiment In the experiment, GM-soy was used to determine the extent of exposure of white non-white rats to colon microbiocenosis. It was found that in the normal microflora of the colon of rats fed with GM-free soybeans, different reliable quantitative differences were found compared to intact laboratory animals Bifidobacterium spp (1.28-fold decrease), Lactobacillus spp (1.53-fold decrease), Enterobacter spp and Proteus spp (4.1 and an increase of 6.25 times). This did not indicate the development of complete dysbiosis, as no intergroup distinction was found between lactose-negative and lactose-positive strains of Escherichia coli. While GM-shadow-fed laboratory animals contained all 5 cited elements of dysbiosis, they did not show up clearly in rats consuming GM-shade. No signs of dysbiosis were detected in intact laboratory animals, dysbiosis symptoms were poorly developed in GM-free soy feeding (DI I), and dysbiosis symptoms were evident in GM-shadow-fed animals (DI II).

Keywords GMO soy, White outbred rats, Microbiocenosis of the large intestine, Intestinal dysbiosis, Dysbacteriosis index

1. Introduction

In addition to the various microorganisms that enter the body from the external environment, there are also microorganisms in the human body that live in symbiosis with it and form the normal human microflora. They are located in different biotopes and are important for the functioning of the organism. One such biotope is the colon, whose normal microflora, consisting of indigenous and facultative microorganisms, is essential for life activity. It is known that the microbiocenosis of the colon consists of more than 450 microorganisms and is involved in the metabolism of the "host" organism and the formation of colonization resistance in the intestine.

Disruption of the normal microflora of the colon under the influence of various external and internal factors is characterized by a qualitative and quantitative imbalance of the indigenous and facultative microflora in it and is called intestinal dysbiosis. Many physical, chemical, and biological factors can be examples of factors that lead to intestinal dysbiosis.

Today, a lot of scientific work has been done on the different effects of genetically modified (GM) products on the human body, and experts are divided on this issue, along with the opinion that these products have no adverse effects on the human body [2,10]. There are many proven works [37,9]. Subsequent scientific studies have shown that GM products have a negative effect on the immune system [1], liver and pancreas [8], thymus and spleen [11], as well as hematological, biochemical changes, mutagenic and reproductive activity [5,6], there are studies showing that it has a negative effect on bone marrow cells [12].

An analysis of many scientific literatures shows that there are few studies to determine the degree of impact of GM products on the microbiocenosis of human biotopes, including the microbiosis of the colon, and they are scattered.

In view of the above, the aim of the study was to determine the degree of effect of GM-shadow on the microbiocenosis of the colon in white rats in the experiment.

2. Materials and Methods

To study the effects of GM products on the body obtained using new technologies, experimental studies were

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conducted in laboratory animals (white rats). For this purpose, we used soy flour (GM-product) grown abroad.

For this purpose, a total of 90 male white rats were involved in the study, which were divided into 3 groups: Group 1 - intact white rats with a standard vivarian diet, not fed with GM or without GM (n = 30); Group 2 - white non-GM rats included in the standard vivariate diet without GM (n = 30); Group 3 - white pedigree rats fed with GM-shade on a standard vivariate diet (n = 30).

These groups were representative and differed from each other by only one character. Emphasis was placed on whether the studies were randomized and that principles of evidence-based medicine were followed. The study strictly adhered to the ethical principles and biological safety rules of working with laboratory animals [4].

After the white mass of rats was delivered to the bacteriological laboratory, bacteriological examinations were performed using Bergy's Manual Systematic Bacteriology (1997) using appropriate nutrient media (Blaurokk, SRM-4 (MRS-4), Endo, Saburo media, egg yolk agar, etc.). microorganisms were identified and differentiated: Bifidobacterium spp, Lactobacillus spp, Escherichia coli, Enterobacter spp, Proteus spp, Staphylococcus spp, Streptococcus spp, Candida spp. Intergenerational and interspecific identification was performed using food media from HiMedia (India).

Statistical processing of the results was carried out using traditional variational statistical methods, and the principles of evidence-based medicine were followed in organizing and conducting the research.

3. Results and Their Discussion

The results obtained showed that there were convincing differences in the quantitative indicators studied between the groups being compared (Table 1).

Table 1. Quantitative analysis of quantitative indicators of colon microbiocenosis in GM-free shade-fed and intact laboratory animals, lg KXQB / ml (M ± m)

Microorganisms	Group 1, n = 30	2- group, n = 30
Bifidobacteriumspp	5.10 ± 0.2	4.00 ± 0.1 * ↓
Lactobacillus spp	6.10 ± 0.2	4.00 ± 0.1 * ↓
Escherichia coli(lactosapositive)	5.15 ± 0.2	5.00 ± 0.2 ↔
Escherichia coli(lactose-negative)	0	0 ↔
Enterobacterspp	1.20 ± 0.1	5.00 ± 0.2 * ↑
Proteus spp	0.80 ± 0.1	5.00 ± 0.2 * ↑
Staphylococcus spp	4.10 ± 0.1	5.00 ± 0.2 * ↑
Streptococcus spp	6.30 ± 0.3	4.00 ± 0.2 * ↓
Candida spp	3.60 ± 0.1	7.00 ± 0.1 * ↑

Note: * - a sign of a reliable difference between groups; ↑, ↓ - directions of changes; ↔ - There is no convincing difference.

