The Effect of Probiotic Strains on Myocardial Infarction Size, Biochemical and Immunological Parameters in Rats with Systemic Inflammatory Response Syndrome and Polymorbidity

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Abstract—Numerous experimental and clinical studies have shown the effectiveness of various probiotic strains in metabolic disorders, gastrointestinal and liver diseases, immune system pathology. The effects of probiotics on cardiovascular dysfunction are less well known. The development and validation of a new experimental model in rats, including obesity, acute colon inflammation and antibiotic-induced dysbiosis, with common characteristics of systemic inflammatory response syndrome (SIRS), became the basis for investigating the effects of probiotic drugs on myocardial resistance to ischemic-reperfusion injury (IRI) using an in vivo model of infarction after coronary occlusion. A 24% increase in myocardial infarction compared to intact animals (\(p < 0.05\)) and significant changes in leukogram, biochemical and immunological parameters were found in Wistar rats with SIRS modelling. Introduction of a mixture of strains of \textit{Lactobacillus acidophilus} (LA-5) and \textit{Bifidobacterium animalis} subsp. \textit{lactis} (BB-12) to animals with SIRS reduced infarct size to a value close to the control. Rats treated with LA-5 and BB-12 also showed normalization of the leukocyte count, bile acids, transforming growth factor-\(\beta\), interleukins: IL-1\(\alpha\), IL-2, IL-6, IL-8, tumor necrosis factor-\(\alpha\), lipopolysaccharide and monocyte chemoattractant protein-1 in blood in comparison with the SIRS group and with the groups treated with other probiotic strains. The obtained data convincingly show the prospects for further study of the cardiotropic potential of probiotic microorganisms in translational studies.

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Obesity is now considered not only as an independent immunoinflammatory process [1], but is also recognized as one of the predictors of COVID-19 mortality [2] with a characteristic pat-
tern of hypercytokinemia. It is also known that the presence of cardiovascular diseases (CVD’s) and their risk factors in a patient increases vulnerability to COVID-19, which, in turn, can aggravate CVD’s and provoke new cardiovascular complications [3]. One of the common pathogenetic links of the above pathologies is systemic inflammatory response. The most important markers of low-intensity systemic inflammatory response are IL-6, TNF-α, IL-1α, IL-10 and MCP-1, whereas cytokine storm is characterized by a significant increase in blood concentration of IL-2, IL-7, IFNγ as well [4, 5]. The pathogenesis of cytokine storm is associated with an excessive effect of cytokines on cells and the resulting secondary damage to target organs: the liver, lungs, kidneys, and central nervous system. The clinical significance of the problem gives additional impetus to the search for common therapeutic targets and tools for the prevention of multiple organ failure formed in systemic inflammation and cytokine storm.

In the context of the association of obesity, inflammation and changes in the composition of the intestinal microbiota [6] with the development of CVD’s, we proposed a new experimental model of systemic inflammatory response syndrome (SIRS) [7] to develop a strategy for myocardial protection from ischemic and reperfusion damage, based on controlled changes in the composition of the intestinal microbiota with probiotic drugs. This model of SIRS in rats includes chemically induced inflammation of the colon on the background of primary visceral obesity and antibiotic-induced dysbiosis as the basic damaging effect. According to the results of the study of the effect of various antimicrobial drugs [8] and the qualitative composition of the fat-carbohydrate diet [9] in rats with SIRS, we obtained data confirming the positive effect of probiotic strains on myocardial resistance to ischemic-reperfusion damage in the model of the isolated Langendorff-perfusion heart [10].

The aim of this work was to study the therapeutic potential of probiotic microorganisms in relation to myocardial tolerance to ischemic-reperfusion injury (IRI) in vivo, against the background of intragastric administration of a mixture of probiotic lacto- and bifidobacteria as well as Saccharomyces and enterococci. In this work, the technique of coronary occlusion myocardial infarction was used in the model of SSRI in Wistar rats. The main objective of the study was to test the hypothesis about the effect of probiotic strains on the course of local and systemic inflammatory reactions with a cardioprotective effect. An additional task was to evaluate the effect of probiotic drugs on biochemical and immunological parameters.

