

PROBIOTIC STRAINS AS SYSTEMIC BIOLOGICAL ANTI-INFLAMMATORY AGENTS

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Abstract. *Therapeutic control of the intensity, duration, and quality of immune-inflammatory processes is fundamental to the successful treatment of most infectious and non-infectious diseases, including autoimmune disorders. The aim of this study was to investigate the anti-inflammatory potential of probiotic strains. In an experiment on male Wistar rats using a model of systemic inflammatory response syndrome (SIRS), significant reductions in key inflammatory markers such as TNF- α , IL-1, IL-12, IFN- γ , and MCP-1 were observed following the administration of a probiotic mix of *L. acidophilus* (LA-5) and *B. animalis* subsp. *lactis* (BB-12), as well as the isolated strain *L. delbrueckii* TS1-06. Improvements in myocardial functional indicators, behavioral responses, and accelerated reparative and regenerative processes were noted, suggesting the potential of these probiotic strains as candidates for therapeutic agents used as slow-acting anti-inflammatory drugs.*

Key words. *Probiotics, immunity, inflammation, cytokines.*

Introduction

The COVID-19 pandemic has advanced sanitary-epidemiological services at all levels and enriched clinical infectious institutions, while providing fundamental biomedical science with new insights into the nature of systemic inflammatory responses [4]. Terms like „cytokine storm” [5], „cytokine release syndrome”, and hypercytokinemia (HC) describe the process of excessive activation of immune cells with uncontrolled production of pro-inflammatory cytokines. HC is characteristic of severe cases of various pathologies: sepsis, viral infections, transplantation, oncology, extracorporeal fertilization, pancreonecrosis, bronchial asthma, and others [16]. As a predictor of

poor prognosis [8], HC is associated with clinical manifestations such as fever, diarrhea, headaches, muscle, and joint pain, and elevated laboratory indicators like ferritin, C-reactive protein, D-dimers, troponins, and lactate dehydrogenase.

The key cytokines implicated in the development of HC include pro-inflammatory (TNF- α , IL-1 β , IFN- γ , IL-2, IL-7, IL-12, IL-18, IL-33, IL-6, CXCL8/IL-8, CXCL1/GRO α , CXCL2/GRO- β , CXCL9/Mig, CXCL10/IP-10, CCL2/MCP-1, CCL3/MIP-1 α , CCL5/RANTES), anti-inflammatory (IL-1RA, TNF-sRI, IL-4, IL-10), and the hematopoietic granulocyte-macrophage colony-stimulating factor (GM-CSF). While leaving aside extensive material on the role of the renin-angiotensin-aldosterone system (RAAS) [10], the kallikrein-kinin system, the complement system, and hyaluronic acid [15], we focus on the potential modulation of inflammatory status through oral administration of probiotic strains in a SIR'S model in rats [1].

Given that the list of cytokines involved in various HC scenarios is largely similar, and recognizing that the primary pro-inflammatory mediators associated with complications, including cardiovascular diseases, are TNF- α , IFN- γ , IL-6, GM-CSF, and MCP-1, this study aims to test the hypothesis that it is possible to control the balance of key cytokines and factors through the administration of probiotic strains.

Materials and methods

Wistar rats (280 ± 25 g) were randomized into 5 groups (n = 8 per group):

1. Control (CTR): Rats received standard feed and drinking water ad libitum.

2. SIRS modeling: Animals with primary visceral obesity were chemically induced with colonic inflammation by administering 1 ml of saline per os.

3. SIRS + Probiotic Mix (SPM): Rats underwent procedures according to the previous protocol and were given a mixture of probiotic strains *L. acidophilus* (LA-5) and *B. animalis* subsp. *lactis* (BB-12) at a concentration of 10^8 CFU per animal in 1 ml of saline for 8 days instead of saline.

4. SIRS + *L. delbrueckii* (SLD): Rats in this group received the probiotic strain *L. delbrueckii* TS1-06 according to a similar protocol.

One day before the end of the experiment, blood samples (1.5 ml) were collected from the rats under short-term anesthesia from a large

subcutaneous vein for hematological and immunological analysis. Clinical blood analysis was performed using an automatic veterinary hematological 3-diff analyzer (URIT-3000 Vet Plus, URIT Medical Electronic, China). Levels of TNF- α , IL-1 α , IFN- γ , MCP-1, IL-2, GM-CSF, G-CSF, and M-CSF were measured by ELISA (MR-96A, Mindray, China). Throughout the experiment, the clinical status of the animals, feed and water consumption, and body weight were monitored daily from 9 to 10 am.

Statistical analysis of the experimental data was conducted using the STATISTICA 12.0 software package. Statistical analysis of discrete values was performed using the non-parametric Kruskal-Wallis H-test to detect statistically significant differences, followed by a posteriori comparison using the Mann-Whitney U-test. Median values (Me) and interquartile ranges (25%; 75%) are provided in the tables. Differences were considered statistically significant at $p < 0.05$.

Results and discussions

Throughout the observation period, the body weight of rats in the control group (CTR) increased by an average of 0.60 ± 0.05 g/day. In contrast, body weight in the SIRS, SIRS + Probiotic Mix (SPM), and SIRS + *L. delbrueckii* (SLD) groups decreased by 1.59 ± 0.81 , 1.27 ± 0.51 , and 0.95 ± 0.59 g/day, respectively ($p < 0.05$ vs CTR). Water consumption per 100 g of body weight in the CTR group was 10.6 ± 1.2 ml/day, which was significantly lower than in the other groups (14.3 ± 2.2 , 13.8 ± 2.5 , and 13.8 ± 0.7 ml/day for the SIRS, SPM, and SLD groups, respectively, $p < 0.05$ vs CTR). Feed consumption in these groups was 2.2 ± 0.8 , 1.8 ± 0.4 , and 2.2 ± 0.5 g/day, respectively ($p < 0.05$), which was significantly lower than in the CTR group (5.2 ± 0.6 g/day).

An increase in spleen mass index (MI) by 24% ($p < 0.05$) compared to CTR was observed in the SIRS group. In the SPM group, the kidney mass index increased by 15% compared to CTR ($p < 0.05$). Significant increases in cecum mass were observed in the SIRS, SPM, and SLD groups ($p < 0.05$ compared to CTR). The visceral fat deposit mass index in the SIRS group was 144% lower than in the CTR group ($p < 0.05$), and in the SPM group, it was 65% lower ($p < 0.05$) (Table 1).

Table 1. Organ Mass Indices, Me (25%;75%).

Group organ	CTR	SIRS	SIRS+SPM	SIRS+SLD
Cecum	1.8 (1.7;2.0)	5.7 (5.3;5.9)*	4.9 (4.2;5.2)*	4.6 (4.4;5.3)*
Liver	2.9 (2.8;3.0)	2.8 (2.7;2.9)	2.7 (2.6;2.9)	2.7 (2.6;2.9)
Spleen	0.17 (0.16;0.19)	0.21 (0.19;0.22)*	0.16 (0.15;0.18)	0.17 (0.15;0.19)
Kidneys	0.61 (0.60;0.64)	0.65 (0.64;0.68)	0.70 (0.68;0.71)*	0.63 (0.61;0.67)
Visceral Fat	2.1 (1.9;2.2)	0.86(0.68;1.05) *	1.27 (0.98;1.32)*	1.62 (1.31;1.95)

Note: * – $p < 0.05$ compared to KTR (U test).

No significant changes in the number of erythrocytes and platelets compared to the control were observed in the SIRS+SPM and SIRS+SLD groups. In the SIRS group, the number of platelets and leukocytes was significantly higher by 19% and 139%, respectively, with lymphocytes increased by 26%, MID-fraction cells by 50%, and granulocytes by 106% ($p < 0.05$). In the SIRS+SPM and SIRS+SLD groups, changes in the blood cell composition mostly differed from the control insignificantly (Table 2).

Table 2. Hematological Parameters, Me $\pm$ SD.

Group Parameter	CTR	SIRS	SIRS+SPM	SIRS+SLD
WBC 10*9/L	5.8 (5.4;6.4)	9.9 (8.1;11.9)*	7.5 (6.1;9.4)	6.8 (5.8;9.2)
LYM 10*9/L	2.3 (1.9;2.7)	2.9 (2.4;3.6)	1.9 (1.4;2.6)	1.7 (1.2;2.5)
MID 10*9/L	0.6 (0.4;0.7)	0.9 (0.6;1.2)*	0.8 (0.7;0.9)*	0.7 (0.4;0.9)
GRAN 10*9/L	2.9 (2.5;3.3)	6.0 (5.7;6.4)*	4.8 (4.3;5.5)*	4.4 (4.0;5.8)*
RBC 10*12/L	6.7 (6.3;7.5)	6.9 (6.5;7.4)	6.3(5.7;6.6)	6.5 (6.2;7.3)
PLT 10*9/L	575 (522;596)	683 (604;715)*	596 (572;638)	612 (590;642)

Note: * – $p < 0.05$ compared to control (U-test). $\uparrow$ – Increase in parameter.

In Table 3, data on several key markers of SIRS are presented: TNF- α , IL-1 α , IL-12 α , IFN- γ , MCP-1. In the SIRS group, compared to the CTR group, all indicators were significantly increased: TNF- α by 96%, IL-1 α by 87%, IFN- γ by 100%, and MCP-1 by 91% ($p < 0.05$). In the SIRS+SPM and SIRS+SLD groups, the indicators were close to control levels in the vast majority of cases (Table 3).

