Original Research Article

Effect of bifidobacteria on intestinal injury and flora in a mouse model of ulcerative colitis

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Abstract

Purpose: To investigate the effect of bifidobacteria on intestinal injury and flora in a mouse model of ulcerative colitis (UC).
Methods: Mouse model of UC was produced using dextran sulphate sodium (DSS). The mice were divided into seven groups, viz, reference group, MRS-L medium-negative control group, mesalamine-positive control group, high dose bifidobacteria (MIMBb75) group, middle dose MIMBb75 group and low dose MIMBb75 group. Normal mice were used as control. All mice were sacrificed at day 7 of treatment, and colon length, hemoglobin concentration and intestinal flora were determined.
Results: Bifidobacteria inhibited UC-induced decreases in mice hemoglobin and UC-induced colon shortening. In addition, it augmented the diversity of intestinal flora and increased the number of bacteroides and Clostridium leptum.
Conclusion: Bifidobacteria plays a therapeutic role in UC via regulation of intestinal microflora.

Keywords: Bifidobacteria, MIMBb75, Ulcerative colitis, Intestinal injury, Intestinal flora

INTRODUCTION

Ulcerative colitis (UC) is a human disorder characterized by multiple intestinal inflammations due to injuries to the mucosa and submucosa of colon and rectum [1]. The disease which is not associated with geographical and racial differences, generally manifests clinically with symptoms such as abdominal pain, diarrhea, bloody mucopurulent stool and various general symptoms of unknown etiology [2]. Currently, it is thought that UC may be related to over-activation of antigen-induced immune responses in the intestinal lumen [3].

With developments in microecology, the intestinal microenvironment (a complex microecological system) has been closely associated with the onset of UC. Alterations in intestinal flora and increases in pathogenic bacteria and opportunistic pathogens in UC patients enhance intestinal mucosa permeability and induce inflammation by shifting bacterial metabolites to lamina propria mucosa, thereby activating immunocytes [4,5].

Studies have found that UC patients have decreased number of beneficial bacteria such as bifidobacteria and *Lactobacillus,* and increases in the number of harmful germs such as *Colibacillus* and *Enterococcus*. This indicates that intestinal flora may be involved in the occurrence and progression of UC. Bifidobacteria, which have been recognized as probiotics, enhance immunity, and possess anti-aging and anti-tumor properties. In addition, they maintain a balance in intestinal bacteria. The purpose of the current study was to investigate the effect of bifidobacteria on intestinal injury and intestinal microflora of UC mice.

EXPERIMENTAL

Animals

Seventy (70) clean grade Kunmin mice of both sexes provided by Animal laboratory of Jilin University were used in this study. They weighed between 18 and 22 g. The research was approved by the Animal Ethical Committee of Department of Gastroenterology, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang (approval no. 20182217). The study was carried out in accordance with the guidelines of "Principles of Laboratory Animal Care" (NIH publication no. 85 - 23, revised 1985) [6]. All mice were acclimatized for seven days to laboratory environment prior to the study.

Consumables and instruments

Mesalazine (enteric-coated) tablets were purchased from Sunflower Pharmaceutical Co., Ltd.; Bifidobacteria (MIMBb75) was kindly provided by Jilin University Pharmaceutical Co., Ltd. and adjusted to various concentrations (10^8, 10^7, and 10^6 CFU/mL). HiCN-transformed methemoglobin conversion solution was purchased from Shanghai Rongbai Biological Technology Co., Ltd. Dextran sodium sulfate (DSS, molecular weight range 36000 - 50000) was purchased from MP Biomedicals, USA. Low-speed centrifuge was obtained from Shanghai Anting Scientific Instrument Factory, while vertical pressure steam sterilizer was product of Shanghai Shenan Medical Instrument Co., Ltd. Water bath was from Shanghai Biochemical Equipment Co., Ltd.; PCR instrument was bought from Thermo SCIENTIFIC, and electrophoresis apparatus was purchased from Beijing Junyi Electrophoresis Equipment Co., Ltd. Denaturing Gradient Gel Electrophoresis (DGGE) apparatus was product of Beijing Junyi Electrophoresis Equipment Co., Ltd., while gel-imaging apparatus was obtained from Shanghai Qiren Instruments Co., Ltd.

