SELECTION OF THE AUTOPROBIOTICS FOR CORRECTION OF INTESTINAL DYSBIOSIS

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Introduction:
Intestinal dysbiosis accompanies many intestinal and extraintestinal diseases negatively affecting different systems of the organism. The use of probiotics for the treatment of dysbiosis is not always effective due to various reasons including antagonism to the indigenous microbiota. In this respect autoprobiotics - bacterial preparations created from indigenous microbiota of the patient have a number of benefits such as immunological tolerance and lack of strong antagonistic activity against the host microbiota. Until now, the methodology of creating autoprobiotics is not fully developed. The aim of the study was to find the optimal composition of autoprobiotics for the correction of experimental dysbiosis considering their effects on immunity and intestinal microbiocenosis.

Methods:
Intestinal dysbiosis of male Wistar rats was induced by antibiotics (ampicillin® and metronidazole®) for 3 days. Indigenous enterococci, lactobacilli, bifidobacteria were isolated from feces before the antibiotic treatment and grown separately. The anaerobic consortium was prepared by growing the complex of fecal bacteria in anaerobic conditions. Indigenous enterococci (E), lactobacilli (L), bifidobacteria (B), their mixture (M) or anaerobic consortium (AC) were given for 4 days after inducing dysbiosis. Rats from control group 1 (C1) didn’t receive autoprobiotics. Animals from control group 2 (C2) didn’t receive antibiotics and autoprobiotics. The study of fecal samples, collected on 4th and 9th days of experiment, was performed bacteriologically, by RT-PCR and by metagenomics 16S rRNA analysis. Immune status was assessed on the final stage of experiment by flow cytometry and ELISA with monoclonal antibodies. Culture of Staphylococcus aureus (strain 209) was used to study phagocytic activity of peripheral blood leukocytes.

Results:
Dysbiosis characterized by excessive growth of Gammaproteobacteria, and decrease in the number of beneficial bacteria. Elimination of atypical Escherichia coli, Proteus spp., Klebsiella spp., Enterobacter spp. was more effective in groups B, E and AC relatively to groups C1, L and M. Restoration of Firmicutes (Faecalibacterium sp., Lactobacillus spp.) and Actinobacteria (Bifidobacterium spp.) populations was more significant in rats receiving AC, B and E. The content of NKT (CD3 + CD161 +) in peripheral blood was higher in animals with dysbiosis which did not take autoprobiotics (group C1). The lowest content of CD3+CD25 + FoxP3 + lymphocytes was also found in the spleens of animals from the group C1. The quantity of this cluster of lymphocytes after consumption of AC, B and E increased comparing with initial level (C2 group). A decrease in the B lymphocyte pool (CD45RA +) was revealed in the spleen of animals in groups E and AC. The level of anti-inflammatory and regulatory cytokine - IL-10 was elevated in the blood serum of rats treated with AC, B and E. The phagocytic activity of peripheral blood leukocytes was the least in rats from C1 group. Group AC was characterized by the highest phagocytic activity of peripheral blood leukocytes.

Discussion:
The most pronounced positive changes in intestinal microbiocenosis and innate and adaptive immunity were provided by autoprobiotics based on enterococci and on the consortium of indigenous anaerobic bacteria. The study was supported by Russian scientific foundation grant 16-15-10085.

Keywords: Probiotics, Phagocytic activity, Cytokines, Immunity, Microbiota, Lymphocytes
Citation: