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Research article

ANTI-INFLAMMATORY POTENTIAL OF *LACTOBACILLUS PLANTARUM* LS/07 IN ACUTE COLITIS IN RATS

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To evaluate the efficiency of the probiotic strain *Lactobacillus plantarum* LS/07 in dextran sulphate sodium (DSS) induced acute colitis selected biochemical (activity of β-glucuronidase), microbiological (counts of lactobacilli and coliforms), and immunological (IL-6, IL-8, IL-13, NF-ϰB, MPO) parameters were assessed. Sprague-Dawley rats were divided into groups: Control, Acute colitis, and Probiotic. Acute colitis was induced using 5% DSS in drinking water for 7d. DSS induced an inflammatory process in the colonic tissue, increased the activity of β-glucuronidase ($p<0.001$), increased the counts of coliform bacteria and decreased lactobacilli counts ($p<0.05$), and activated the production of the measured parameters (NF-ϰB, MPO, IL-6, IL-8) except of IL-13. *Lactobacillus plantarum* LS/07 in the diet alleviated the DSS induced inflammatory process by inhibiting the production of IL-6, IL-8, the activities of NF-ϰB and MPO, and stimulated the production of IL-13. The probiotic reduced the activity of β-glucuronidase ($p<0.05$), increased lactobacilli counts and decreased coliform bacteria. These results indicate that dietary intake of *Lactobacillus plantarum* LS/07 suppressed the expression of markers playing an important role in the inflammatory process. We conclude that the anti-inflammatory properties of *Lactobacillus plantarum* LS/07 makes its suitable for the prevention or treatment of colitis.

Key words: Sprague-Dawley rats, Colitis, Inflammation, *Lactobacillus plantarum* LS/07

INTRODUCTION

Ulcerative colitis (UC) as a nosological unit within inflammatory bowel diseases (IBD) represent important chronic multifactorial diseases affecting the gastrointestinal tract in humans, as well as in animal species [1-3]. The etiology of IBD, as well as ulcerative colitis is unknown. Contributing factors to the pathogenesis of IBD include disturbance of the intestinal microbiota and its metabolites, the host´s genetic susceptibility, and the host´s innate and acquired immunity [4,5]. Ulcerative colitis is manifested by alternating of acute inflammation (relapse) and inflammation in decline

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(remission). UC is a serious medical and socio-economic problem, an increasing incidence and prevalence, and the decreased quality of life are the reasons for seeking new possibilities for the prevention and treatment. In general, the treatment of UC or IBD usually involves both dietary and pharmacologic intervention, as well as therapeutic manipulation of the enteric microbiota through the use of antibiotics, probiotics, and prebiotics may attenuate the inflammatory process and beneficially modulate gut microbiota and intestinal health and possibly protect humans and animals from ulcerative colitis. For the treatment of IBD probiotic products are currently used as supplements, and not as alternatives or substitutes for conventional therapy [6,7]. Animal studies have confirmed that probiotics and prebiotics enhance the intestinal mucosal immunity, recover the intestinal microbial community structure, and improve chronic intestinal inflammation [8-10].

The aim of the presented study was to obtain information about the effects of the probiotic strain *Lactobacillus plantarum* LS/07 on the activity of β-glucuronidase, counts of coliform and lactobacilli in fresh caecal digesta, cytokine levels (IL-6, IL-8, IL-13), nuclear factor kappa beta (NF-kB) and myeloperoxidase (MPO) activities in the colon tissue and blood samples of rats with dextran sulphate sodium (DSS) induced acute colitis.

