MICROBIOLOGICAL CHARACTERISTIC OF THE EXPERIMENTAL USE OF AUTOBIOTIC ON THE MODEL OF ANTIBIOTIC – INDUCED DYSBIOSIS IN OLD RATS

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Annotation. *The aim of this work is the determination of the condition of gastrointestinal tract's microbiocenosis changes of laboratory rats in dynamics of the reproduction of experimental dysbacteriosis of the II degree, and the use of "Autobiotic" with the medical purpose, – the agent, which contained the representatives of its own indigenous intestinal microflora of laboratory animals. The obtained results demonstrate the that in all old rats dysbacteriosis of the II degree was observed; the quantity and frequency of identification of the representatives of both obligate and transitory microflora was changed. Oral application of eubiotic strains of Bifidumbacterium spp. and Lactobacillus spp. ("Autobiotic") against the background of intestines dysbiosis of the II degree in laboratory rats promotes the faster homeostasis restoration of the gut microflora, overcoming dysbiosis and its consequences.*

Key words: *Autobiotic, dysbiosis, gastrointestinal tract, rats.*

Introduction. The human microbiota is the aggregate and a ratio of the symbiotic microorganisms, inhabiting an organism – bacteria, archaeal and eukaryotic microorganisms, which have (take) a considerable effect on the human physiology and health. The gut microbiota (a part of the gastrointestinal tract, GIT) is the most numerous and studied. The intestinal microbe forms the collective system of genes, consisting of the trillions of microorganisms, which constantly exist and changes in a gastrointestinal ecosystem. The interaction between a human body and microbiomes is verycomplicated and multifactorial [1, 5].

The gut microbiota actively affects the transformation processes of the molecules of proteins, fats and carbohydrates, synthesis of vitamins in the GIT, intestinal peristalsis regulation of, takes part in the processes of detoxication, regulates permeability of some substances through the mucous membrane of the gut. Studying of the interrelation between the gut and the brain, that is the so-called gastrobrain axis (gut-brain-axis), by means of which the brain has an impact of the function of the gastrointestinal tract and the last one – vice-versa, is modern and relevant [1, 3]. At the same time, the main basic components of the microbiota-gut-brain axis is the central nervous system, neuroendocrinal and the neuroimmune systems, the autonomic nervous system, and the system of the nerve ganglia of the intestines and the gut microbiota. These components

form a complex multiple-factor network, by means of which the signals from the brain can affect not only the motor, sensory and secretory activity of the intestines, but also its microbiota. And on the contrary, visceral signals from the microbiota-mediated area of intestines, significantly influence brain functions [6].

The signaling pathway of the microbiome – CNS axis is functioning by means of the studied regulatory mechanisms of nourishment and saturation. Changes in the dietary intake of the organism affect the availability of various nutrients for the intestinal microflora and, its qualitative and quantitative structure changes appropriately [1].

It is important to emphasize that the probiotics bacteria, applied at certain human diseases and morbid conditions colonize the intestines, can affect the central nervous system through the products of a number of neurotransmitters and biological substances: serotonin, melatonin, gamma-aminobutyric acid (GABA), catecholamines, histamine and acetylcholine. A part of these substances is capable to take effect not only on the mesenterial, but, first of all, on central nervous system [4].

The results of practical studying of the interaction of the microbe-intestines-brain are also extremely important in development of the principles of prevention and treatment of not only intestinal disorders, but also such pathogenetic difficult diseases of the central nervous system as schizophrenia, Alzheimer's disease or Parkinson's disease [8].

But at the present stage of medical science intestine dysbacteriosis (dysbiosis) is considered first of all, as a clinical set of disorders of the macroorganism that is characterized by the changes of the quantitative and qualitative structure of the microbiota (microbiocenosis disorders). Our research was devoted to studying of the separate tasks of the issue. The determination of the condition of the GIT microbiocenosis changes of laboratory rats in dynamics of the reproduction of experimental dysbacteriosis of the II degree, and the use of "autobiotic" with the medical purpose, – the agent, which contained the representatives of its own indigenous intestinal microflora of laboratory animals, became the purpose of this work.