**MATERIALS AND METHODS**

The experiments were performed on 80 male Wistar SPF-category rats (Pushchino) in conditions of an improved conventional vivarium, weighing 300–350 g, according to the European Council Directive on observance of ethical principles in work with laboratory animals, according to the experiment design (Fig. 1) approved by the decision of the Bioethics Committee of the V.A. Almazov National Medical Research Center, Application Protocol 21-09PZ#V1 of May 21, 2021.

Animals were randomly assigned to one of five groups (n = 16 in each group):

1) control (CTR)—rats received standard food and drinking water ad libitum;

2) systemic inflammatory response syndrome (SIRS). Rats with primary visceral obesity induced by a fat-carbohydrate diet, based on a controlled intake of unsaturated fatty acids (2 g) and sucrose (1 g) per day, respectively, were simulated for 28 days before modeling of SIRS [7] and then until the end of the experiment [9]. The animals were injected once rectally with a mixture of 3% acetic acid solution and 3% ethanol solution in physiological solution, with a total volume of 1 mL, inducing acute colon inflammation. Starting from the next day, these animals were intragastrically injected with a mixture of antimicrobials (amoxicillin, metronidazole, and clarithromycin) in the following mode: 1 mL of antimicrobial solution at a daily dose of 15 mg of each drug per rat for three days, which induced antibiotic-induced dysbiosis;

3) SIRS with administration of mixture of probiotic lacto- and bifidobacteria (SIRS + LBS)—rats with SIRS received 1 mL of mixture of probi-
otic strains *Lactobacillus acidophilus* (LA-5) and *Bifidobacterium animalis* subsp. *lactis* (BB-12) at a concentration of at least $10^8$ colony-forming units (CFU) of each strain per animal;

4) SIRS with *Saccharomyces boulardii* (SIRS + SB)—rats in this group were injected 25 mg of lyophilized *Saccharomyces boulardii* per animal in 1 mL of saline;

5) SIRS with enterococci (SIRS + EF)—rats of this group were given lyophilized Enterococcus faecium L3 at a dose of $10^8$ CFU per animal as probiotic microorganisms. Brief details of the strains used are given in the discussion.

On day 8 after the simulation of SIRS, 40 randomly selected rats for the respective groups ($n = 8$) were experimented with the technique of left coronary artery occlusion under isoflurane anesthesia, which included the following steps: anesthesia induction, tracheostomy, carotid artery catheterization, femoral vein catheterization, thoracotomy, simulation of myocardial ischemia-reperfusion injury, including a period of baseline stabilization, ischemia (30 min) and reperfusion (120 min). At the end of the experiment, anesthesia was deepened from 2% to 5% isoflurane solution, coronary artery reocclusion was performed, and 2.5 mL of Evans blue (2% solution) was injected. After 10 s, after a clear border between the perfused and ischemic region of the heart was identified, it was rapidly excised, washed in saline, and cut into 5 transverse fragments 1.5–2.0 mm thick. Planimetric evaluation of infarct size was performed by staining heart slices with 1% triphenyltetrazolium chloride solution at 37°C for 15 min. We determined the size of risk area and infarct size, which were expressed as a percentage of the total slice area and risk area, respectively, and calculated the average values for a given heart according to the results of analysis of 5 slices [11].

In the remaining 40 rats of the respective groups, whole blood (2 mL) was taken from a large saphenous vein under short-term anesthesia for hematological, biochemical and immunological analysis. Clinical blood analysis was performed on an automatic veterinary hematology analyzer (URIT-3000 Vet Plus, URIT Medical Electronic, China). Calcium (Ca$^{2+}$), bile acids (BA), alkaline phosphatase (ALP), uric acid, and

![Experimental design](image-url)
urea concentrations were assessed using a biochemical analyzer (BioChem Analette, HTI, USA). The levels of transforming growth factor-β (TGF-β), tumor necrosis factor-α (TNF-α), proinflammatory cytokines IL-1α, IL-2, IL-6, IL-8, lipopolysaccharide (LPS), and monocyte chemoattractant protein-1 (MCP-1) were assessed by enzyme immunoassay (MR-96A, Mindray, China).

