Original Article

Lactobacillus Casei Downregulates the Expressions of Inflammatory Markers in Lipopolysaccharide-induced Colitis

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Objective: Infectious colitis, which is a global disease with potential morbidity and mortality, is an inflammatory process involving a myriad of pro-inflammatory cytokines. Although probiotics are widely used in gastrointestinal disorders, such as necrotizing enterocolitis, inflammatory bowel disease, and infectious enterocolitis, whether probiotics can alleviate colitis remains unclear.

Methods: Sprague Dawley rats were randomly separated into a control group, a group that received lipopolysaccharide (LPS) intraperitoneal injection without probiotic administration, and a group that received LPS intraperitoneal injection after *Lactobacillus casei* administration. Colon tissues were later sampled and total RNA was extracted. The expressions of transforming growth factor-β (TGF-β) and interleukin 6 (IL-6) were measured using a real-time reverse transcription polymerase chain reaction.

Results: LPS induced colonic inflammation and significantly increased IL-6 level compared with that in the control group (4.69 ± 1.22, n = 4, vs. 1.00 ± 0.07, n = 4; $p < 0.05$), but TGF-β levels showed no significant difference (1.41 ± 0.07, n = 2, vs. 1.00 ± 0.10, n = 4; $p = 0.064$). In the LPS group pre-fed with *L. casei*, the IL-6 level was significantly lower than that in the LPS only group (2.47 ± 0.43 vs. 4.69 ± 1.22, $p < 0.05$). TGF-β expression in the LPS group pre-fed with *L. casei* tended to decrease compared to that in the LPS only group (0.97 ± 0.16, n = 4, vs. 1.41 ± 0.07, n = 2; $p = 0.05$), but the difference was not significant.

Conclusions: Pre-feeding of rats with *L. casei* might downregulate the expressions of inflammatory markers in colon tissues induced by LPS.

Key words: colitis, probiotics, *Lactobacillus casei*, lipopolysaccharide

Introduction

Not only is infectious enterocolitis a common global disease that may cause

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malabsorption and adversely affect nutritional status, but it may also lead to further morbidities and even mortality.[1,2] The infectious process releases numerous proinflammatory factors and cytokines, such as tumor growth factor beta (TGF-β), interleukin (IL)-1, and IL-6, that cause additional symptoms and signs.[3]

Lipopolysaccharide (LPS), a major component of the cell wall of certain gram-negative bacteria including *Escherichia coli*, *Shigella sonnei*, and *Salmonella enterica* serovar Typhimurium,[4] is an endotoxin known to trigger inflammation in patients with infectious enterocolitis.

Probiotics have been therapeutically used in many gastrointestinal disorders, including necrotizing enterocolitis, inflammatory bowel disease, infectious enterocolitis, and even chemotherapy-induced diarrhea.[5-9] Probiotics are widely used with established safety[10] in children in the acute phase of infectious diarrhea as well as for its prevention.[6] The mechanisms by which probiotics alleviate the symptoms of colitis include (1) downregulation of proinflammatory processes, (2) alteration of gut bacterial flora, and (3) enhancement of mucosal function.[11] However, the precise mechanism by which probiotics affect the inflammatory cascade in infectious colitis remains unclear. Therefore, the aim of this study was to investigate the effect of probiotics on the expressions of proinflammatory factors in LPS-induced colitis.

Materials and Methods

LPS was purchased from Sigma-Aldrich (Missouri, USA). *Lactobacillus casei* was purchased from Laboratoires Lyocentre (Aurillac, France). The dose of *L. casei* was 12.5 mg (10^{10} colonies)/dose. Male Sprague Dawley (SD) rats (weighing approximately 400 g) were obtained from BioLASCO (Taipei, Taiwan). All procedures were performed according to relevant laws and institutional guidelines. The Institutional Animal Care and Use Committee of E-Da Hospital approved the protocol for this work (Permit Number: IACUC-100019). All rats were sacrificed with CO_2 and all efforts were made to minimize suffering.

Administration of lipopolysaccharide and probiotics in rats

The SD rats were kept in an animal facility with 12-hour day/night rhythm and were protected from excessive noise and vibrations. The rats were divided into 3 groups: a control group (n = 4), an LPS exposure only group (n = 4), and an LPS exposure with probiotic use group (n = 4). The control group was given standard animal chow and water ad libitum without probiotic administration or LPS injection. The LPS exposure only group received the same treatment except for intraperitoneal injection of LPS (8 mg/kg) on day 6.[12] The treatment of the combined LPS-probiotic group was the same as that of the LPS only group except for the oral administration of *L. casei* (12.5 mg, 10^{10} colonies/dose) for 5 consecutive days, starting from day 1.[13] All animals were sacrificed on day 7 (Fig. 1). Changes in body weight were recorded daily.

