THE INFLUENCE OF LOCAL STRAINS OF LACTOBACILLI ON EXPERIMENTAL MODEL OF ULCERATIVE COLITIS

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Abstract: The article is devoted to one of the most important effects of local strains of lactic acid bacteria - the identification of anti-inflammatory and protective action. Investigations have been carried out on an experimental model of ulcerative colitis caused in experimental animals (mice). Presented data proved that the action of various probiotic bacteria were unequal. The ability of probiotic lactic acid bacteria in invitro and invivo systems to influence the production of pro- and anti-inflammatory cytokines, stimulate the antimicrobial, anti-inflammatory, protective effect has been shown. The necessity of studying the specific effect of local strains of lactobacilli on anti-inflammatory properties has been shown for a more adequate, effective selection of probiotic drugs in the treatment of intestinal ulcers.

Key words: inflammatory bowel disease, ulcerative colitis, probiotics, lactobacilli, intestinal micro flora, dysbiosis, conditionally pathogenic bacteria, IBD pathogens, biological products, prevention, treatment.

Introduction. Inflammatory bowel diseases (IBD), which include ulcerative colitis and Crohn's disease, are autoimmune, chronic, recurrent diseases and represent one of the most serious problems in modern gastroenterology and coloproctology [2]. According to the severity, frequency of complications and mortality, these diseases occupy one of the leading places among the diseases of the gastrointestinal tract all over the world. Studying the mechanisms of development of ulcerative colitis is an urgent biomedical problem, since its constant growth is observed throughout the world. So, according to foreign data, the incidence of UC is from 35-100 people per every 100,000 people, the prevalence reaches 505 per 100,000 people. [3, 4]. The course of IBD leads to serious complications. The frequency of the formation of the pathological process at an early age increased 1.5 times. More severe extra intestinal manifestations appeared. Resistance to traditional therapy is more often observed. The development of new therapeutic and biotherapeutic approaches is required because of the early patients' disability and high mortality from these diseases [6]. Inflammatory bowel disease is one of the factors that increase the risk of colorectal cancer. Inflammatory bowel diseases due to the above mentioned features have not only medical but a great social importance as well. The lack of clear ideas about the etiology and pathogenesis of IBD leads to the problems in the diagnosis and treatment of these diseases using a wide range of drugs.

The chronic nature of IBD requires constant anti-relapse therapy and during exacerbations the use of drugs to induce remission. The main classes of drugs used currently to treat IBD are 5-aminosalicylic acid preparations, antibiotics, glucocorticosteroids, immunosuppressants and monoclonal antibodies to tumor necrosis factor. Most of these drugs have pronounced side effects: impaired glucose tolerance, inhibition of adrenal function, osteoporosis and ulcerative lesions of the stomach and duodenum, the development of opportunistic infections, allergic or

toxic reactions. Therefore, it is very important, appropriate and extremely necessary to evaluate the effectiveness of a new and potentially safe class of drugs - probiotics, which are living microorganisms that have a multidirectional beneficial effect on the digestive system functioning.

This problem is of great interest for specialists microbiologists, since the colon is a reservoir for many microorganisms and food antigens. Under physiological conditions the colon epithelial barrier prevents the penetration of bacteria and toxic substances into the internal environment of the body and prevents the development of the inflammatory process. Disturbances of the structure and function of the epithelial barrier can lead to the development of a number of colon diseases, including ulcerative colitis [9].

