医生電顕技術誌 30 (1): 1-4 (2017)
J. Electr. Microsc.Technol. Med. Biol. 30 (1): 1-4 (2017)

【原著：Original Work】

洗剤によるマウス小腸微絨毛障害に対する乳酸菌混合発酵産物の保護効果

Protective Effect of a Lactic Acid Bacteria-Fermented Soybean Extract from Detergent-Induced Damage to Murine Small Intestinal Microvilli

阿部一彦[1)]、西川俊郎[1)]、関口守衛[2)]

Abe, K.[1)], Nishikawa, T.[1)], Sekiguchi, M.[2)]

(Received: August 8, 2016, Accepted: August 25, 2016)

ABSTRACT

BACKGROUND & AIMS: A lactic acid bacteria-fermented soybean extract (LFS) is believed to have a protective effect on the gastro-intestinal system. This current study was undertaken to evaluate the ultrastructural changes of the jejunal microvilli following exposure to a detergent containing surfactant and determine whether such mucosal changes would be diminished by prior treatment with LFS. **METHODS:** Twenty mice were divided into two broad experimental groups that were fed either an ordinary diet (group A; n = 15) or one supplemented with LFS (group B; n = 5). The detergent known to induce mucosal damage was administered by oral gavage to group B and a subgroup in group A (n = 5 each), which were sacrificed ten minutes afterwards. Distilled water was given in the same manner as a negative control to another subgroup in group A (n = 5). The ultrastructure of the jejunal mucosa was then investigated using electron microscopy. **RESULTS:** The subgroup in group A given the detergent showed severe damage to the mucosal ultrastructure with fragmentation of the microvilli in all animals. However, in mice given LFS, jejunal microvilli of the jejunum showed an almost normal ultrastructural appearance in all animals following treatment with the detergent. As such, LFS appeared to have protective effects on the intestinal mucosa at the ultrastructural level. **CONCLUSIONS:** Our findings suggest that LFS protects the intestinal mucosa and may thus be examined in a clinical setting against mucosal damage caused by conventional drugs known to induce mucosal irritation, such as aspirin and non-steroidal anti-inflammatory drugs.

INTRODUCTION

The jejunal mucosal surface is composed to numerous finger-like projections called villi that are covered by absorptive cells having fine microvilli on their surface. The microvilli are present on the luminal surface of absorptive cells. Electron microscopy (EM) of the surface of absorptive cells shows as many as 3000 microvilli on each villus that are approximately 1μm tall containing actin filaments, whose tips are covered with a glycocalyx layer[1, 2)].

A lactic acid bacteria-fermented soybean extract (LFS) is a health-promoting supplement that has been shown in our clinical experience to have protective effects in the gastro-intestinal (GI) tract against irritable bowel syndrome as well as against bronchial asthma. As a type LFS contains the fermentation products of sixteen kinds of lactobacilli and yeast cultured in soy milk that are then enriched with cyclodextrin and xylo-oligosaccharide[3)].

With established treatments for the prevention of intestinal irritation by conventional drug therapies not yet established, the aim of the current study was to observe the microvilli of the intestine at the ultrastructural level and evaluate the effectiveness of a health-promoting supplement made from LFS against detergent-induced damage.

MATERIALS AND METHDS

Animal experiments

Eight week-old male BALB/c mice (Japan SLC, Inc., Japan) were divided into four subgroups as illustrated in Fig. 1: group A-1, a healthy control group fed an ordinary diet (MF-powdery diet, Oriental Yeast Co., LTD., Tokyo) (n = 5); group A-2, mice fed an ordinary diet who were forcibly injected with distilled water by oral gavage to exclude the effects of physical injury by oral gavage injection (n = 5); group A-3, mice fed an ordinary diet to which 0.5ml of 0.1% detergent (JOY dishwashing liquid, P&G far-east inc., Kobe, Japan) was forcibly injected by oral gavage; and group B-4, mice fed a LFS-enriched diet for one week starting at the age of seven weeks to which 0.5ml of 0.1% detergent was forcibly injected by oral gavage.

The animals in groups A-2, A-3 and group B-4 were sacrificed ten minutes after treatment by cervical vertebrae dislocation and jejunal mucosal specimens were obtained for EM evaluation. Samples were immediately washed with 0.1M cacodylate buffer solution and processed for scanning electron microscopy (SEM) or transmission electron microscopy (TEM).