Materials and methods. Modeling of thee gut microbiota dysbiosis or intestinal dysbiosis of the II degree was caused by the results of the previous researches of the gut microbiota of elderly people and the patients, suffering from Alzheimer's disease, according to which in these patients the development the intestinal dysbiosis of the II-III degree dominated [9-11].

Experiments have been made with the quantity (n = 25) of white not pedigree rats (at the age of 26 ± 1 month), with respect for all norms and rules of carrying out experiments with animals (reference). The control group consisted of the white rats aged 25 ± 0.3 months (n = 16). Before the beginning of the experiment (dysbacteriosis modeling), inoculation of excrements of intact white rats of differential and diagnostic, selective and special media for the purpose of determination of the quantitative and qualitative structure of their gut microbiota were carried out. The animals before the beginning of the experiment, taking part in research conditions of intestines dysbiosis served as control. The animals, included in the experiment were divided into 3 groups. The first group was made by old rats (n = 6) with the physiological dysbacteriosis of the II degree,

associated with the age. They kept a standard diet of the vivarium, and in addition, within two weeks (daily once up to 12 h. per day) they were orally taking the autobiotic solution (108 CFU), made of their own strains bifidus- and/or lactobacteria, isolated from the animals before carrying out experiments with the animals.

In the experimental animals of the 2nd and the 3rd groups the development of experimental intestinal dysbiosis of the II degree was induced. For this purpose a therapeutic dose (0.2 ml) of the solution of ciprofloxacin antibiotic was the white rats were intraperitoneally introduced within 7 days. After that, in addition, the animal of the second experimental group (n = 8) within two weeks (daily, once time during the first half of the day) was orally taking a mix of the pharmaceutical pro-biotic drugs, containing lactobaciteria (the microbic mass of live lactobacteria of L. plantarum or L. fermentum, frozen-dried) and bifidus bacteria in the volume of 15 ml, containing bifidus bacteria and lactobacteria in the number of 108 CFU/ ml.

In the third group (n = 8) instead of a probiotic within two weeks the solution of an autobiotic was introduced in the volume of 15 ml; it was made of its own strains bifidum- and/or the lactobacteria (108, CFU each strain), isolated from the animals to modeling of intestinal dysbiosis [patent № 10/2366].

The choice of a pro-biotic agent for the animals of the second group and an autobiotic – for the animals of the other groups was based on the results of the bacteriological research of the quantitative and qualitative structure of the indigenous microbic gut flora on the 3rd day of determination of the clinical symptoms of dysbacteriosis. According to the results of disco-diffusive method pro-biotic strains of Bifidumbacterium spp. and Lactobacillus spp., were used as bioindicators, while modeling dysbacteriosis (in the animals of the 2nd and 3rd groups), who were sensitive to the effect of ciprofloxacin (d of the zone of growth inhibition of cultures $\geq$ 22 mm).

For the purpose of treatment of dysbiotic changes a probiotic (the 2nd group) or an autobiotic agent (the 3rd group) with the quantity of bacteria of the indigenous microbiota, corresponding to 0.5 units, diluted in the sterile distilled water was applied. (According to McFarland's standard).

During the whole experiment the physical activity, body weight of the animals, their appetite (an amount of the eaten food), the nature of their stools were studied. On the 3rd, 5th, 9th, 14th and 17th day of the experiment the samples of animal's excrements for carrying out microbiological by the intestines microbiocenosis research were collected.

The samples of excrements (one from each rat of all three groups) were collected in sterile containers and bacteriological researches on a standard technique were conducted at once [11]. The sample of excrements was weighed, homogenized in 0.85% sodium chloride solution, obtaining the initial cultivation 10-1. 9 tenfold dilutions in physiological solution (before cultivation 10-10) were made of it. Then the cultivation with tenfold dilution of excrements right after their preparation was carried out.

The statistical data processing was carried out by means of the computer STATISTICA (Statsoft) program. The reliability of differences between the studied groups was determined by Student's t-test after the analysis of the division on normality.

The distinctions, corresponding to the value of the error $p<0,05$, were considered statistically reliable.