MATERIAL AND METHODS

Animals and experimental design

Male Sprague-Dawley rats (n = 24, 7 weeks old, 220–290 g body weight) were placed at the Laboratory of Research Bio-models of the Faculty of Medicine, University of P. J. Šafárik, Slovak Republic (SK PC 4013) under standard laboratory conditions. All animal experiments were conducted in accordance with the principles outlined in Law No. 377/2012 and No. 436/2012 of the Slovak Republic for the Care and Use of Laboratory Animals, and were approved by the Ethical Committee of the University of P.J. Šafárik, Faculty of Medicine and State Veterinary and Food Administration of the Slovak Republic. The rats were randomly assigned to groups: **Control** (control group) received the conventional feed (Snina, Slovak Republic) for 14 days, **Acute colitis** (acute colitis group) received conventional feed without DSS for 7 days followed by 7 days of feed with DSS, and **Probiotic** (probiotic group) received conventional feed with probiotic *Lactobacillus plantarum* LS/07 given in pasteurized milk containing 0.5% fat at a daily dose 1.5×10^9 CFU/1 mL, without DSS for 7 days and then 7 days simultaneously with DSS (Figure 1). Animals were weighted and clinically monitored daily with free access to water and feed. At the end of the experimental diets, the animals were euthanized under anesthesia (Zoletil, Virbac S.A., France) administered at a dose of 50 mg/kg body weight with Xylazin (Riemser, Germany) at a dose of 15 mg/kg body weight, intramuscular). Samples of blood, caecal digesta and tissue were recovered for biochemical, microbial and immunological analysis.

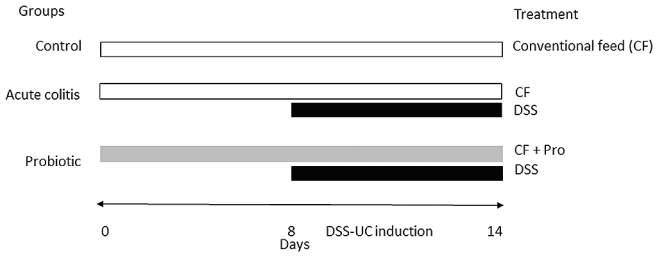

Figure 1. Experimental design of the study. The Control group received CF during the 14-day period. Acute colitis group received CF from day 0 until day 14. At day 8 UC was induced via DSS administration in drinking water. The Probiotic group started administration of probiotic with CF from day 0 until day 14, and at day 8 UC was induced via DSS.

Acute colitis induction

Acute colitis was induced using DSS (molecular weight 40 000, TdB Consulting AB, Uppsala, Sweden) added to drinking water at a final concentration of 5% (wt/vol) for 7d. Rats in the control group received drinking water only. The DSS solution was replenished daily and DSS consumption was noted per cage at the end of 7d treatment.

Disease activity index

Disease activity index (DAI) is the combined score of animal weight loss, stool consistency and bleeding present in the stool. To these parameters a score was assigned and calculated as average daily disease activity index for each rat as described [11].

Probiotic strain

The probiotic strain of *Lactobacillus plantarum* LS/07 was isolated from rectal human swabs reported by Strojný and coworkers [12]. The strain was cultured in MRS broth (Merck, Germany) prepared as night cultures at 37ºC aerobically and provided in a dose of $3x10^9$ CFU of strain/1 mL. Then 0.5 mL of lactobacilli strains mixed with 9 mL of pasteurized milk (20-22ºC) was filled into bottles and administered every day. Each rat received approximately $1.5x10^9$ CFU lactobacilli via the oral route.

Bacteriological examination

Microbial analyses (total counts of lactobacilli and coliform) of the fecal samples were carried out after completion of the experiment. For coliforms culturing selective McConkey agar plates (Merck, Germany) incubated aerobically at 37ºC for 16-18 h. were used, and for lactobacilli culturing Rogosa agar plates (Biokar Diagnostics, France) under anaerobic condition incubating at 37ºC for 48 h were used. The numbers of colony forming unis (CFU) are expressed as $\log_{10}$ CFU per gram of feces.

Measurement of caecal β-glucuronidase activity

The activity of β-glucuronidase (β-GLUCUR) was measured in fresh caecal digesta by determining the rate of *p*- or *o*-nitrophenol as previously described by Juskiewicz et al. [13]. The reaction contained 0.3 mL substrate solution (5 mM, Sigma Aldrich, USA) *p*-nitrophenyl-β-D-glucuronide for β-glucuronidase (β-GLUCUR), and 0.2 mL of 1:10 (v/v) dilution of the caecal digesta in 100 mM phosphate buffer (pH 7.0). After incubation *p*- or *o*-nitrophenol was quantified after addition of 0.25 M cold sodium carbonate and absorption was measured at 400 nm. A measurement unit of enzymatic activity is expressed as μmol of *p*–nitrophenol per min per gram digesta.