Postmortem weight of some internal organs was measured—cecum as a macroscopic indicator of antibiotic-induced dysbiosis, liver—a polynutritional organ producing bile, kidneys—the basis of the urinary system, and spleen as the largest lymphoid organ. Mass ratio of organs as a percentage ratio of organ weight to body weight [12] in this model was calculated without mass of cecum due to multiple increase of intraluminal contents induced by dysbiosis as a result of antibiotic administration. Throughout the experiment, feed and water consumption and body weight of the animals were assessed daily from 9 to 10 a.m.

Statistical processing of the experimental data was performed using the STATISTICA 10.0 software package. Statistical analysis of discrete values was performed using nonparametric Kruskal-Wallis H-criterion to detect statistically significant differences, followed by a posteriori comparison using Mann-Whitney test. For repeated measures of continuous type, hypothesis testing for equality of mean values in several independent samples was performed by ANOVA analysis of variance with post-hoc test (Tukey HSD). Median values (Me) and values for the lower and upper quartiles (25%; 75%) were used in the tables. Mean values and standard deviations (SD) were used to construct graphical representations. Differences were considered statistically significant at a significance level of \( p < 0.05 \).

RESULTS

Weight of the body and internal organs

The body weight of rats in the CTR group increased on average by 1.5 ± 0.6 g/day from the day of modeling SIRS until the end of the experiment, whereas in the SIRS, SIRS + LBS, SIRS + SB and SIRS + EF groups a weight reduction of 3.9 ± 0.4; 3.6 ± 1.4; 3.9 ± 1.1 and 2.3 ± 0.2 g/day was observed respectively (\( p < 0.05 \) vs. the CTR group). Animal water consumption per 100 g body weight during the same period in the CTR group was 7.1 ± 0.6 mL/day, which did not differ significantly from that in the other groups (7.5 ± 0.6; 7.3 ± 1.3; 8.3 ± 0.8 and 6.3 ± 1.2 mL/day in the SIRS, SIRS + LBS, SIRS + SB and SIRS + EF groups, respectively). Feed intake per 100 g body weight per day in the groups from the day of simulation was 3.9 ± 0.2 for rats from the CTR group; SIRS was 1.5 ± 0.03; SIRS + LBS—1.6 ± 0.1; SIRS + SB—1.8 ± 0.1; and SIRS + EF—1.6 ± 0.2 g/100g/day, showing a reduction of 1.9–2.5 times (\( p < 0.05 \)) in the experimental groups relative to control (Figs. 2a, 2b, 2c).

There was a 16% (\( p < 0.05 \)) decrease in liver mass ratio in the SIRS group, 10% (\( p < 0.05 \)) in the SIRS + LBS group, 19% (\( p < 0.05 \)) in the

![Fig. 2. Dynamics of changes in body weight (a), water (b) and food (c) consumption starting from the day of SIRS modeling till the end of the experiment, mean value (± SD). *—\( p < 0.05 \) in relation to the CTR group. CTR—Control; SIRS—systemic inflammatory response syndrome; SIRS + LBS—SIRS and a mixture of LA-5 and BB-12; SIRS + SB—SIRS and S. boulardii; SIRS + EF—SIRS and E. faecium L3.](image-url)
SIRS + EF group relative to the CTR group, whereas the SIRS + SB group did not differ from the CTR group. For the spleen, there was a 12% ($p < 0.05$) decrease in the mass ratio in the SIRS group compared to the CTR group. In the SIRS + SB group, the spleen mass ratio was 12% higher than in the CTR group. A significant 12% increase in renal mass ratio was observed in the SIRS group (Table 1).

Clinical blood analysis

There were no significant changes in the number of erythrocytes and their characteristics in the experimental groups with respect to the control (Table 2). The SIRS and SIRS + EF groups showed a 25% increase in platelets ($p < 0.05$ compared to the CTR group). The description of leukogram in this work is limited by the capabilities of the used class II analyzer, therefore, the focus is made on the percentage ratio of the main leukocyte fractions: LYM, MID, GRAN. The SIRS group showed a 13% increase in total leukocyte count, with a significant contribution of the lymphocyte population (LYM), with an increase of