Currently, a modern hypothesis has been put forward, which presents a complex of genetic factors, local immunity, diet and environmental factors as a direct or indirect effect, characterized by a change in the composition of the intestinal microbiota. Studies of the intestinal microflora of patients with inflammatory bowel disease (IBD) have shown that the most typical pathogens of these diseases are: *Salmonella*, *Shigella*, *Campylobacter*, *Clostridium difficile*, *Escherichia coli* O157: H7, *Yersinia* and some others. Numerous studies have demonstrated that there is a close relationship between the composition of the microbiota and various aspects of human health, including the physiological state, metabolism, and immunological response [1]. Summarizing many studies, we can draw a conclusion that subpopulations of *E.coli* can play an important role in the pathogenesis of ulcerative colitis. It was established that patients with Crohn's disease and ulcerative colitis have an increased concentration of *Enterobacteriaceae* and *Bacteroides* species [12]. Patients suffering from IBD differ from healthy patients in the altered composition of commensal intestinal bacteria and, as a rule, an increased number of adhesive invasive *E.coli*, *Enterococci*, and an underestimated number of species of *Bifidobacterium* and *Lactobacillus*. To date, a few microbiological studies have been carried out to identify inducers of IBD diseases and methods of inhibiting these bacteria [7]. Taking into consideration that the basis of pathogenesis is gastrointestinal tract microbiome and intestinal immunity balance disturbance, the use of probiotics as an addition to standard anti-inflammatory and immunosuppressive therapy has its own expediency and validity. For the prevention and treatment of inflammatory bowel disease the use of probiotics supports strong biological importance. Many probiotic strains exhibit anti-inflammatory properties due to their effect on various immune cells, decreased secretion of pro-inflammatory cytokines and the induction of anti-inflammatory cytokines. For the prevention or relapse of postoperative pouchitis in patients there is very convincing evidence supporting the use of the multi-species probiotic VSL # 3. For the treatment of active ulcerative colitis, as well as for maintenance therapy, clinical efficacy data are most effective for VSL # 3 and *Escherichia coli* Nissle 1917. Pei LY, Ke YS et al. (2019) investigated the regulatory effect of garidisan from wild poppy *Artemisia frigida* Willd on microbiota dysbacteriosis intestines in a mouse model of ulcerative colitis and studied the possible mechanism of therapeutic effects of garidisan on UC. It was noted that the therapeutic effect of garidisan on ulcerative colitis is associated with a direct

effect on the Lachnospiraceae family of bacteria [9-10].

The aim of this work is to find new methods for the prevention and treatment of inflammatory diseases of human gastrointestinal tract using preparations consisting from probiotic microorganisms by selecting strains that exhibit antimicrobial activity against dominant harmful microorganisms of intestinal origin and by studying the anti-inflammatory mechanism of probiotics action and local strains of lactobacilli on an experimental model of ulcerative colitis.

Materials and methods. Strains of probiotic cultures were isolated from environmentally pure products (cheese, feta cheese, pickles and tarragon plant). They were identified in the sanitary-hygienic laboratory at the Ministry of Health of the Republic of Uzbekistan by mass spectrometry method (MALDI TOF) [5].

Scientific investigations have been carried out on experimental animals - 48 sexually mature male and female mice of 21-26 g body weight, according to the requirements of the Pharmacological Committee of the Republic of Uzbekistan from 2000. Experimental animals have been previously quarantined for two weeks in conditions of room temperature 18-25°C with natural light. Qualitative and quantitative indicators of stool, motor activity and condition of the coat have been determined in mice during the experiment; their death has been separately recorded.

After the administration of the preparations, a visual examination has been carried out, including an assessment of the condition of the colon mucosa and determination of the number of ulcerative lesions. A necessary condition for this study was the measurement of body weight of experimental animals on the third, sixth and ninth day after taking the drugs.

An experimental model of ulcerative colitis was caused by a single injection of 4% acetic acid according to the method of Fitzpatrick et al.

The object of the research were local strains of lactobacilli: *Lactobacillus casei* K7/3, *Lactobacillus plantarum* ET-2, *Lactobacillus plantarum* AB-1, *Lactobacillus plantarum* CO-1 and IVXL - a composition of four probiotics developed by scientists from the Institute of Microbiology of the Academy of Sciences of the Republic of Uzbekistan.

To assess the effectiveness of the lactobacilli strains effect animals have been divided into 6 groups: group 1 - control, animals with model pathology, group 2 - animals treated with K7/3 of 1 ml dose per a mouse, group 3 - animals treated with ET-2 of 1 ml dose per a mouse, group 4 animals treated with AB-1 of 1 ml dose per a mouse, group 5 animals treated with CO- 1 of 1 ml dose per a mouse, group 6 animals treated with IVXL of 1 ml dose per a mouse and control group without pathology.

Results and its discussion. The following results have been obtained during the influence with various strains of lactobacilli on experimental animals: in animals of the control group, regeneration processes have been slower than in other groups tested by local strains, as evidenced by changes in the affected areas of the large intestine mucosa and ulcer area; starting from the second day up to the sixth day, diarrhea with an admixture of blood has been observed

in experimental mice induced by ulcerative colitis.