Real-time reverse transcription polymerase chain reaction (real-time RT-PCR)

Briefly, after the rats were sacrificed, the colon was removed and washed. The tissue samples were stored in RNAlater storage solution (Applied Biosystems Inc., Foster City, CA, USA) for 2 days at 4°C. The GeneJET RNA Purification Kit (Thermo Fisher Scientific Inc., Waltham, MA, USA) was used to isolate RNA from tissue. The quality and purity of the isolated RNA were assessed with a UV/Vis spectrophotometer (DU800, Beckman Coulter, CA, USA). According to the manufacturer's recommendations. High-Capacity cDNA reverse transcription kit (Applied Biosystems Inc., CA, USA) was used to obtain cDNA, which was used for subsequent RT-PCR. The

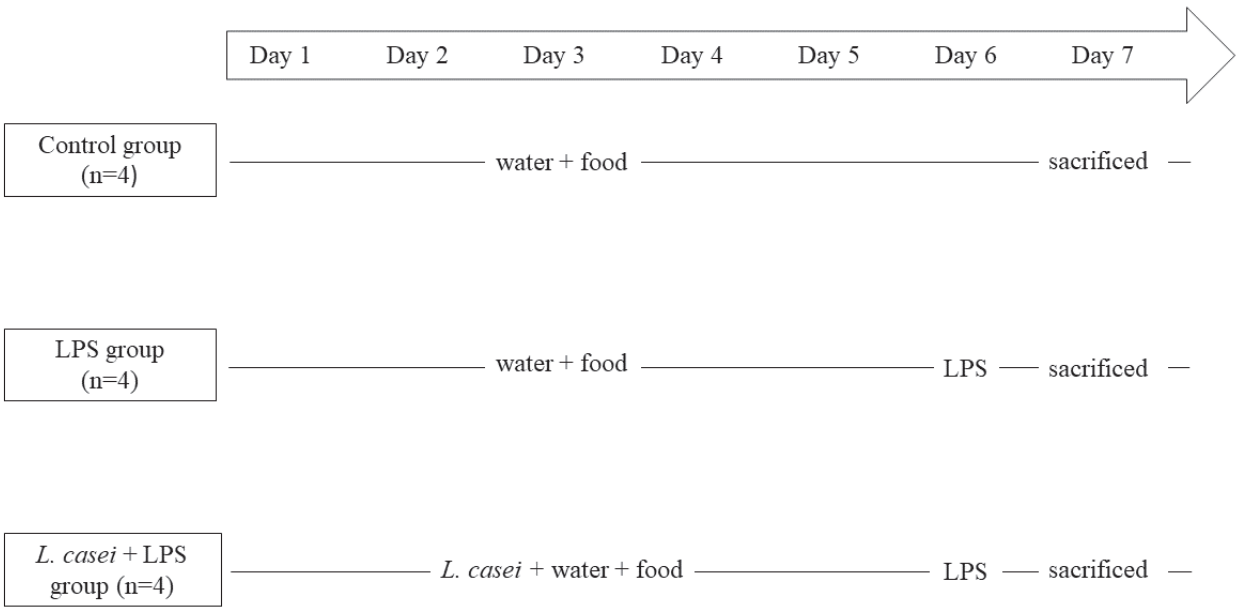

Fig. 1 The probiotic and lipopolysaccharide protocol used before the rats were sacrificed
(c) LPS: Intraperitoneally injected lipopolysaccharide 8 mg/kg,
(d) L. casei: Lactobacillus casei, 12.5 mg (10^{10} colonies)/dose

Table 1. Primers for PCR amplification

Cytokines	Forward	Reverse
β-actin	5' -TCGGTTGGATGGAGCATCCCC-3	5' -GGGAAGGCAGGGACTTCCTGTAA-3'
TGF-β	5' -CGTCAGACATTCGGGAAGC-3'	5' -CAGCCACTCAGGCGTATCA-3'
IL-6	5' -ATGAAGTTCCTCTCTGCAAGAGACT-3'	5' -CACTAGGTTTGCCGAGTAGATCTC-3'

TGF-β- and IL-6-specific sequences were amplified via RT-PCR using the ABI PRISM® 7000 Sequence Detection System (Applied Biosystems Inc., CA, USA) according to the following course: one cycle of 50°C for 2 min, one cycle of 95°C for 10 min, 42 cycles of 95°C for 15 sec, 60°C for 70 sec, and one cycle of 95°C for 15 sec, 60°C for 1 min, 95°C for 15 sec. Amplification of β-actin was used as an internal control. The primer sequences are shown in Table 1 and the methods are based on a previous study.[14] In addition, the colonic tissues were fixed in 4% paraformaldehyde for 1 day, washed in phosphate-buffered saline before being embedded in paraffin. The sections of tissues were stained with hematoxylin and eosin following standard procedures.