| Organ | CTR | SIRS | SIRS + LBS | SIRS + SB | SIRS + EF |
|-------|-----|------|------------|-----------|-----------|
| Colon | 1.41# (1.39; 1.45) | 5.92* (4.64; 6.9) | 2.6# (2.66; 2.68) | 3.29*# (3.03; 3.32) | 3.25*# (3.2; 3.29) |
| Liver | 3# (2.96; 3.08) | 2.69* (2.68; 2.7) | 2.78* (2.77; 2.79) | 3.08# (3.01; 3.17) | 2.53# (2.51; 2.57) |
| Spleen | 0.18# (0.175; 0.182) | 0.138* (0.136; 0.145) | 0.172# (0.17; 0.175) | 0.2*#, (0.197; 0.212) | 0.168# (0.165; 0.178) |
| Kidneys | 0.523# (0.509; 0.527) | 0.575* (0.574; 0.576) | 0.535 (0.532; 0.544) | 0.558 (0.535; 0.566) | 0.524# (0.522; 0.531) |

*—$p < 0.05$ in relation to CTR; #—$p < 0.05$ in relation to SIRS (U-test). CTR—Control, SIRS—systemic inflammatory response syndrome, SIRS + LB—SIRS and a mixture of LA-5 and BB-12, SIRS + SB—SIRS and S. boulardii, SIRS + EF—SIRS and E. faecium L3.

| Parameter | CTR | SIRS | SIRS + LBS | SIRS + SB | SIRS + EF |
|-----------|-----|------|------------|-----------|-----------|
| WBC, $10^9$/L | 7.5 (6.7; 8.5) | 8.5 (7.0; 11.3) | 7.3 (5.8; 8.1) | 5.1# (4.3; 6.2) | 7 (6.4; 8) |
| LYM, % | 27# (26; 35) | 49* (40; 55) | 35 (26; 51) | 49* (43; 54) | 36 (24; 44) |
| MID, % | 9.5 (8.3; 11.4) | 7.3 (6.5; 9.5) | 8.2 (4.5; 8.9) | 9.7 (9.1; 10.6) | 5.2* (4.2; 9.3) |
| GRAN, % | 61 (54; 68) | 47 (40; 67) | 58 (47; 65) | 53 (44; 58) | 58 (55; 65) |
| RBC, $10^{12}$/L | 7.9 (7.6; 8.6) | 7.6 (7.3; 7.9) | 7.3 (6.9; 7.9) | 8 (7.4; 8.4) | 7.9 (7.8; 8) |
| PLT, $10^9$/L | 657# (646; 767) | 876* (860; 1010) | 883 (812; 918) | 735 (639; 849) | 857* (731; 118) |

*—$p < 0.05$ in relation to CTR; #—$p < 0.05$ in relation to SIRS (U-test). CTR—control, SIRS—systemic inflammatory response syndrome, SIRS + LB—SIRS and a mixture of LA-5 and BB-12, SIRS + SB—SIRS and S. boulardii, SIRS + EF—SIRS and E. faecium L3. WBC—white blood cells, LYM—lymphocytes, MID—percentage of the other types of white blood cells not classified as lymphocytes or granulocytes, GRAN—granulocytes, RBC—red blood cells, PLT—platelets.
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Table 3. Blood biochemical parameters, Kruskal–Wallis test, Me (25%;75%), n = 8

| Parameter       | CTR    | SIRS   | SIRS+LBS | SIRS+SB | SIRS+EF |
|-----------------|--------|--------|----------|---------|---------|
| Urea, mg/dL     | 25.1#  | 14.8*  | 14.2*    | 12.3*   | 21.2#   |
|                 | (22.5;25.4) | (12.2;15.5) | (11.5;17.2) | (10.3;12.8) | (20.1;25.3) |
| Uric acid, μmol/L | 19.8#  | 42.8*  | 40.6*    | 25.4#   | 32.1    |
|                 | (11.8;27.8) | (28.8;60.4) | (33.8;45.1) | (21.9;34.7) | (19.9;35.5) |
| Ca²⁺, mmol/L    | 2.05#  | 3.1*   | 2.55     | 2.35    | 2.5     |
|                 | (0.95;2.75) | (2.25;3.5) | (1.40;2.85) | (2.05;2.65) | (2.20;3.05) |
| Bile acids, μmol/L | 12.8#  | 3.7*   | 9.5*,#   | 3.3*    | 4*      |
|                 | (11.3;19.8) | (2.2;6.1) | (8.6;11.6) | (2.4;4.2) | (1.9;6.8) |
| ALP, U/L        | 22.8#  | 36.3*  | 21.5#    | 21.3#   | 25#     |
|                 | (18.2;31.7) | (35.2;37.8) | (14.6;25.5) | (20;28) | (19.9;29.1) |
| LDG, U/L        | 555#   | 835*   | 913*     | 396#    | 739     |
|                 | (486;615) | (746;852) | (909;950) | (210;421) | (389;872) |

