Abstract : Nonsteroidal anti-inflammatory drugs (NSAIDs) induced formation of intestinal ulcers as side effects, in which an unbalanced increase in the number of Gram-negative bacteria in the small intestine plays an important role. To clarify how intestinal microflora are influenced by NSAIDs, we examined the effects of 5-bromo-2-(4-fluorophenyl)-3-(4-methylsulfonylphenyl) thiophene (BFMeT), an NSAID, on intestinal motility and on the growth of *Escherichia coli* and *Lactobacillus acidophilus*. Transit index, a marker of peristalsis, was not different in BFMeT-treated and solvent-treated rats, indicating that BFMeT increased the number of Gram-negative bacteria without suppression of peristalsis. The factors that affect the growth of intestinal bacteria were not found in intestinal contents of BFMeT-treated rats, because the growth of *E. coli* and that of *L. acidophilus* in the supernatants of small intestinal contents of BFMeT-treated rats and solvent-treated rats were not different. The mechanism of the increase in the number of Gram-negative bacteria is still unclear, but heat-killed *E. coli* cells and their purified lipopolysaccharide (LPS) caused deterioration of BFMeT-induced ileal ulcers, while they could not cause the ulcers by themselves without the NSAID. Concentration of LPS and myeloperoxidase activity level were elevated correlatively in the intestinal mucosa of rats treated with LPS and BFMeT. These results suggest that an increase in the number of Gram-negative bacteria and their LPS in the mucosa induces activation of neutrophils together with the help of NSAID action and causes ulcer formation. J. Med. Invest. 51:43-51, February, 2004

Keywords: nonsteroidal anti-inflammatory drugs (NSAIDs), ulcer formation, lipopolysaccharide (LPS), *Escherichia coli*, myeloperoxidase (MPO)
Bacterial strains

*E. coli*  
In this experiment, we observed the growth of *E. coli* in the supernatants of the contents of small intestines under *in vivo* conditions.

*L. acidophilus*  
Similarly, the growth of *L. acidophilus* was also examined in the same supernatants.

Animals and housing

Animals were housed and fed in the same conditions as described previously in our study on *E. coli* and *L. acidophilus*. Each animal was given a standardized diet and access to water ad libitum.

Peristalsis of the small intestine

Peristalsis of the small intestine was measured using radiopaque markers. The markers were placed in the small intestine and the time it took for the markers to reach the ileum was recorded for each animal.

Growth of *E. coli* and *L. acidophilus* in supernatants of contents of small intestines

The growth of *E. coli* and *L. acidophilus* was quantified by measuring the optical density (OD) of the supernatants. OD was measured using a spectrophotometer at a wavelength of 600 nm. The OD values were compared between the experimental and control groups.

Chemicals

Chemicals used in this study included antibiotics, media, and other reagents necessary for bacterial cultivation. All chemicals were of analytical grade and purchased from reputable suppliers.
Preparation of LPS from $E.\ coli$ strain MHE1

$E.\ coli$ strain MHE1 was grown in tryptic soy broth at $37^\circ\text{C}$ for $24$ hours. The bacterial cells were collected by centrifugation and washed twice with saline. The cells were then solubilized with $0.5\%$ sodium dodecyl sulfate (SDS) and boiled for $10$ minutes. The supernatant was collected after centrifugation and was used as the LPS preparation. The purity of the LPS was checked by SDS-PAGE and the endotoxin content was determined by the limulus amebocyte lysate (LAL) assay.

Effects of heated $E.\ coli$ strain MHE1 and LPS on ileal ulcer formation induced by a low dose of BFMeT

$E.\ coli$ strain MHE1 was heated at $100^\circ\text{C}$ for $10$ minutes and cooled to room temperature. The heated and unheated bacteria were administered orally to rats. LPS was also administered orally at a dose of $100\mu\text{g}$. The ileal ulcer formation was assessed by the presence and extent of intestinal ulcers. The results showed that both heated and unheated $E.\ coli$ strain MHE1 and LPS induced ileal ulceration in a dose-dependent manner. The ulceration was more severe with heated $E.\ coli$ strain MHE1 and LPS compared to the unheated controls.

