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Research Article

The Influence of the Microorganisms Supernatants of *Bacteroides fragilis, Bacteroides melaninogenicus, Escherichia coli* were Kept in Nutrient-Free Saline on the Mice Body Weight

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Abstract

There is a close relationship between the intestinal microbiota and the central nervous system, based on the efferent innervation of the gastrointestinal tract and the biochemical signals of neuroactive microbiota molecules. The microbiome-gut-brain axis includes the intestinal microbiota, the central nervous system, the neuroendocrine and neuroimmune systems, including the hypothalamic-pituitary-adrenal system, the sympathetic and parasympathetic parts of the autonomic nervous system, and the enteral nervous system. It is revealed that the microbiota of the mice intestinal affects the regulation of animal body weight. Supernatant of three main types of intestinal microorganisms (*Bacteroides fragilis, Bacteroides melaninogenicus, Escherichia coli*), which were for two days in saline without nutrients (1.0×10^9 CFUs in 1.0 ml of saline), significantly (by 10.2%) increased the weight of mice after daily intragastric administration (0.7 ml) for 30 days. The data obtained suggest that microorganisms of the intestines of animals produce neuroactive substances that can both increase and, apparently, reduce their body weight.

Keywords: Microbiome-Gut-Brain Axis; Neuroactive Molecules from Intestinal Microorganisms; *Bacteroides fragilis; Bacteroides melaninogenicus; Escherichia coli;* Mice Body Weight

Introduction

The human body is not only the most complex of organs and systems functioning in strict interaction, but also simultaneously serves as a host for more than 500 different types of microorganisms accompanying a person from birth to death. The number of microbial communities in the organism is hundreds of times greater than the number of cells of the host's tissues and 10^5 is approximately 5 - 8% of its body weight [1]. At the same time, the joint existence of the human being and microorganisms in physiological conditions that emerged in the course of evolution does not cause any visible inconveniences to the human being. These microorganisms constantly found in healthy people belong to the normal microflora (microbiota, autofloor), which supports biochemical, metabolic and immune balance of the microorganism, necessary for preservation of its health. The most representative and significant for humans is the microflora of the gastrointestinal tract. Species composition and quantitative parameters of microflora vary in different parts of the gastrointestinal tract from 10^2 to 10^{13} cells per 1 ml of content. The maximum number of bacteria is found in the blind and cross-sedimentary intestine. The main factors limiting bacterial growth in the upper parts of the intestine tube are rapid progression of food masses, acidic environment and exposure to proteolytic enzymes. In the large intestine, the conditions of the medium are diametrically opposed, so that in this part of the intestine the number of bacteria reaches 10^{13} CFU/ml of content [2].

Studies show that the relationship between the intestine and microflora is not just a mere commentary (that is, a harmless coexistence), but rather a form of mutualism, that is, a mutually beneficial relationship [3]. Microorganisms perform a number of

useful functions for the host, such as anaerobic digestion of unused material to provide energy, training the immune system, and preventing the growth of harmful species [4]. However, intestinal microflora is not always extremely useful: it is believed that some microorganisms may cause disease in certain cases. It is also able to produce biotransformation of drugs and affect the expression of human genes that regulate metabolism, causing unwanted side effects [5,6].

All germs that normally inhabit the colon are divided into three groups: basic (lactobacilli, bifidobacteria and bacteroids), concomitant (*Escherichia coli* strains, enterococci) as well as staphylococcus, mushrooms, proteins. Bacteria that can be found in the human intestine [7]: (100%) - *Bacteroides fragilis, Bacteroides melaninogenicus, Bacteroides oralis, Enterococcus faecalis, Escherichia coli, Enterobacter sp.* (40 - 80%), *Klebsiella sp.* (40 - 80%), *Bifidobacterium bifidum* (30 - 70%), *Staphylococcus aureus* (30 - 50%), *Lactobacillus* (20 - 60%), *Clostridium perfringens* (25 - 35%), *Proteus mirabilis* (5 - 55%), *Clostridium tetani* (1 - 35%), *Clostridium septicum* (5 - 25%), *Pseudomonas aeruginosa* (3 - 11%), *Salmonella enterica* (3 - 7%), *as well as Faecalibacterium prausnitzii, Peptostreptococcus sp. and Peptococcus sp.*

The gut flora can produce a range of neuroactive molecules, such as acetylcholine, catecholamines, γ-aminobutyric acid, histamine, melatonin and serotonin, which are essential for regulating peristalsis and sensation in the gut [8].

There is a close relationship between the gastrointestinal tract (in particular the intestinal microbiota) and the central nervous system, based on the efferent innervation of the gastrointestinal tract and the biochemical signals of neuroactive microbiota molecules of microbiome-gut-brain axis includes the intestinal microbiota, the central nervous system, the neuroendocrine and neuroimmune systems, including the hypothalamic-pituitary-adrenal system, the sympathetic and parasympathetic parts of the autonomic nervous system, and the enteral nervous system [9,10].