*—p < 0.05 in relation to CTR, #—p < 0.05 in relation to SIRS (U-test). CTR—control, SIRS—systemic inflammatory response syndrome, SIRS + LB—SIRS and a mixture of LA-5 and BB-12, SIRS + SB—SIRS and S. boulardii, SIRS + EF—SIRS and S. faecium L3.

45% (p < 0.05 compared to the CTR group), with a corresponding relative decrease of 23% (p > 0.05) in the MID and granulocyte representation, although their number, like MID, also increased in absolute terms. In the SIRS + SB group the leukocyte count decreased by 32% relative to the CTR group and by 40% relative to the SIRS group (p < 0.05), and there was also a 45% increase in the MID population (p < 0.05). The SIRS + LBS and SIRS + EF groups did not differ significantly with respect to controls, although the latter group significantly decreased the number of MID by 45% (p < 0.05).

Biochemical parameters of blood

The results of the analysis of biochemical blood parameters are shown in Table 3. The SIRS group showed a significant decrease in urea and bile acids compared to the CTR group (p < 0.05). In contrast, the levels of uric acid, calcium, ALP and LDG were significantly higher in this group (p < 0.05 compared with the CTR group). In the SIRS + LBS group, calcium, bile acids and ALP levels did not differ from the CTR group, i.e. there was normalization of these parameters. At the same time, elevated LDG activity and elevated uric acid concentration, as well as decreased urea level observed in the SIRS group persisted (p < 0.05). In the SIRS + SB group, uric acid, calcium, and LDG levels did not differ from the CTR group, but decreased urea and bile acids remained (p < 0.05). In the SIRS + EF group, urea, calcium, ALP and LDG levels did not differ from those in the CTR group, while the uric acid concentration and bile acid levels remained elevated (p < 0.05). Thus, SIRS was accompanied by significant deviations of all assessed biochemical parameters from the norm, and application of different probiotic correction regimens led to partial normalization of these parameters, but the nature of normalization depended on the type of probiotic.

Immunological parameters

Hyperproduction of all immunological blood indices presented in Table 4 occurred in the SIRS group with a significant (p < 0.05) increase in their blood concentrations compared to controls. The increase in TNF-α by 28% and TGF-β by 29%, IL-1α by 26%, IL-2 by 33%, and MCP-1 by 41% in the SIRS group can be considered moderate compared to the increase in IL-6 by more than 2-fold, IL-8 by 3-fold, and LPS by almost 10-fold (p < 0.05). The SIRS + LBS group showed a decrease of IL-2, IL-8, MCP-1, TNF-α concen-
tration to approximately control values, indicating the blocking of pleiotropic cytokines and metabolites production under the influence of probiotic therapy, expressed in the stabilization of inflammatory status indicators. The SIRS + SB group also showed normalization of IL-2, MSR-1, TNF-α and TGF-β levels, while the SIRS + EF group showed normalization of all but IL-1α, IL-6 and MSR-1.

Infarct size and hemodynamic parameters

A nonparametric analysis of differences in hemodynamic indices using a software package for continuous repeated measurements (ANOVA) showed that baseline indices, data for the 5th minute of ischemia, and for the 90th and 120th minutes of reperfusion did not differ between the groups. In the SIRS group, from the 10th minute of ischemia to the 60th minute of reperfusion, mean arterial pressure (MAP) and heart rate (HR) from the onset of ischemia to the 90th minute of reperfusion were significantly lower relative to controls ($p < 0.05$). In the SIRS + SB group during the entire follow-up, HRs were significantly lower than in the CTR group, and for CAD they were close to controls. In the SIRS + LBS group, SAD values were lower than controls from minute 5 to minute 40 of reperfusion, with HR decreasing from minute 30 of ischemia to minute 60 of reperfusion, and in the SIRS + EF group, HR decreased from minute 10 of ischemia to minute 40 of reperfusion ($p < 0.05$), with HR close to controls (Figs. 3a, 3b).

