Preventive effect of Lactobacillus mixture on inflammation and cytokines in lipopolysaccharide-induced cystitis in mice

Version 3

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Abstract

This study aimed to investigate the effects of lactic acid bacterial fermentation extract (LABEF) on cystitis induced by *Escherichia coli* lipopolysaccharide (LPS) in the mouse bladder by pathological analyses and measurement of the levels of tumor necrosis factor-alpha (TNF-α) and interleukin-18 (IL-18). LABEF was administered orally (5 μg/L) to mice for 10 days following which, the study group (n = 12) was injected intravesically with 5 μg/L LPS. The bladder tissue was then harvested after 24 hours and subjected to hematoxylin and eosin (H-E) staining. A semiquantative score was used to evaluate inflammation (bladder inflammation index; BII). TNF-α immunohistochemical staining and multiplex cytokine assay were also performed. TNF-α and IL-18 levels were determined. The results were compared with those of the control group (n = 12). The BII in the control and study groups was 2.7 ± 0.5 and 1.1 ± 0.7, respectively, with the control group scores significantly differing from the study group scores (p value < 0.001). TNF-α immunohistochemical staining showed similar results. The TNF-α levels determined by the multiplex cytokine assay were 2.82 ± 1.35 pg/mg and 1.55 ± 0.56 pg/mg for the control and study groups, respectively, and the difference in values between these groups was statistically significant (p value = 0.007). Thus, oral administration of LABEF appears to have a preventive effect against the inflammatory responses and TNF-α expression induced by intravesical instillation of LPS in the mouse bladder. Further studies are required to determine the clinical application of this finding.

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Protocol

Step 1.

Twenty-four 8-week old female black mice (C57BL/6, Orient Bio, Korea) were acclimatized for 1 week. The mice were kept in groups of two in cages with sawdust bedding. Water and feed was supplied freely, and temperature (21–24°C) and humidity (50–60%) conditions were controlled. The mice were subjected to 12-h light/12-h dark cycles.

Step 2.

Control group (n = 12): Mice were orally administered with normal saline 5 times/week for 2 weeks. On day 14, bladders were injected with LPS and harvested after 24 hours.

Step 3.

Study group (n = 12): Mice were orally administered SL 16 (B&S Corporation, Japan) 5 times/week for 2 weeks. On day 14, bladders were injected with LPS and harvested after 24 hours.

Step 4.

SL 16 comprised 66.5% distilled water, 33% LABEF (soy milk 32%, *Lactobacillus curvatus*, *Lactobacillus casei, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus salivarius, Lactobacillus brevis,* and *Lactobacillus rhamnosus*), 1% citrate, and 0.5% lactate).

Mice were anesthetized with Zoletil (0.6 g/kg, Virbac S.A., France) and Ropun (0.4 g/Kg, Bayer AG, Leverkusen, Germany) prior to bladder injection. A sterilized polyethylene vascular catheter (24G, 19 mm, Angiocath Plus, Becton Dickinson Medical(s) Pvt. Ltd., Singapore) was inserted through the urethra into the bladder and the bladder was emptied by applying pressure to the abdomen. Next, 150 µL of *E. coli* LPS strain 55:B5 mixture (100 µg/mL, Sigma, St. Louis, USA) was injected in both groups. For enhanced effects, this injection was repeated after 30 minutes and the catheter was blocked with a 1 mL syringe to avoid urine leakage for 30 minutes before removal.

Step 5.
The animals were sacrificed by CO2 inhalation and the lower abdomen was disinfected with 70% ethanol, after which the abdominal cavity was exposed through a vertical midline incision of 5 cm. The bladder tissue was harvested and divided vertically. One half of the specimen was preserved at -70°C, and the other half was preserved in 10% formaldehyde.

Step 6.
The tissue fixed in formaldehyde was used to prepare 5-µm vertical tissue sections after paraffin embedding. Hematoxylin and eosin (H-E) staining was performed per standard procedure. TNF-α immunostaining was done using TNF-α antibody (Anti-TNF-alpha antibody ab6671, Abcam, Cambridge, UK) as recommended by the manufacturer, with eosin staining as the control. Histopathology and TNF-α immunostaining of the bladder wall was observed through optical microscopy.

Step 7.
The severity of bladder inflammation was categorized according to the BII proposed by Jerde et al., by considering leukocyte infiltration into the lamina propria and interstitial tissue edema. Leukocyte infiltration was assessed in 1-mm2 areas of tissue sections. The score was considered 0 when there was no infiltration, 1 when there were less than 20 leukocytes, 2 with 20–45 leukocytes, and 3 when there were more than 45 leukocytes. With regard to edema in the interstitial tissue, scoring was as follows: a score of 0 was given for no edema; 1 for mild edema, thinner than the mucosa; 2 for moderate edema, thinner than twice the thickness of the mucosa; and 3 for severe edema, thicker than twice the thickness of the mucosa. The scores for leukocyte infiltration and interstitial edema were added and the average scores from three sites (bladder neck, lateral wall, and bladder dome) in each of the bladder specimens were compared.

Step 8.
Frozen bladder specimens were prepared as described previously. The buffer solution containing 2.66% Tris (hydroxymethyl) aminomethane HCl, 0.985% Tris (hydroxymethyl) aminomethane, 0.5 mM pepstatin A, 0.3 M aprotinin (all of the above were obtained from Tocris, Bristol, UK)), phenylmethylsulfonyl fluoride, and 1 M leupeptin, 1 M (both from EMD Millipore, Temecula, CA, USA) was applied, the specimen was pulverized on ice, and incubated at 4°C for 30 minutes. This preparation was subjected to centrifugal separation at 10,000 × g for 10 minutes at 4°C. The TNF-α and IL-18 levels in the supernatant were measured using the MILLIPLEX MAP Non-Human Primate

Cytokine Magnetic Bead Panel-Immunology Multiplex Assay Kit (Merck Millipore, Billerica, MA, USA) and Luminex (Luminex, Austin, TX, USA), according to the manufacturers' recommendations.