Statistical analysis

Data were analyzed using SPSS statistical software (IBM Corp., Armonk, NY, USA). The results are presented as mean ± standard deviation for continuous variables. The Mann-Whitney U test was used for determining the significance of difference among different animal groups. Statistical significance was defined as $p < 0.05$.

Results

Among the three groups, significant reduction in body weight was noted only in the LPS exposure only group ($p < 0.05$) (Table 2). Compared to that in animals without LPS injections, the histopathology of the proxi-

mal and distal colon showed mucosal damage and cracking in addition to neutrophil infiltration (Fig. 2A and B). The findings suggested LPS-induced colonic inflammation which was also reflected in a significant increase in the IL-6 level (4.69 ± 1.22, n = 4, vs. 1.00 ± 0.07, n = 4; $p < 0.05$) compared to that in the control group, but there was no significant difference in the TGF-β level (1.41 ± 0.07, n = 2, vs. 1.00 ± 0.10, n = 4; $p = 0.064$) (Table 3). In the group pre-fed with probiotic before LPS administration, the IL-6 level decreased significantly compared with that in the LPS group (2.47 ± 0.43 vs. 4.69 ± 1.22, $p < 0.05$, n = 4). TGF-β in the LPS group pre-fed with probiotic tended to have a lower expression than that in the LPS only group (0.97 ± 0.16, n = 4, vs. 1.41 ± 0.07, n = 2; $p = 0.05$). The results suggested that LPS-induced inflammation and release of proinflammatory factors in colonic tissue were suppressed by pre-feeding the animals with *L. casei*.

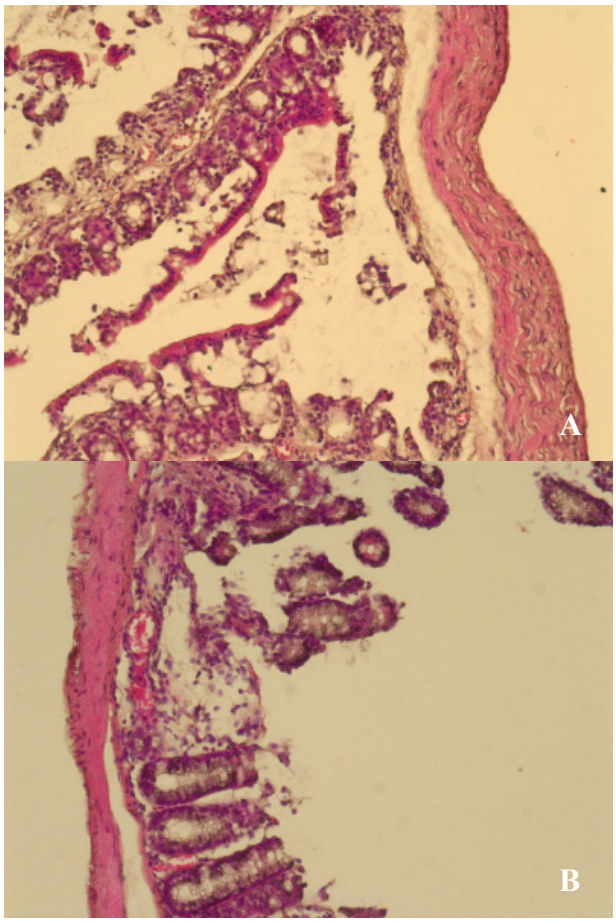

Fig. 2 Mucosal damage and cracking in addition to neutrophil infiltration in (A) proximal, and (B) distal colon in rats with intra-peritoneal lipopolysaccharide (LPS) injection (Hematoxylin-eosin staining, 200x).

Discussion

Infectious enterocolitis is common in both developing and developed countries. The pathophysiological mechanisms include increased fluid and electrolyte secretion as well as decreased absorption. Supportive management with adequate fluid and electrolyte replacement is recommended for infectious diarrhea.[15] In addition to supportive management, relief of symptoms and signs is important, especially in children who are vulnerable to dehydration.