The median values of risk area size from the total area of the myocardial portion examined were 47% (39; 48), 45% (36; 48), 44% (38; 53), 43% (42; 50), 46% (40; 55) in the CTR, SIRS, SIRS + LBS, SIRS + SB and SIRS + EF groups respectively ($p > 0.05$) (Fig. 2), which shows equality of methodological conditions in all experimental groups. Infarct size in the CTR group was 42% (41; 46) and in the SIRS group was 55% (53; 59) of the risk area, a significant increase of 24%, whereas in the SIRS + LBS group 47% (41; 50) of this value was close to control. In the SIRS + SB and SIRS + EF groups, the

| Parameter       | CTR      | SIRS     | SIRS+LBS  | SIRS+SB   | SIRS+EF   |
|-----------------|----------|----------|-----------|-----------|-----------|
| IL-1α, pg/mL    | 74# (66;78) | 100* (93;109) | 42*,# (31;51) | 48*,# (39;50) | 47*,# (45;49) |
| IL-2, pg/mL     | 4.01# (3.15;4.62) | 6.01* (4.43;6.54) | 3.02# (2.90;4.93) | 3.8 (3.75;4.24) | 3.6# (3.12;3.81) |
| IL-6, pg/mL     | 21# (19;25) | 48* (41;64) | 7.8*,# (7.6;8.5) | 5.4*,# (3.7;7) | 4.1*,# (3.3;5.3) |
| IL-8, pg/mL     | 19# (18;24) | 57* (53;66) | 31# (30;35) | 67* (30;93) | 15# (14;21) |
| MCP-1, pg/mL    | 4.87# (3.54;5.68) | 8.31* (7.26;11.5) | 4.11# (3.71;4.68) | 5.45# (4.09;6.55) | 6.91* (6.61;8.22) |
| TNF-α, pg/mL    | 18# (17;19) | 25* (22;26) | 17# (16;18) | 17# (16;18) | 17# (16;17) |
| TGF-β, pg/mL    | 4.35# (3.98;4.59) | 6.13* (5.63;6.39) | 6.76* (4.32;7.78) | 3.26# (3.05;3.3) | 4.93 (3.57;4.93) |
| LPS, pg/mL      | 8.3# (7.9;10) | 71* (68;75) | 11.5* (10.7;12.8) | 50*,# (46;54) | 8.9# (8.0;10.5) |

*—$p < 0.05$ in relation to CTR, #—$p < 0.05$ in relation to SIRS (U-test). CTR—Control, SIRS—systemic inflammatory response syndrome, SIRS + LB—SIRS and a mixture of LA-5 and BB-12, SIRS + SB—SIRS and S. boulardii, SIRS + EF—SIRS and E. faecium L3.
myocardial infarction size was 59% (47; 61) and 58% (50; 59), respectively, which was 40% and 38% greater than controls ($p < 0.05$) and almost equal to that in the SIRS group, indicating the absence of cardioprotection in these groups, (Figs. 4a, 4b).

The primary analysis of the results allows to conclude that SIRS significantly increased infarct size against the background of significant decrease of BP and HR, whereas after probiotic therapy in the group with lacto- and bifidobacteria administration this index was close to the control, which suggests a possible cardioprotection in conditions of SIRS. For this SIRS + LBS group, after stability during ischemia, a decrease in blood pressure at the beginning of the reperfusion period with a concomitant drop in HR in relation to CTR, with a subsequent rise, which seems to be of compensatory nature, was shown. Administration of the other two probiotics had no effect on infarct size after modeling SIRS, despite the stability of BP in the SIRS + SB group, and the stability of HR in the SIRS + EF group, which shows the specific features of the effect of different probiotic strains on the coordination of regulatory mechanisms of myocardial functionality. All three probiotics normalized, to varying degrees, hematological, biochemical, immunological and functional parameters, but only in the SIRS + LBS group besides stabilization of most biochemical and immunological parameters, there was a decrease in infarct size to control values.