Assessment of IL-6, IL-8, IL-13, NFkB and MPO

Samples of blood were left to clot for two hours at room temperature before centrifugation for 15 minutes at 1 000 × g. The removed serum samples were stored at –20ºC. Colon tissue samples were rinsed in ice cold PBS (pH 7.0–7.2) to thoroughly remove excess blood, cut longitudinally and homogenized in PBS with a homogenizer on ice (Disperser T10 Basic Ultra Turrax, Germany) and stored overnight at –20ºC. Thereon, two freeze-thaw cycles were performed to break the cell membranes. Next, the homogenates were centrifuged for 5 min at 5 000 × g at 4ºC, the supernatant was removed and stored at –20ºC. All endpoints were measured by enzyme-linked immunosorbent assay (ELISA) as follows: nuclear factor kappa beta (NF-kB) in the tissue by USCN Life Science, Inc., USA; interleukin 6 (IL-6) in the blood and tissue by eBioscience, interleukin 8 (IL-8) and interleukin 13 (IL-13) in the tissue by Cusabio Biotech Co., Ltd. China, and MPO in the tissue and blood by BlueGene Biotech Co., China. The final values of each parameter were measured on the Synergy H4 multiplate reader (BioTek Instruments, Inc. USA).

Statistical analysis

Results are expressed as mean ± standard deviation (SD). Statistical analysis was performed using analysis of variance (ANOVA) with p values ($p<0.05$) considered to be statistically significant.

RESULTS

The final mean body weight of the animals compared to the initial was in the control group increased by 30.92%, in the acute colitis group by 9.90%, and in the probiotic group by 21.74%.

The average value of all daily disease activity index scores showed that the maximum DAI score = 6.5 was in the acute colitis group, a decline in DAI score was observed after application of probiotic *Lactobacillus plantarum* LS/07 = 3.5. Changes in the

monitored parameters correspond to the changes in weight, as well as the results of the DAI score.

Within a short experimental period preventive dietary supplementation with *Lactobacillus plantarum* LS/07 positively modified activity of β-glucuronidase which is believed to be a biomarker of increased risk of neoplasm was significantly decreased ($p<0.05$) in the probiotic group (Table 1). Acute colitis elevated (non significantly) the number of coliforms and significantly ($p<0.05$) decreased the number of lactobacilli in comparison to the control group (Table 1). *Lactobacillus plantarum* LS/07 nonsignificantly increased lactobacilli counts and decreased counts of coliform bacteria.

Table 1. Activity of β-glucuronidase and total count of lactobacilli and coliform

Parameters	Control	Acute colitis	Probiotic
β-GLUCUR μmol/min/g	0.14 ± 0.02	0.54 ± 0.05 ***	0.39 ± 0.21+
Lactobacilli log10CFU/g	7.78 ± 0.17	7.15 ± 0.90 *	7.40 ± 0.44
Coliforms log10CFU/g	5.18 ± 0.56	5.74 ± 1.03	5.19 ± 0.57

Values are expressed as mean ± SD. Statistical significance is between Control/Acute colitis and + Acute colitis/Probiotic: */+ $p<0.05$; *** $p<0.001$

In Table 2 are shown changes in nuclear factor kappa beta and myeloperoxidase activities in serum (s) and tissue (t) in the control group, acute colitis group and in groups treated with the probiotic.

Table 2. Serum and tissue changes in MPO and NF-ϰB activities

Parameters	Control	Acute colitis	Probiotic
NF-ϰB t ng/mL	41.73 ± 7.41	60.21 ± 10.31 ***	50.52 ± 17.56
MPO s pg/mL	53.38 ± 30.53	98.78 ± 18.26***	69.94 ± 32.95 +
MPO t pg/mL	362.53 ± 71.53	424.12 ± 73.46	346.04 ± 92.13 +

Values are expressed as mean ± SD. Statistical significance is between * Control/Acute colitis and + Acute colitis/Probiotic: + $p<0.05$; ***/+++ $p<0.001$

Serum and tissue cytokine levels of IL-6, IL-8 and IL-13 in the control group, acute colitis group, and probiotic groups are shown in Table 3.