Table 3. Levels of TNF- α , IL-1 α , IL-12 α , IFN- γ , MCP-1. Me $\pm$ SD.

<i>Group</i> Analyte	CTR	SIRS	SIRS+SPM	SIRS+SLD
TNF- α	4,6(3,2;4,0)	8,3(8,1;10,9)*	3,8(3,5;4,2) #	4,9(4,1;5,6) #
IL-1 α	78(64;89)	160(128; 184)*	85(76;91) #	94(88;99) #
IL-12b	2,7(2,6;2,8)	4,7(2,8; 7,7)*	2,6(2,3;3,1) #	4,0(2,4;4,8)
IFN- γ	15(9;20)	60(35;129) *	14 (11;18) #	17(15;19) #
MCP-1	34(25;65)	80(60;140)	21(15;40) #	31(20;40) #

Note: * – $p < 0.05$ compared to control; # – $p < 0.05$ compared to SIRS; (U-test).

The results of studies using this experimental model regarding myocardial resistance in isolated hearts [2] and in cases of left coronary artery occlusion [13], corroborated by histological data on chemically induced damage to the large intestine [14] and behavioral tests [12], convincingly demonstrate the effectiveness of probiotic strains in the context of systemic inflammatory response syndrome (SIRS). The reduction of visceral fat deposits, characteristic of SIRS, combined with increased levels of pro-inflammatory cytokines [1], particularly cachectin TNF- α , produced by monocytes and lymphocytes [6], endothelial and epithelial cells, smooth muscle cells of blood vessels, and cardiomyocytes [9], aligns with the known role of this cytokine in lipid metabolism [7]. Data on TNF- α cytokines as a key cytokine stimulating the expression of genes necessary for controlling inflammation and tissue damage help understand the importance of maintaining its functions in various immune responses during inflammation, differentiation, and the regulation of cell proliferation and apoptosis [11]. The increase in all studied markers, along with the shift in the leukocyte formula observed in this study, forms the basis for evaluating the impact of probiotic strains on the intensity of cytokinemia. It is known that some cytokines (IL-1, IL-6, TNF- α , and IFN- γ) have systemic effects associated with the development of pronounced inflammation; however, the possibility of modulating their production at the local and systemic levels using probiotic strains remains an open question. Inflammation development, for example, IL-1, TNF- α , and IFN- γ , enhances the adhesive and procoagulant activity of endothelial cells in post-capillary venules, IL-2 stimulates neutrophil migration, IL-1, IL-6, TNF- α , GM-CSF, and IFN- γ potentiate the effector functions of macrophages, etc., demonstrating the pleiotropy of the studied cytokines. Neutrophils are programmed by activated

macrophages through IL-1, IL-8, TNF, IFN- α , G-CSF, and others, as well as by migrating Th1 cells to the site of inflammation, with IFN- γ being a predominant inducer among cytokines [17].

Currently, research is underway to determine the molecular mechanisms of interaction between the macroorganism and individual probiotic strains, including their modulation of the entire intestinal microbiota. Undoubtedly, probiotic modulation of immune-inflammatory responses in the macroorganism represents the most physiological and evolutionarily justified approach for overall control of the pathological process. The search for new, safe therapeutic and preventive tools, used both individually and as part of comprehensive treatment regimens for managing inflammation and pain, is an undoubtedly promising direction. This is because prolonged use of steroidal and non-steroidal drugs, included in clinical treatment guidelines for a wide range of diseases, is associated with undesirable reactions, side effects, and reduced efficacy. Additionally, alongside the expansion of the nomenclature of classical anti-inflammatory and anti-allergic drugs, there has recently been rapid development in genetically engineered biological drugs and selective cytokine blockers, which, despite their efficacy and high cost, also have the aforementioned drawbacks. This further underscores the need for continued research into probiotic regulators of immune-inflammatory responses.

Conclusions

1. Changes in clinical status and blood parameters: Rats subjected to the SIRS model exhibited weight loss and decreased food intake, while water consumption increased. Significant changes in hematological parameters were also observed.

2. Anti-inflammatory potential of probiotic strains: Probiotic strains *L. acidophilus* (LA-5), *B. animalis* subsp. *lactis* (BB-12), and *L. delbrueckii* TS1-06 have demonstrated significant anti-inflammatory potential. This is evidenced by the reduction in key inflammatory markers such as TNF- α , IL-1, IL-12, IFN- γ , and MCP-1 in rats, along with the normalization of hematological parameters in the SIRS model.

3. Improvement in functional indicators and behavioral responses: The administration of probiotics led to improvements in myocardial functional indicators, accelerated reparative and regenerative processes, and enhanced behavioral responses in experimental animals. This indicates the therapeutic potential of probiotic strains.

4. Need for further research: The study underscores the necessity for further research into probiotic strains as effective therapeutic agents for managing inflammatory and immune reactions without causing undesirable side effects.

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