**DISCUSSION**

In this work we investigated the cardiotropic effects of three probiotic drugs in a model of regional myocardial ischemia-reperfusion in rats with SIRS including primary visceral obesity, antibiotic-induced dysbiosis and acute colon inflammation. Clinical studies on strains LA-5 and BB-12 have shown beneficial effects on gut microflora and local immunity in healthy individuals and people with gastrointestinal diseases. Under the influence of BB-12, there is an increase in the production of immunoglobulin A in the intestinal mucosa, which contributes to its anti-infective resistance [13, 14]. *Saccharomyces boulardii* exhibits antagonism to a number of pathogenic and conditionally pathogenic microorganisms, suppresses their development; increases local immune protection due to increased production of immunoglobulin A and other immunoglobulins, has immunobiological and antidiarrheal effects [15]. The probiotic strain *Enterococcus faecium* L3 is a nonpathogenic enterococcus isolated from milk starter with a pronounced antagonism to pathogenic and conditionally pathogenic flora with immunomodulatory and vitamin-forming properties [16, 17].

The maximum increase in the mass of the cecum in our work was noted in the SIRS group when compared with the CTR group, which confirms the antibiotic-induced impairment of the digestive and evacuatory functions of the small intestine [18, 19]. The overall pattern of changes
in leukocyte composition obtained in this study is generally characteristic of burn injury and is accompanied by a decrease in granulocyte count with an increase in the representation of lymphocytes and eosinophils in the SIRS group [20]. Under conditions of modeling SIRS against the background of an increase in the main subpopulations of leukocytes (LYM and MID), specific features of the effect of probiotic preparations on changes in the composition of leukocytes were revealed. In relation to the SIRS group, it is interesting to note a significant decrease in LYM and MID in the SIRS + LBS and SIRS + EF groups, with the most significant decrease in MID subpopulation observed in the latter group, indicating the specific features of the effect of the probiotic drugs studied.

It is known that enhancement of leukopoiesis occurs under the influence of such proinflammatory cytokines as IL-1, IL-6, IL-8, as well as under the influence of several inflammatory mediators. Universal stimulators of granulocytic-monocytic leukopoiesis are adaptation hormones—catecholamines and glucocorticoids, which realize their effects on bone marrow through increased formation of colony-stimulating factors and interleukins. Leukopoiesis stimulators include vitamin B₁₂, ascorbic acid, folic acid, and iron. Suppression of medullary hematopoiesis is possible under the influence of a number of inflammatory mediators, such as prostaglandins, IL-10, IL-13, TNF-α, TGF-β, as well as lactoferrin and acid isoferritin [21]. However, the analysis of the results of experimental modeling of inflammation in our case is complicated by the introduction of probiotic strains with their own pro- and anti-inflammatory potential into the internal environment of the body with local chemically induced damage to the colon [22]. In our work, we observed an increase in most proinflammatory cytokines in the SIRS simulated group and a decrease in myocardial resistance to IRI, whereas in the SIRS + LBS group with infarct size not different from controls, there was a decrease in proinflammatory cytokine levels in all cases studied, and in the SIRS + SB group—no normalization of LPS and IL-8 levels, which may be indirectly related to the lack of cardioprotection effect. However, there is no reason to claim that it is LPS or IL-8 that is the direct cause of myocardial resistance to IRI, because no cardioprotection effect was observed in the SIRS + EF group, while the values of these markers were close to the control ones. The information about possible suppression of leukopoiesis under the influence of TNF-α, TGF-β, etc., as a contour of
reverse regulation during hyperexcitation in the inflammation system, provides a background for similar reasoning. It can be assumed that the maximum TGF-β index in SIRS + LBS group explains the conjugate reduction of mediator complex to near normal values, which contributes to the regulation of leukopoiesis and leukocyte death, as well as a certain differentiation stage of myeloid, lymphoid and megakaryocytic elements with preservation of myocardial resistance regulatory mechanisms to IRI.