Table 3. Serum and tissue changes in cytokine levels

Parameters	Control	Acute colitis	Probiotic
IL-13**t** pg/mL	62.81 ± 5.99 [a]	47.87 ± 6.91 ***	53.20 ± 14.52
IL-6 **s** pg/mL	49.31 ± 15.83	61.89 ± 15.33	43.00 ± 6.09 +++
IL-6 **t** pg/mL	47.00 ± 8.53	62.65 ± 12.19 **	41.27 ± 14.88 +++
IL-8 **t** pg/mL	37.78 ± 7.42	50.12 ± 7.32 ***	39.34 ± 5.39 +++

Values are expressed as mean ± SD. Statistical significance is between * Control/Acute colitis and + Acute colitis/Probiotic: ** $p<0.01$; ***/+++ $p<0.001$

DISCUSSION

Probiotic bacteria have been defined as live microorganisms, which when consumed in adequate amounts, confer a health benefit for the host. In recent years, the therapeutic and preventive application of probiotics for several gastrointestinal disorders has received increasing attention [14-16]. The categories of probiotics used today include: bacteria such as lactic acid bacteria (LAB) and *Escherichia coli* strains (such as *E. coli* Nissle 1917, which is one of the few examples of non-LAB probiotic), as well as yeast species including most predominantly *Saccharomyces boulardii* among other.

The goal of this study was to obtain information about the effect of the probiotic strain *Lactobacillus plantarum* LS/07 administered to DSS-induced acute colitis rats. Experimental and clinical data so far support the hypothesis that probiotic strains can offer the opportunity to prevent or mitigate intestinal inflammatory lesions and modulate the intestinal microflora and the host immune system in gastrointestinal disorders to provide an adjuvant approach to conventional therapy. Ulcerative colitis is the result of interactions between genetical, immunological, microbial and environmental factors, however the exact etiology and pathogenesis is still unclear.

Of the signaling pathways involved in colonic inflammation, the one triggered by NF-κB plays a key role. The nuclear factor NF-κB pathway has long been considered a prototypical proinflammatory signaling pathway, largely based on the role of NF-κB in the expression of proinflammatory genes including cytokines, chemokines, and adhesion molecules. NF-κB controls apoptosis, cell-cycle progression, cell proliferation, and differentiation. Normal functioning of NF-κB is essential for the maintenance of epithelial cell homeostasis in the gut. But, the antiapoptotic functions of NF-κB can both protect against inflammation, in the case of epithelial cell survival and mucosal barrier integrity, and also maintain the inflammatory response through persistent leukocyte activation [17].

NF-ϰB expression in colonic tissues samples was markedly increased in acute colitis groups ($p<0.001$) and it may provide a sensitive mean of assessing the state of activation of the mucosal immune response. Application of *Lactobacillus plantarum* LS/07 decreased colon tissue NF-ϰB activity slightly. Activated NF-ϰB in the acute colitis group significantly activated serum and colon tissue levels of pro-inflammatory cytokines (IL-6 and IL-8) compared with the control group. *Lactobacillus plantarum* LS/07 significantly down-regulates the synthesis of IL-6 and IL-8 ($p<0.001$) in the serum and colon tissue compared to the acute colitis group. Probiotic *Lactobacillus plantarum* LS/07 stimulated tissue IL-13 production thus demonstrating its anti-inflammatory activity and immune enhancing effect. Dietary *Lactobacillus plantarum* LS/07 interferes with signal transduction pathways related to the carcinogenesis process, thereby acting as chemopreventive agents. They include the suppression of NF-ϰB, induction of apoptosis and anti-inflammatory effects [18].