In the next stage of the research, the quantitative indicators of colon microbiocenosis of white pedigree rats with GM-shade added to the standard vivarian diet were studied comparatively and the obtained data were analyzed. Intact (not consuming GM-soy) laboratory animals were obtained for comparison. All results are presented in Table 2.

Table 2. Quantitative analysis of quantitative indicators of colon microbiocenosis in GM-shade-fed and intact laboratory animals, lg KXQB / ml (M ± m)

Microorganisms	Group 1, n = 30	3- group, n = 30
Bifidobacteriumspp	5.10 ± 0.2	2.10 ± 0.1 * ↓
Lactobacillus spp	6.10 ± 0.2	2.00 ± 0.2 * ↓
Escherichia coli(lactosapositive)	5.15 ± 0.2	0 ↓
Escherichia coli(lactose-negative)	0	5.30 ± 0.3 * ↑
Enterobacterspp	1.20 ± 0.1	5.45 ± 0.2 * ↑
Proteus spp	0.80 ± 0.1	3.00 ± 0.1 * ↑
Staphylococcus spp	4.10 ± 0.1	6.15 ± 0.2 * ↑
Streptococcus spp	6.30 ± 0.3	4.30 ± 0.2 * ↓
Candida spp	3.60 ± 0.1	7.00 ± 0.4 * ↑

Note: * - a convincing sign of difference between groups; ↑, ↓ - directions of changes; ↔ - There is no convincing difference.

The next phase of the study was a comparative study of quantitative indicators of indigenous and facultative microflora of laboratory animals with GM-free soy (group 2) and GM-soy (group 3) added to the standard vivarian diet. The results obtained are presented in Table 3.

Table 3. Comparative analysis of quantitative indicators of colon microbiocenosis in laboratory animals fed without GM and with GM, lg KXQB / ml (M ± m)

Microorganisms	Group 2, n = 30	3- group, n = 30
Bifidobacteriumspp	4.00 ± 0.1	2.10 ± 0.1 * ↓
Lactobacillus spp	4.00 ± 0.1	2.00 ± 0.2 * ↓
Escherichia coli(lactosapositive)	5.00 ± 0.2	0 ↓
Escherichia coli(lactose-negative)	0	5.30 ± 0.3 * ↑
Enterobacterspp	5.00 ± 0.2	5.45 ± 0.2 * ↑
Proteus spp	5.00 ± 0.2	3.00 ± 0.1 * ↑
Staphylococcus spp	5.00 ± 0.2	6.15 ± 0.2 * ↑
Streptococcus spp	4.00 ± 0.2	4.30 ± 0.2 * ↓
Candida spp	7.00 ± 0.1	7.00 ± 0.4 * ↑

Note: * - a convincing sign of difference between groups; ↑, ↓ - directions of changes; ↔ - There is no convincing difference.

In order to summarize all the results obtained, we found it necessary to compare the performance of all three groups (Table 4).

In this Table 4, a convincing difference between the groups is clearly seen, the analyzed figures show that there are practically no changes in the normal microflora of the colon of GM-free soy-fed (intact, control) laboratory animals, no signs of dysbiosis were detected; In laboratory animals consuming soy without GM (group 2), the balance between indigenous and facultative microorganisms is partially disturbed, there are signs of dysbiosis, but it is not formed

and developed; In GM-soy-fed animals (group 3), the balance of indigenous and facultative microorganisms was disturbed, signs of dysbiosis were clearly observed, all 5 elements were identified, total dysbiosis of the colon was developed. This condition has been interpreted as a result of GM-soy exposure to white rats.

Table 4. Quantitative status of colonic microflora in GM- and GM-free rats, lg (M ± m) (KXQB / ml)

Microorganisms	Group 1, n = 30	Group 2, n = 30	Group 3, n = 30
Bifidobacterium spp	5.10 ± 0.2	4.00 ± 0.1	2.10 ± 0.1 * ^ ↓
Lactobacillus spp	6.10 ± 0.2	4.00 ± 0.1	2.00 ± 0.2 * ^ ↓
Escherichia coli (lactosapositive)	5.15 ± 0.2	5.00 ± 0.2	0 ↓
Escherichia coli (lactose-negative)	0	0	5.30 ± 0.3 * ↑
Enterobacterspp	1.20 ± 0.1	5.00 ± 0.2	5.45 ± 0.2 * ^ ↑
Proteus spp	0.80 ± 0.1	5.00 ± 0.2	3.00 ± 0.1 * ^ ↑
Staphylococcus spp	4.10 ± 0.1	5.00 ± 0.2	6.15 ± 0.2 * ^ ↑
Streptococcus spp	6.30 ± 0.3	4.00 ± 0.2	4.30 ± 0.2 * ↓
Candida spp	3.60 ± 0.1	7.00 ± 0.1	7.00 ± 0.4 * ↑

Note: * - a convincing difference sign between groups 1 and 3; ^ Is a convincing difference sign between groups 2 and 3; ↑, ↓ - directions of changes; ↔ - There is no convincing difference.