For the integrated assessment of the microecological microflora characteristics the following indicators were used: species richness index (SRI) – the average quantity of the species, which are a part of the biocenosis; stability indicator (S) – identification of a share of different types in the structure of the biocoenosis ($S = (p / P) x100\%$, where S – the stability indicator, p – the number of examinations, containing the studied species; P – the total number of examinations). Interpretation was carried out according to the following data: >50% – the constant species; 25-50% – additional species view; <25% –accidental species, and a range of the opportunistic microorganisms (OM) [2].

Results and their discussion. In the analysis of the data of bacteriological and mycologic researches of the gut microbiota of research age animals the significant changes in the structure of the microbiocenosis in comparison with the results of the study of intact animals have been revealed. In all old rats dysbacteriosis of the II degree was observed; the quantity and frequency of identification of the representatives of both obligate and transitory microflora changed. In spite of the fact that bifido-and lactobacteria, had appeared as a part of the gut microflora in all experimental animals (S = 100%), their quantitative contents in compliance decreased – bifidobacteria to lg 6.9 ± 0.2 against lg 8.7 ± 0.3 CFU /g in intact animals, and lactobacteria – lg 5.4 ± 0.1 against lg 7.5 ± 0.3 CFU /h. Except such changes in the quantitative and qualitative structure of the indigenous microbiota, namely bifido- and lactobacilli, in all studied rats of the second (II) group against the background of ciprofloxacin antibiotic application, the reduction of the quantity of Escherichia coli with the normal enzymatic activity by 1.23 times (to lg 5.3 ± 0.2 CFU /h) ($p<0,05$) in comparison with the indicators of intact animals was observed and at the same time ($p<0,05$) the quantity of microorganisms of Escherichia coli with the reduced enzymatic activity and quantity of OM – Proteus spp., Klebsiellaspp., Pseudomonas spp., S. aureus and opportunistic pathogenic fungi of the genus Candida considerably increased in 55% of the studied species of animals.

Studying the results of the observations, concerning the physical activity, body weight of animals, appetite (an amount of the eaten food), the nature of stools it should be noted that in the majority of laboratory rats with intestinal dysbiosis, , reproduced by using of the ciprofloxacin and other research groups, the phenomenon of polyfecalia was observed, in 62 5% of cases – a change in the consistence of stools, in 86.4% – bad appetite, in 68.2% – weight reduction by 30 ± 1.2% ($p<0,05$).

In the rats of the first (I) research group after introduction of per os of the autobitic strains of Bifidumbacterium spp. and Lactobacillus spp. their quantity increased on the 9th day of the experiment to the next indicators: bifidobacteria to lg 7.3 ± 0.4 CFU /g; lactobacteria – lg 6.2 ± 0.3 CFU/g, that is 1.15 times more than before autobiotic treatment. The complete restoration of the studied indicators of the indigenous gut microflora of the rats from the first (I) group to the indicators of intact animals was observed only on the 14th day. Besides, in the rats of the first (I) research group ($p<0,05$) the quantity of OM of the genus Klebsiella, Proteus and Escherichia coli with the low

enzymatic activity authentically decreased on the 9th day of the experiment. On the 14th day of the experiment in 66.7% of the rats of this group in the gut microbiota the representatives of Escherichia coli with the low enzymatic activity were completely absent. The evident facts testify to the high level of the antagonistic activity of autobiotic strains of Bifidumbacterium spp. and Lactobacillus spp. in relation to OM that has been proved in in vitro experiments. Besides, when studying the level of the adhesive activity of Bifidumbacterium spp. and Lactobacillus spp. strains, separated from the rats of the first group on the 14th day of the experiment it hadbeen determined that 87.3% from them showed the average or high level of adhesion to cells.

While examining the general condition of the white rats of the first (I) group, it has been revealed that on the 16th day from the start of the autobiotic intake all the animals of this group were observed to have their completely restored weight, dyspepsia symptoms disappeared, and the other functions of animals were within norm.