On the third day the death of mice was observed in all experimental groups - 65%, with the exception of the control group (without pathology). Within 36 hours after the reproduction of acute colitis, a decrease in the motor activity of animals, a shaky gait and a painful posture have been noted, the coat was disheveled and dull. On the tenth day, the animals had similar external signs with the control group, that is, the coat was smooth, clean, active gait and the nature of the stool normalized.

Table 1

Group		Mass of animals			
		Initial	3-day of treatment	6- day of treatment	9- day of treatment
1	Control group without pathology	20±1,7	20,6±1,3	21±1,1	23±1,5
2	Control group with pathology	24±1,3	16±1,5	18±1,7	18,5±1,3
3	Lactobacillus casei K7/3	20±1,5	18±1,8	18±1,3	19,5±1,6
4	Lactobacillus plantarum ET-2	21±1,3	16±1,4	17±1,5	17,5±1,2
5	Lactobacillus plantarum AB-1	23±1,4	21±1,1	23±1,3	24±1,6
6	Lactobacillus plantarum CO-1	21±1,5	16±1,3	17±1,5	17,5±1,2
7	IVXL – composition of four probiotics	25±1,7	20±1,3	23±1,5	24±1,7

According to the table 1 in the treatment with the tested strains AB-1, K7/3, IVXL in appropriate doses, an increase in weight is noted in comparison with the control group without pathology and the tested strains, both the control one with pathology and strains ET-2 and CO-1.

In addition, a test screening was performed within 10 days. On the basis of induction, which was caused by the introduction of 4% acetic acid, the development of pronounced destructive changes in the mucous membrane of the colon with edema, hyperemia and hemorrhages has been observed.

As a result of experimental studies presented in table 2, it was found that in animals treated with IVXL - a composition of four probiotics, the area of ulcers decreased 3.15 times faster in comparison with the control (without treatment) group, where the degree of mucosal damage decreased 2.05 times.

Table 2

Group	Dose	Mucous membrane impairments, score	The area of ulcers, mm ²
Control group (without treatment)	-	5,5±0,25	135,04±24,53
Lactobacillus casei K7/3	0,5ml/per a mouse	3,50±0,04*	61,66 ±24,16*
Lactobacillus plantarum ET-2	0,5ml/per a mouse	4,13±0,17	71,53±23,14
Lactobacillus plantarum AB-1	0,5ml/per a mouse	3,33±0,12*	65,10 ±21,03*
Lactobacillus plantarum CO-1	0,5ml/per a mouse	4,57±0,15	74,71±21,07
IVXL – composition of four probiotics	0,5ml/per a mouse	2,67±0,33*	42,75±5,05*

* — declination authentically in comparison with control (P < 0,05)

Treatment with the tested strains have shown that with the introduction of Lactobacillus casei K7/3, the ulcer area decreased 2.19 times, Lactobacillus plantarum ET-2 - 1.89 times, Lactobacillus plantarum AB-1 2.07 times, Lactobacillus plantarum CO-1 1.81 times in comparison with the control group. While studying the condition of the mucous membrane of the colon, it has been found that among the groups receiving local strains there was an improvement in the condition of the mucous membrane of 1.57; 1.33; 1.65 and 1.20 times higher in comparison with the control group.

According to the results presented in tables 1 and 2 in the treatment with local strains of all the studied drugs, IVXL - a composition of four probiotics, has the most marked anti-inflammatory properties in the experimental model of ulcerative colitis in a comparative aspect.

Conclusions. Thus, IVXL - a composition of four local strains of probiotics has pronounced therapeutic properties and is one of the effective drugs for the correction of disorders caused by ulcerative lesions of the large intestine mucosa. Based on the results of test screening the local strain (IVXL) - a composition of four probiotics also showed high activity compared to strains of Lactobacillus casei K7/3, Lactobacillus plantarum ET-2, Lactobacillus plantarum AB-1, Lactobacillus plantarum CO-1, which confirms its practicability of use for disturbances of ulcerative colitis and gives an effective opportunity to treat the disease conservatively. In addition, local strains during the studies showed a long preservation of their biochemical activity, depending on external factors (composition of the nutrient medium, temperature, etc.), which contributed to the sustainable viability of the microorganism population, its practical value. So, the effectiveness and safety of various bacterial therapies require further evaluation in the form of experiments and new studies. These studies will help identify possible new therapeutic strategies aimed at

improving the effectiveness of methods for the prevention and treatment of ulcerative colitis.

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