Modulation of the activity of bacterial enzymes was described as one of the mechanisms through which probiotic *Lactobacillus plantarum* exhibits beneficial effect. This enzyme, β-glucuronidase, characteristic of harmful bacteria species, has deconjugative properties that support transformation of xenobiotics into more toxic substances. This can lead to high local concentrations of carcinogenic compounds within the gut, thus increasing the risk of carcinogenesis. Furthermore, reuptake of the deconjugated compounds from the gut and reglucuronidation in the liver leads to an enterohepatic circulation of xenobiotic compounds, which increases their retention time in the body. Within a short experimental period preventive dietary supplementation with *Lactobacillus plantarum* LS/07 positively modified counts of lactobacilli and coliforms and activity of β-glucuronidase (summarized in Table 1).

Myeloperoxidase is important to understand its effects on inflammation, as it is the most abundant proinflammatory enzyme stored in the azurophilic granules of neutrophilic granulocytes. Neutrophil accumulation in the inflamed intestinal mucosa is a prominent feature of ulcerative colitis. Extracellular MPO activity gives an estimate of oxidative stress in inflammatory diseases, while intracellular MPO activity correlates well with tissue neutrophil content [19]. The determination of MPO can be used as one of the non–invasive markers of disease and prediction of relapse, and is also considered as an index of inflammatory damage. Increased activity of MPO recorded in the serum and colon tissue homogenate of rats was significantly suppressed by the probiotic. The release of MPO correlated to an enhanced local release of the neutrophil activating interleukin-8 [20].

The presented experimental data demonstrate the ability of food supplements containing probiotic strain *Lactobacillus plantarum* LS/07 to intervene and affect the pathophysiological process of the development of acute colitis. The exact etiology and pathogenesis of ulcerative colitis is not yet known, and in that regard the use of probiotics can be a suitable form of prevention of acute colitis in humans and in animals.

CONCLUSION

Probiotic *Lactobacillus plantarum* LS/07 exerted anti-inflammatory effects and contributed to a rapid recovery of DSS-induced acute colitis. Dietary *Lactobacillus plantarum* LS/07 interfere with signal transduction pathways related to the carcinogenesis process, thereby acting as chemopreventive agents.

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Authors' contributions

Author´s contribution were substantial to the article and herein describe their contributions: HE – animal welfare, concept and design of the study; BI, ŠJ and CA – analysis and interpretation data; SL – animal welfare, aplication of DSS; BA – concept and design of the study.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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ANTIINFLAMATORNI POTENCIJAL *LACTOBACILLUS PLANTARUM LS/07* U SLUČAJEVIMA AKUTNOG KOLITISA PACOVA

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U cilju evaluacije efikasnosti probiotskog soja *Lactobacillus plantarum LS/07* kod pacova kod kojih je akutni kolitis izazvan sa Na-dekstran sulfatom (DSS), procenjivani su biohemijski (aktivnost β-glukuronidaze), mikrobiološki (brojevi laktobacila i koliforma) i imunski (IL-6, IL-8, IL-13, NF-*κ*B, MPO) parametri. Pacovi rase *Sprague-Dawley* su

podeljeni u grupe: kontrolna, akutni kolitis i probiotik. Akutni kolitis je indukovan sa 5% DSS u vodi za piće, u trajanju od 7 dana. DSS je izazvao inflamatorni proces u tkivu kolona, povećana je bila aktivnost β-glukuronidaze ($p<0,001$), povećan je broj koliformnih bakterija uz smanjenje laktobacilusa ($p<0,05$) uz aktiviranu proizvodnju parametara koji su posmatrani (NF-*κ*B, MPO, IL-6, IL-8), sa izuzetkom IL-13. Probiotski preparat je smanjio aktivnost β-glukuronidaze ($p<0,05$), povećao je broj laktobacilusa uz smanjenje broja koliformnih bakterija. Ovi rezultati ukazuju da dijetetsko unošenje *Lactobacillus plantarum LS/07* suprimira ekspresiju markera koji imaju značajnu ulogu u zapaljenskom procesu. Može da se zaključi da antiinflamatorne karakteristike *Lactobacillus plantarum LS/07* čine da je ovaj mikroorganizam pogodan za prevenciju ili tretman kolitisa.