Mice grouping and drug administration

Seven groups of mice were used (10/group). These were normal control group, DSS reference group, MRS-L medium-negative control group, mesalamine-positive control group, and high, middle and low MIMBb75 groups. Mice in each group were fed adaptively for seven days and fasted for 4 h. Except for the blank group, all mice were given 3 % DSS and administered 0.4 mL MRS-L medium, mesalamine, and MIMBb 75 culture solutions at various concentrations via gavage.

Determination of colon injury-related indices

Determination of colon length: Entire colon length of each mouse was measured and recorded. *Determination of hemoglobin*: Hemoglobin concentration of each mouse was determined using Wen-Qi's solution according to the instructions.

Determination of intestinal flora

Samples for determination of intestinal flora were taken out from -80 °C freezer and placed in a -20 °C freezer for 1 h, and then thawed for 20 min on an ice box. A germ-free 2-liter centrifuge tube was weighed by peeling, and about 0.2 g of the mixture was placed in the centrifuge tube. The DNA of intestinal flora contents was extracted using phenol/chloroform/isoamyl alcohol method, and the diversity of the intestinal flora was determined with PCR-DGGE.

Statistical analysis

The data obtained were processed with SPSS 23.0 software, and expressed as mean ± SD. Differences in orthogonal test outcomes were analyzed using *F*-test, and were taken as significant at $p < 0.05$.

RESULTS

Colon injury-related indices

Following intake of DSS, the blood hemoglobin concentration and colon length of mice were significantly changed in the reference group and the MRS-L negative control group, when compared with the normal control group. In addition, the DSS intake significantly decreased their hemoglobin levels and significantly shortened their colons, relative to the control group *(p < 0.05*; Table 1). These results indicate

that there were some changes in colon injury-related indices in the reference group, and that MRS-L had no obvious positive effect on DSS-induced colon injury. In contrast, hemoglobin concentration and colon length were significantly increased in the mesalamine-positive group and in each bifidobacteria (MIMB 75)-treated group, when compared to the reference group $(p < 0.05)$. This suggests that MIMB 75 suppressed the DSS-induced colon injury.

Table 1: Hemoglobin concentration and colon length of mice in the various groups

Group	Hemoglobin (g/dL)	Colon length (cm)
Control	13.01±0.31	10.01±0.11
Reference	10.11*±0.26	7.62*±0.21
MRS-L negative control	9,96*±0.29	7,58*±0.18
Meslamine-positive control	12.84#±0.18	9.92#±0.16
10^8 CFU/mL. MIMBb75	12.89#±0.13	9.95#±0.11
10^7 CFU/mL. MIMBb75	12.93±0.24#	9.90#±0.17
10^6 CFU/mL. MIMBb75	12.96±0.27#	9.87#±0.19

*$P < 0.05$, compared with the blank group; #$p < 0.05$, compared with the reference group

Effect of MIMBb75 on intestinal flora diversity in DSS-induced colitis mice

The number of DNA bands was significantly raised in the high- and middle-dose MIMBb75 groups, when compared with the control group $(p < 0.05)$, and was significantly elevated in the three MIMBb75 groups and mesalamine group, relative to the DSS reference group $(p < 0.05)$.

However, there was no significant difference in the number of DNA bands between DSS control group and MRS-treated group.

Effect of MIMBb75 on the similarity of intestinal flora in DSS-induced colitis mice

As shown in Table 3, since the size of the V3 region fragment of the 16SrDNA that amplified bacteria was 200 bp, the clone can only measure about 170 bp. No dominant microflora such as *Lactobacillus*, bifidobacteria and *Colibacillus* were found in this study. The results showed that the common bands were 1, 6, 12, 14 and 16, corresponding to *Barnesiella intestinehominis*, bacteroides, and *Citrobacter*, respectively. The specific bands in the MIMBb75-treated groups were 4, 5, 7, 8, 9, 10, 11, 13, 15, 16, 17, 19, and 20, which were mainly bacteroides *Barnesiella intestinehominis*, *Clostridium leptum* strain, and *Clostridium polysaccharolyticum* strain. *Clostridium leptum* and *Clostridium* were present in all groups, but they had more advantage in the MIMBb75-treated group.