To determine the state of the normal microflora of the colon, the degree of development of dysbiosis, the appearance of its depth, criteria for the assessment of dysbiosis have been developed by many studies and presented in practical health care. Among these methods, we selected the one that we considered most appropriate and used it to assess dysbiosis.

This method was developed by Uzbek researchers Garib F.Yu., Adilov Sh.K. and Narbaeva I.E. Recommended by the researchers in 1995, changes in the microflora of the colon are assessed at 2 levels:

In grade I dysbiosis - changes are observed only among representatives of the indigenous group, Bifidobacterium spp and Lactobacillus spp are reduced compared to lactose-positive Escherichia coli, intestinal dysfunction is not manifested.

In grade II dysbiosis - with a decrease in indigenous microorganisms, the amount of facultative conditionally pathogenic microorganisms increases, the balance between them is disturbed, the symptoms of intestinal dysfunction are clearly visible. These levels are determined using the dysbacteriosis index (DI):

DI I = E.coli KXQB / g / Indigenous microorganisms, KXQB / g < 0,1;

DI II = Facultative microorganisms, KXQB / g / Indigenous microorganisms, KXQB / g ≤ 0,5.

Agar DI I > 0.1; If DI II is ≤ 0.5, it is grade I dysbiosis, if DI II is> 0.5, it is grade II dysbiosis regardless of how much DI I is.

The results of our study were as follows:

In group 1 - 0.31 <0.1 (DI I); 0.37 <0.5 (DI II);
In group 2 - 0.38 <0.1 (DI I); 0.77 <0.5 (DI II);
In group 3 - 1.29 <0.1 (DI I); 3.56 <0.5 (DI II).

The results fully confirmed the above, that is, intact laboratory animals (group 1) have no signs of dysbiosis, in GM-free soy-fed (group 2) dysbiosis symptoms are poorly developed (I-degree), in GM-soy-fed white rats The symptoms of dysbiosis are obvious (grade II).

4. Conclusions

1. GM-free soy-fed white rats in the normal microflora of the colon compared with reliable intact laboratory animals Bifidobacterium spp (1.28-fold decrease), Lactobacillus spp (1.53-fold decrease), Enterobacter spp, and Proteus spp (4, An increase of 16 and 6.25 times, respectively). These are the initial signs of dysbiosis and do not indicate the development of complete dysbiosis, as no group differences between lactozanegative and lactosapositive strains of Escherichia coli have been identified.

2. Quantitative indicators of Bifidobacterium spp and Lactobacillus spp in laboratory animals fed with GM-soy were significantly reduced by 2.43 and 3.05 times compared to intact rats. This decrease was an external factor negatively affecting them, and in this experiment it was interpreted as a GM-soy. This condition was interpreted as the first element of dysbiosis formed in the colon biotope.

3. In contrast to intact ones, white lactating rats fed with GM-soy did not produce lactose-negative Escherichia coli, while lactose-positive Escherichia coli did not produce flour, and vice versa. Lactation of lactosanegative strains, the absence of lactose-positive strains, has been shown to be a second element of colonic dysbiosis.

4. In laboratory animals fed with GM-soy, Enterobacter spp and Proteus spp were found to be 4.54 and 3.75 times higher, respectively, than in the control group, proving to be the third element of colon dysbiosis.

5. No significant changes in gram-negative cocci were observed in elements 1-3 of colonic dysbiosis with obvious manifestations of this condition - Streptococcus spp in the main group decreased 1.47 times compared to intact laboratory animals, coagulazapositive Staphylococcus spp 1.50 times more reliable. increased significantly. This intergroup incompatibility was interpreted as the fourth element of colon dysbiosis.

6. The quantitative index of Candida spp in white-bred rats fed with GM-soy was significantly increased by 1.94 times compared to those not fed with this product, as the fifth element of colon dysbiosis.

7. In laboratory animals fed GM-soy, all 5 of the cited elements of dysbiosis were present, whereas in rats consuming soy without GM, they were not evident.

8. Determination of dysbacteriosis index, indicating the I and II degrees of dysbacteriosis, gave the following results: in group 1 - 0.31 <0.1 (DI I); 0.37 <0.5 (DI II); In group 2 -

0.38 <0.1 (DI I); 0.77 <0.5 (DI II); In group 3 - 1.29 <0.1 (DI I); 3.56 <0.5 (DI II). There were no signs of dysbiosis in intact laboratory animals, the symptoms of dysbiosis were poorly developed in GM-free soy feeding (grade I), and the symptoms of dysbiosis were pronounced in GM-soy-fed animals (grade II).

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