Toll-like receptor 4 (TLR4) is known to trigger inflammation.[16] LPS, a major component of Gram-negative bacterial cell wall, has been shown to induce an infectious or inflammatory cascade via the TLR4 (a receptor for

Table 2. Body weight changes before and after intervention among different animal groups

	Initial BW[a]	BW[a] before sacrifice	BW[a] change	*p**
Control	378.25 ± 14.44	381.75 ± 18.03	3.5 ± 5.07	0.564
LPS[b]	370.5 ± 15.01	342.2 ± 14.47	-28.5 ± 1.14	0.043
L. casei[c] + LPS	378.25 ± 23.31	375.38 ± 9.46	-2.88 ± 14.04	0.773

[a]BW: Body weight (grams)
[b]LPS: Lipopolysaccharide 8 mg/kg, intraperitoneal injection
[c]*L. casei*: *Lactobacillus casei*, Laboratoires Lyocentre (Aurillac, France), 12.5 mg (10^{10} colonies)/time
*Significance of difference determined by Mann-Whitney U Test

Table 3.The expression level of TGF-β and IL-6 in colon epithelial cell by real time RT-PCR[a]

	Control group	LPS[b] group		*L. casei*[c] + LPS group	
	mean± SD[g]	mean ± SD	*p*	mean ± SD	*p*
TGF-β[d]	1.00 ± 0.10 (n = 4)	1.41 ± 0.07 (n = 2)[f]	> 0.05*	0.97 ± 0.16 (n = 4)	> 0.05**
IL-6[e]	1.00 ± 0.07 (n = 4)	4.69 ± 1.22 (n = 4)	< 0.05*	2.47 ± 0.43 (n = 4)	< 0.05**

[a]RT-PCR: Reverse transcription polymerase chain reaction; [b]LPS: Lipopolysaccharide 8 mg/kg, intraperitoneal injected; [c]*L. casei*: *Lactobacillus casei*, Laboratoires Lyocentre (Aurillac, France), 12.5 mg (10^{10} colonies)/time; [d]TGF-β: transforming growth factor-beta; [e]IL-6: Interleukin 6
[f]Insufficient sample amount; [g]standard deviation; **p* value compared between LPS group and control group; ***p* value compared between *L. casei* + LPS group and LPS group

LPS)-mediated pathway.[17] Accordingly, Gram-negative bacteria such as *E. coli*, *S. sonnei*, and *S. enterica* serovar Typhimurium, which are common pathogens in infectious diarrhea, can trigger an inflammatory cascade in intestinal epithelial cells by activating the TLR4-mediated pathway. TLR4 has been shown to ignite inflammation through the upregulation of TGF-β and IL-6 expressions.[18,19] Furthermore, TGF-β has been found to enhance IL-6 expression in intestinal epithelial cells, thereby aggravating diarrhea.[20]

Probiotics, which have been shown to modulate inflammation, are widely used for treating many disorders.[8,21] Probiotics contain *Lactobacillus*, *Bifidobacterium*, *Clostridium*, *Streptococcus*, and other bacterial species. Among these bacteria, *L. casei* reportedly can downregulate inflammation in intestinal mucosa in Crohn's disease.[22] Furthermore, *L. casei* has been demonstrated to be protective against intestinal inflammation and inhibit the secretion of IL-6.[23,24]

Since *L. casei* has been found to modulate inflammation, the therapeutic effects of probiotic pre-administration against infectious diarrhea were investigated. In this animal study, colonic tissue was sampled and analyzed for histological integrity as well as the expressions of TGF-β and IL-6 after intraperitoneal injection of LPS. Despite the lack of significant change in TGF-β expression after LPS exposure, mucosal damage and cracking as well as a significant elevation of IL-6 expression were noted. Compared to the animals with LPS exposure only, those having received *L. casei* showed significantly suppressed IL-6 expression and relatively lower TGF-β expression. The findings highlighted the modulating effect of *L. casei* on LPS-induced colonic inflammation following infection with LPS-producing bacteria.

The limitation of this study is the small sample size. Therefore, further studies may be considered to support our findings. Furthermore, a group being fed with *L. casei* alone was not included in the present study for comparison so that the effect of probiotics *per se* in this experimental setting remains unclear. In addition, enzyme-linked immunosorbent assay or western blot analysis of the specimens may have provided further information in this study.

In conclusion, LPS, a component of Gram-negative bacterial cell wall, triggered colonic inflammation that could be suppressed by pre-feeding with *L. casei* in an experimental setting.

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Disclosure

The authors declare no conflicting financial interests.

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