DISCUSSION

The pathogenesis of UC is unclear at present, although with recent advances in genetics, immunology, and molecular biology, more information on UC is now available [7]. It has been established that the onset of UC is closely related to environment, genetics, immunity and intestinal microflora. Currently, it is believed that the interaction between the intestinal microbial barrier and the tissues may be one of the important causes of UC [8].

Table 2: Effect of MIMBb75 on the number of DNA bands of intestinal flora of mice

Blank group	DSS group	MRS group	MIMBb75 groups			Mesalazine group
			High	Middle	Low	
32.16±1.78	29.43±1.06*	28.95±1.27*	42.73±2.62*#	36.24±2.28*#	33.06±1.97#	32.68±2.04#

*$P < 0.05$, compared with the blank group; #$p < 0.05$, compared with the reference group

Table 3: Serial numbers of bands of the gene fragment sequences in the DGGE profile

Band number	Sequence size	Closest species names from GenBank database NCBI BLAST match (accession no.)	Similarity (%)
1,4,5	176	*Barnesiella intestinihominis* [NZ-JH815206.1]	99.61
9,11,17,20	172	Clostridum leptum 753[NR-114789.1]	97.36
3,8,9,12,14	165	Bacteroides [NZ-EQ973630.1]	99.38
7,10,15,18,6,19	178	*Clostridium polysaccharolyticum* strain G17(NR-025651.1)	99.17
2,13,16	176	*Citrobacter*	99.40

It has been reported that probiotics can resist UC by inhibiting the NF-kB signaling pathway [9]. The findings in this study showed that the number of DNA bands was largely increased in the three MIMBb75 groups, and similarity was higher in the low dose MIMBb75 group than in the control, MRS-positive control, mesalamin, and DSS groups. This indicates that the various doses of MIMBb75 had the same regulating effects on intestinal flora of mice. This may be due to the fact that bifidobacteria participate in the synthesis of short-chain fatty acids, thereby enhancing the barrier function of epithelial cells and preventing the invasion of pathogenic *Colibacillus*.

It has been reported that triple viable *Bifidobacterium* can effectively restrain the growth of pathogenic bacteria in the intestinal tract [10]. In addition, studies have shown that the combination of mesalamine and triple viable *Bifidobacterium* is able to ameliorate the clinical manifestations of UC and boost the recovery of intestinal mucosa [11]. The results of the present study suggest that MIMBb75 can compete with the original host microorganisms in the intestine, inhibit the growth of harmful bacteria, promote the growth of probiotics, and increase the diversity of intestinal microflora in mice. Probiotics can provide essential energies for intestinal epithelial cells (IECs), and the metabolites of their symbiotic bacteria can also influence the balance of IECs.

Microorganisms metabolize dietary fiber and carbohydrates to produce short-chain fatty acids such as acetic acid, propionic acid, and butyrate. Butyrate signal induces the expression of IEC IL-18 through G-protein-coupled receptor (GPR) 109A, thereby inhibiting colitis-associated colon cancer (CAC) [12]. Other related studies have also demonstrated that bifidobacteria uses acetic acid to enhance the anti-apoptosis reaction of intestinal epithelial cells, thus protecting the intestine from injury [13]. The present study has demonstrated that *Clostridium leptum*, *Clotridium polysaccharolyticum* strain, and *Citrobacter* specifically lower intestinal pH and inhibit the invasion of pathogenic microorganisms. Hence, the concentration of hemoglobin in each dose group of MIMBb75 and the length of colon were increased, and colon damage was reduced. This may be due to the fact that MIMBb75 reduced the intestinal pH through acetic acid production, and created an acidic environment in the intestine which inhibited the growth of harmful bacteria. It has been suggested that probiotics can mitigate intestinal inflammation in mice and reduce pathological injury of UC through inhibition NF-kB signal pathway.