All the experimental animals of the second (II) and the third (III) research groups after the effect of ciprofloxacin, which was used for the purpose of dysbacteriosis modeling showed bad appetite, and 68.2% – weight reduction by 36 ± 1.4%. The dynamic research of the changes of the structure of the gut microflora of animals, against the background of the carried-out treatment by various agents showed that in rats of the second (II) group, the quantity of Bifidumbacterium spp. and Lactobacillus spp. authentically did not increase on the 9th day of the experiment, that is on the specified scheme of probiotic introduction. The reliable ($p<0,05$) increase was observed only in the animals of this group on the 14th day, but it did not reach the quantitative indices of intact animals. At the same time, on the 9th day in the rats of the second (II) group a reliable ($p<0,05$) decrease in the quantity of opportunistic microorganisms (OM), including the representatives of Escherichia coli with the low specific enzymatic activity in intestines was observed. Besides, in all the animals of this (II) group, having coagulase-positive staphylococcus during the initial stage of the experiment they were already absent on the 17th day of the experiment.

While studying the adhesive and antagonistic activity of pro-biotic strains of Bifidumbacterium spp. and Lactobacillus spp. in relation to OM separated from the excrements of the animals of the second group it has been determined that they owned either the low, or average level of the activity and at the same time the high level of the antagonistic activity concerning Proteus spp., Klebsiella spp., Pseudomonas spp., S. aureus and the low level of antagonism concerning the fungi of genus Candida. The longer period of the gut microbiota restoration of the second group of animals in comparison with other groups is explained by it. It is also necessary to note that in the animals of the second (II) group dyspepsia symptoms were observed up to the 13th -14th day (normalization took place on the 15th -16th day) of the experiment, and appetite restoration – on the 17th day of the study.

In experimental animals of the third (III) group after the oral administration of autobiotic strains of Bifidumbacterium spp. and Lactobacillus spp., that is "Autobiotic", the restorative dynamics of the quantitative and qualitative structure of the gut microbiota

of animals was the following: a reliable ($p<0,05$) increase in the quantitative contents of bifido- and lactobacteria, according to lg 8.1 ± 0.3 CFU/h and lactobacteria – to lg 6.4 ± 0.3, CFU. A reliable ($p<0,05$) reduction of the quantitative contents of OS on the 13-14th days of the experiment. It should be noted that in 25% of the rats of this group, Escherichia coli with the low enzymatic activity was sowed in high concentrations till the 9th day of the experiment, and ($p<0,05$) the indicator of their isolation authentically decreased on the 14th day of the experiment.

The analysis of the adhesive and antagonistic activity of Bifidumbacterium spp. and Lactobacillus spp. strains(autobiotic) in relation to OM, separated from the animals of the third group has showed that 100% of strains of these bacteria owned the average or high level of adhesion and the low level of the antagonistic activity, concerning the studied representatives of OM.

The complete restoration of the consistence of fecal boluses, the appetite of the animals and the physiological activity of rats of the third experimental group was detetermined on average on the 14th day of the experiment (from the 10th to the 15th day of the experiment).

An important microecological indicator that can characterize the intestines microbiocenosis homeostasis in general is species richness index (SRI). In the analysis of the material from the laboratory animal of all the groups SRI was counted for the representatives of OM. After the carried-out treatment it has been determined that in the first (I) group of laboratory animal SRI fluctuated ranging from 1.6 up to 1.8 r.u (reference units).; in the second (II) – from 1.7 to 2 and only in the representatives of the third (III) group – 1.4 to 1.7.

The results of the use of autobiotics in laboratory rats are described. It is shown that early correction of intestinal dysbiosis in rats against the background of using an autobiotic. More pronounced antagonistic properties of autobytic strains of lacto-and bifidobacteria relative to potential-patogenicmicroorganisms – one of the factors in the development of the dysbacteriosis clinic – have been revealed.

Conclusions. Thus, the obtained results demonstrate the that oral application of eubiotic strains of Bifidumbacterium spp. and Lactobacillus spp. (Autobiotic) against the background of intestines dysbiosis of the II degree in laboratory rats promotes the faster homeostasis restoration of the gut microflora, overcoming dysbiosis and its consequences, which confirms the restoration of separate physiological functions of the organism of experimental animals and the activity of separate microorganisms' functioning.

For this reason, the development and use of autobiotic therapy is an urgent task of the present stage of gastroenterology.

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