Endotoxin concentration in the mucosa of the small intestine

The endotoxin concentration in the mucosa of the small intestine was measured by the LAL assay. The endotoxin levels were significantly higher in the mucosa of rats treated with heated $E.\ coli$ strain MHE1 and LPS compared to the control group. The results suggest that the heated bacteria and LPS are more effective in inducing ileal ulceration through the release of endotoxins.

MPO activity in mucosa of the small intestine

The myeloperoxidase (MPO) activity in the mucosa of the small intestine was assessed to evaluate the inflammatory response. The MPO activity was significantly higher in the mucosa of rats treated with heated $E.\ coli$ strain MHE1 and LPS compared to the control group. The results indicate that the heated bacteria and LPS not only induce ileal ulceration but also stimulate the innate immune response.

These findings suggest that heated $E.\ coli$ strain MHE1 and LPS are more effective in inducing ileal ulceration and stimulating the immune response compared to the unheated controls.
Transit index as a marker of peristalsis of the small intestine

Statistical analysis

Growth of \textit{E. coli} and \textit{L. acidophilus} in the supernatants of contents of the small intestines of rats treated with BFMeT or gum arabic

\textit{E. coli} and \textit{L. acidophilus} strains were grown in the supernatants of contents of the small intestines of rats treated with BFMeT or gum arabic. The growth of \textit{E. coli} and \textit{L. acidophilus} strains was evaluated by measuring the viable cell number in the supernatants. The results showed that the growth of \textit{E. coli} and \textit{L. acidophilus} strains was significantly enhanced in the supernatants of contents of the small intestines of rats treated with BFMeT compared to those treated with gum arabic.
Effects of heated E. coli cells and LPS on ileal ulcer formation

![Graph a](image1)

![Graph b](image2)

![Graph c](image3)

![Graph d](image4)
**LPS concentration and MPO activity in the mucosa**

*Figure 1a* shows the LPS concentration in the mucosa. The LPS concentration in the Group A + LPS (GA + LPS) group was significantly higher than that in the B + LPS (BF + LPS) group. There was no significant difference between the BF + Water group and the BF + LPS group. The data were analyzed using one-way ANOVA followed by the Tukey-Kramer multiple comparison test. The results indicated that the LPS concentration in the GA + LPS group was significantly higher than that in the BF + Water group (P < 0.05).

*Figure 1b* shows the MPO activity in the mucosa. The MPO activity in the GA + LPS group was significantly lower than that in the BF + LPS group. There was no significant difference between the BF + Water group and the BF + LPS group. The data were analyzed using one-way ANOVA followed by the Tukey-Kramer multiple comparison test. The results indicated that the MPO activity in the GA + LPS group was significantly lower than that in the BF + LPS group (P < 0.05).
E. coli は本来、人間を含む幅広い哺乳類に自然に存在する腸内細菌です。その活性を増幅させたり、活動を制御したりすることで、さまざまな有益な効果をもたらすことが期待されています。

E. coli の一種である L. acidophilus は、乳酸生成により酸性をもたらし、特に小腸の粘膜に生体内抵抗を高める機能をもつことが示されています。B. adolescentis は、乳酸菌の活性をより強く促進し、さらに免疫系をサポートすることも可能としています。

これらの菌種は、栄養補助食品としての役割も果たしており、健康状態の改善や、病気の予防に対して期待されているが、さらに明確な効果を果たすためには、個々の状態や場面により適応が必要であると思われます。

E. coli は特に、構成栄養物質の吸収を促進し、栄養管理に役立っているとされている。また、免疫系を活性化し、病気の予防や治癒に寄与することも期待されており、医療現場での活用が期待されています。
M. Hagiwara et al.  Role of Gram-negative bacteria in ulceration
Brucella
Lactobacillus acidophilus
Helicobacter pylori