CONCLUSION

The results of this study indicate that MIMBb75 lowers the pH of the intestinal tract by creating a good intestinal micro-environment. This provides intestinal probiotics, reduces harmful intestinal bacteria, and improves the distribution of intestinal flora microecology, thereby inhibiting UC-induced colon damage. Thus, bifidobacteria mitigates ulcerative colitis through regulation of intestinal microflora.

DECLARATIONS

Acknowledgement

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All authors read and approved the manuscript for publication. Lin Xiuqing conceived and designed the study, while Lin Xiuqing, Huang Zhiming, Wu Fang, Huang Erjiong, Chen Xin, Jin Ruifang, Chen Renping and Wu Wei collected and analyzed the data. Lin Xiuqing wrote the manuscript.

REFERENCES

1. He XR, Ward V. Research progress on pathogenesis and drug treatment of ulcerative colitis. China Mod Med 2018; 29: 104-106.
2. Ward H, Fisher KL, Waghray R, Wright JL, Card SE, Cockcroft DW. Constrictive bronchiolitis and ulcerative colitis. Canadian Respir J 2016; 6(2): 197-200.
3. Ward MA, Pierre F, Leal RF, Huang Y, Shogan B, Dalal SR, Weber CR, Leone VA, Musch MW, An GC, et al. Insights into the pathogenesis of ulcerative colitis from a murine model of stasis-induced dysbiosis, colonic metaplasia, and genetic susceptibility. Am J Physiol Gastrointest Liver Physiol 2016; 310(11): G973.
4. Zhang SL, Wang SN, Miao CY. Influence of Microbiota on Intestinal Immune System in Ulcerative Colitis and Its Intervention. Front Immunol 2017, 8: 1674.

5. Zeng QL, Song H, Guo HH, Guo C, Zhen D, Liang F, Zhang TY. Effect of supplementation of Flammulina velutipes stembase on the caecal microflora, short chain fatty acid of broiler. J Chin Agricul Univ 2016; 21(05): 104-114.

6. World Health Organization. Principles of laboratory animal care. WHO Chron 1985; 39: 51-56.

7. Jin B. Intestinal flora transplantation and ulcerative colitis. World Chin J Digestology 2017; (01): 23-30.

8. Bajer L, Kverka M, Kostovcik M, Macinga P, Dvorak J, Stehlikova Z, Brezina J, Wohl P, Spicak J, Drastich P. Distinct gut microbiota profiles in patients with primary sclerosing cholangitis and ulcerative colitis. World J Gastroenterol 2017; 23(25): 4548-4558.

9. Xu M, Zhao L, Du JC, Yu SF, Ding XY; Huo GC. Anti-colitis effect of probiotics mixture by the restrain of the NF-κB signaling pathway. Sci Tech Food Industry 2016; 37(17).

10. Zhang L, Li CP, Jiang Z. Clinical Observation of Bifidobacteria Triple Viable Bacteria Combined with Infliximab in the Treatment of Moderate and Severe Ulcerative Colitis. Chin Pharm 2017; 28(05): 629-632.

11. Xu JY, Wu JY. Observation on effect of mesalamine combined with bifidobacterium tetragenous viable bacteria tablets in preventing lipid peroxidation damage in patients with ulcerative colitis. Chin J Pharmacoepidemiol 2015; (12): 712-714.

12. Singh N, Gurav A, Sivaprakasam S, Brady E, Padia1 R, Shi HD, Thangaraju M, Prasad PD, Manicassamy S, Munn DH, et al. Activation of Gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis. Immun 2014; 40(01): 128-139.

13. Feng G, Liu-Fang HE, Liu SL, Chen YY. The influence of Bifidobacteria on the intestinal γδT lymphocytes of neonatal rats with necrotizing enterocolitis. Guangdong Med J 2016; 32: 203-209.