It should be noted that hemodynamic parameters (BP and HR) in SIRS group were significantly lower than parameters recorded in CTR group. While HR in SIRS + LBS and SIRS + EF groups, and MAP in SIRS + SB group over the observation period significantly differed in increasing direction from SIRS group.

To date, it has been convincingly demonstrated in the literature that blood levels of proinflammatory cytokines and, in particular, TNF-α in patients with chronic heart failure are associated with the severity of clinical manifestations [23]. In connection with the task of research to elucidate the cardioprotective role of probiotic microorganisms against the background of SIRS it is necessary to pay attention to the changes of myocardial infarction size in connection with the concentration of bile acids (BA) in the blood of animals. The question remains open: whether BA are a marker of pathology of enterohepatic circulation or a direct participant of pathophysiological chain “macroorganism—GI tract—microbiota—inflammation—myocardium”. Primary BA are formed in liver, directly involved in digestion and metabolized by enzymes of certain representatives of intestinal microbiota, have direct and indirect antimicrobial action, act as endogenous ligands to receptors of certain hormones, regulatory proteins, vitamin D₃, etc. Secondary BA take part in the processes of obesity, inflammation and carcinogenesis [24]. In a pathophysiological sense, the information about intestinal dysbiosis with inflammatory intestinal diseases in connection with the deficiency of secondary BA, the few known producers of which are representatives of the families Lachnospiraceae and Ruminococcaceae, is interesting, while many intestinal commensals of such extensive genera as Bacteroides, Clostridium and Enterococcus are involved in the deconjugation and hydrolysis of primary BA [25]. In our previous studies on the model of SIRS, we showed a direct correlation between the increase in the area of myocardial necrosis of the isolated heart and the concentration in blood plasma of acetic and propionic acids produced by facultative and obligate anaerobes, including pathogenic and conditionally pathogenic ones [7]. On the other hand, the producers of short-chain fatty acids are representatives of the above families Lachnospiraceae and Ruminococcaceae, which gives rise to an ambiguous and sometimes contradictory interpretation of the role and significance of individual representatives of microbiota, often regarded as unambiguous symbionts [26]. Returning to the results of this study about the combined reduction of values of BA pool, a number of key proinflammatory markers (TNF-α, IL-1, IL-8, MCP-1), normalization of leukogram and reduction of necrosis area in SIRS + LBS group after modeling SIRS, we can assume that certain representatives of gut microbiota and probiotic strains, and their compositions are able to induce both cardioprotective response and, conversely, reduce myocardial resistance to IRI that requires further research.

CONCLUSIONS

An experimental model including primary visceral obesity, acute inflammation of the large intestine and antibiotic-induced dysbiosis is designed to explore the potential of probiotic drugs to explore the possibility of modulating myocardial resistance to ischemia-reperfusion under unbalanced increases in blood cytokine levels. This model reflects the known clinical picture of SIRS and requires a comprehensive study of general and specific body reactions to local and systemic damage. The work confirms that SIRS is accompanied by significant deviations of biochemical, hematological and immunological indices from the norm and leads to an increase in the infarct size, i.e. reduces myocardial resistance to ischemia-reperfusion damage. It was shown that all three probiotics used to varying degrees normalize deviations of biochemical, hematological and immunological parameters arising in
SIRS, while only LA-5 and BB-12 mixture brings the infarct size closer to the control value, i.e. has cardioprotective potential in conditions of SIRS. The other two studied probiotics, despite partial normalization of biochemical and immunological parameters against the background of SIRS, do not have cardioprotective effect. The main conclusion of this work can be considered the fact that in the experimental model of SIRS, different probiotic strains specifically affect myocardial resistance to ischemia-reperfusion damage. The results obtained can be used to investigate the cardioprotective potential of probiotic drugs within the concept of translational medicine.

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AUTHORS’ CONTRIBUTION

Idea of work, planning of the experiment (Yu.Yu.B., M.M.G.), data collection (Yu.Yu.B., D.L.S., I.Yu.B., Yu.V.Ch., O.V.B., M.M.G.), data processing (Yu.Yu.B., D.L.S., I.Yu.B., V.Yu.B.), writing and editing the article (Yu.Yu.B., I.Yu.B., M.M.G.).

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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