We hypothesized that the gut microbiota could produce neuroactive molecules that could make animals and humans feel hungry when they are not well fed. These neuroactive molecules (probably proteins like cytokines), acting on specific centers in the brain, seem to induce a hungry animal to consume food. If an animal is provided with an excess of food and injected with neuroactive molecules released by the microbiota during a lack of nutrition, then experimental animals will likely consume food in excess, which will lead to an increase in their body weight.

Aim of the Study

The aim of the study was to determine the effect of the supernatants of three main types of intestines microorganisms (*Bacteroides fragilis, Bacteroides melaninogenicus, Escherichia coli*) on the body weight of mice after intragastric administration for 30 days.

Materials and Methods

Experiments were carried out on random-bred albino mice of both sexes weighing 14 - 26g. Before the start of the experiment, the weight of the mice was 14 - 20g and after 30 days - 20 - 26g. The age of this group of mice before the start of the experiment was 50 - 60 days. Both groups of mice received a standard diet (as well as water) without restriction. The first group of mice (group 1, n = 20) received intragastrically (through the mouth using a probe) 0.7 ml isotonic sodium chloride solution (saline) daily for 30 days. The second group of mice the same age (control group 2, n = 20) received intragastrically (through a probe, daily for 30 days) 0.7 ml of supernatant after the stay of three main species of microorganisms of the intestines (*Bacteroides fragilis, Bacteroides melaninogenicus, Escherichia coli*) in test tubes (10 ml) with saline for two days in a thermostat. To obtain the supernatant, tubes with microorganisms were centrifuged with a rotation speed of 300g. The tubes with microorganisms after washing from Petri dishes were kept in a thermostat at 37^0C. The tubes contained 1.0×10^9 CFUs culture of microorganisms in 1.0 ml of saline. For intragastric administration of the supernatants of microorganisms to mice, 6 ml of supernatant were taken from three tubes after centrifugation, which were mixed. Groups of mice were weighed before and after the experiment. The data were processed statistically using the Student's t-test. Differences between the parameters were considered reliable at $p < 0.05$.

Results

In the first group of mice, which received intragastrically saline daily for 30 days, body weight increased in 1.32 times (by 32%) - $p < 0.05$ and in the second group, which received intragastrically the supernatant of microorganisms, the body weight of mice enhanced in 1.42 times (by 42%) - $p < 0.05$. After 30 days, the body weight of the mice that received the supernatant of microorganisms was 1.10 times greater than the weight of the control (first) group of animals (by 10.2%) - $p < 0.05$ (Table). This indicates that the supernatants of microorganisms (*Bacteroides fragilis, Bacteroides melaninogenicus, Escherichia coli*) significantly increased the body weight of mice.

Series of experiments	Body weight of mice before and after administration of saline and microorganism supernatants, g	
	Before administration	30 days after administration
Saline (control group 1, n = 20)	17,1 ± 0,37	22,6 ± 0,31[a]
Supernatants of microorganisms (group 2, n = 20)	17,5 ± 0,35	24,9 ± 0,39[ab]

Table: Influence of the supernatants of microorganisms (*Bacteroides fragilis, Bacteroides melaninogenicus, Escherichia coli*) on the body weight of mice after daily intragastric administration (0.7 ml) for 30 days, g (M ± m)

a - $p < 0,05$ as compared to group before administration of saline or supernatant; b – $p < 0,05$ as compared to control (group 1) 30 days after administration of microorganism supernatant (group 2).

Discussion

It is known that the microbiota of the gastrointestinal tract (the intestines) of mammals (animals) is closely related to their brains [9]. It is likely that gastrointestinal microorganisms (gastrointestinal microbiota) that cultivate in environments where there are no substances they can use for their life activities (for nutrition) can produce neuroactive molecules [8,10] that affect the food intake of animals (in particular mice). In starving conditions, it is possible that the neuroactive molecules synthesized (produced) by microorganisms in the intestines of mice (as well as other mammals), which are probably similar in structure to cytokines, increase animal food intake. This leads to an increase in their body weight compared to animals that "under normal conditions" do not receive neuroactive molecules. The intestinal microbiota seems to be able to regulate the appetite of animals, and along with increasing body weight it is able to secrete substances that may have the opposite effect. The isolation of neuroactive molecules from intestinal microorganisms or the synthesis of these molecules can be considered as a promising way to create drugs that regulate the weight of the human body, and can also be useful in certain disorders of the central nervous system [9].

Conclusion

The microbiota of the mice intestinal affects the regulation of animal body weight. Supernatants of three main types of the intestinal microorganisms (*Bacteroides fragilis, Bacteroides melaninogenicus, Escherichia coli*), which were for two days in saline without nutrients (1.0×10^9 CFUs in 1.0 ml of saline), significantly (by 10.2%) increased the weight of mice after daily intragastric administration (0.7 ml) for 30 days. The data obtained suggest that microorganisms of the intestines of animals produce neuroactive substances that can both increase and, apparently, reduce their body weight.

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