The freeze-dried LFS, which contains cyclodextrin and xylo-

Key Words: Lactic acid bacteria-fermented soybean extract, Jejunal microvilli, Detergent, Surfactant, Electron microscopy

1) Department of Surgical Pathology, Tokyo Women's Medical University Hospital（東京女子医科大学病院病理診断科）
2) Japan Research Promotion Society for Cardiovascular Diseases（日本心臓血圧研究振興会）

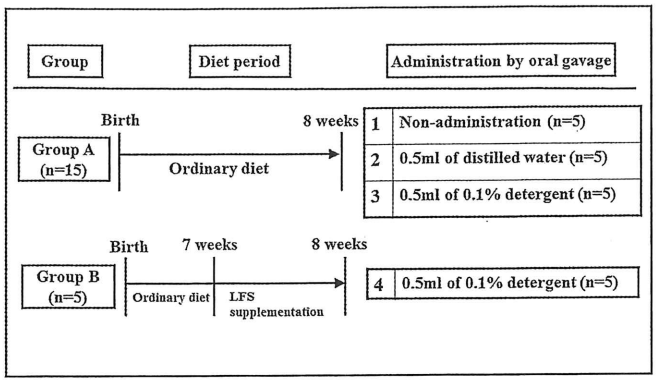

Fig. 1. Test groups for evaluating the effects of a lactic acid bacteria-fermented soybean extract (LFS) on jejunal microvilli against exposure to a detergent.

oligosaccharide, was provided by the Central Institute for Health Science, A.L.A. Corporation (the A.L.A. Institute, Tokyo, Japan) as a favor. The ratio of each component is not specified.

The animal experiments were conducted in compliance with protocol reviewed by the A.L.A. Institutional Animal Care and Use Committee and approved (Permit Number: #43).

The dishwashing detergent "JOY" has three kinds of surfactant: anionic alkyl ethereal sulfate ester sodium, ampholytic alkylamine oxide, and nonionic polyoxyethylene alkyl ether. The proportion of surfactant in the detergent is 41 percent. The ratio of each surfactant is not specified.

SEM ultrastructural observation

Jejunal specimens were prefixed with 3% glutaraldehyde in 0.1M cacodylate buffer solution, pH7.4, for one hour at 4°C. After rinsing, the specimens were postfixed in 1% OsO4 in 0.1M cacodylate buffer solution, pH7.4, for one hour at 4°C. The specimens were then dehydrated through 50% to 100% ethanol gradients and dried with a Critical Point Dryer (Model HCP-1, Hitachi, Japan). After osmium vapor coating with a Neo Fine Osmium Coater (Meiwa, Japan), the jejunal microvilli were visualized with a Model S-4300 SEM (Hitachi, Japan).

TEM ultrastructural observation

Jejunal specimens were cut into small 1 mm^3 pieces with a sharp knife and then prefixed and postfixed in the same manner as that of SEM. After dehydration through 50% to 100% ethanol gradients, the specimens were substituted in propylene oxide and embedded in epoxy resin using standard procedures. Specimen blocks were cut into ultra-thin 80nm sections with an Ultramicrotome (Ultracut N model, Reichert-Nissei, Austria). Jejunal microvilli were then observed by a Model T-7650 digital TEM (Hitachi, Japan).

RESULTS

Control mice that were fed an ordinary diet showed a normal ultrastrucuture under EM (Fig. 2-A). The jejunal microvilli appeared as a regularly-aligned structure similar to that of a corncob, and the inner ultrastructure as shown in Fig. 3-A showed a typically normal appearance. The jejunal microvilli that were given distilled water also showed no apparent damage and presented a normal luminal and cross-sectional ultrastructure (Fig. 2-B and Fig. 3-B).

Jejunal microvilli that were treated with detergent showed extreme damage to microvilli, appearing as disarranged and destroyed microvilli (Fig. 2-C and Fig. 3-C). However, in mice treated with LFS for a one week period, EM analysis showed a microvilli ultrastructure that was virtually identical to that of controls, which suggested a protective effect against such insult (Fig. 2-D and Fig. 3-D).

DISCUSSION

The jejunal microvilli are covered by the glycocalyx containing disaccharidase and pepitidase. For that reason, the microvilli play an important role in the absorption of amino acids, monosaccharides, and other nutritional elements[1, 2]. The membranous surface of the microvilli is easily damaged by extrinsic agents, so it is believed that protection of intestinal microvilli integrity is essential for proper nutrition and homeostasis.

This study was undertaken to investigate whether LFS could protect against extrinsic tissue damage with a commercial dishwashing detergent. This detergent contains three kinds of surfactant, and has been shown to have a disruptive effect on intestinal surfaces in a matter of minutes. Here, application of the detergent caused severe fragmentation of the microvillous ultrastructure. The exact mechanism of cell membrane solubilization by detergents has been described elsewhere[4]; briefly, microvilli cell membrane proteins are embedded in the lipid bilayer via fatty acid hydrophobic side chains[5]. When the hydrophobic bonds of the microvilli cell membrane are weakened by the detergent, the proteins are drawn out of the cell membrane. Thereafter, many holes are opened in the microvilli cell membrane, core actin filaments become depolymerized, and the microvilli structure becomes fragmented into small particles. This phenomenon could be clearly observed in the non-LFS -treated group.

On the contrary, in intestinal samples from mice given LFS for one week prior to detergent administration, microvilli damage was not detectable, likely due of the protective effect imparted by the supplement. Thus, it can be considered that LFS inhibited weakening of the hydrophobic bonds of the microvilli cell membrane by the detergent.

Currently, the usage of anti-platelet agents, such as low-dose aspirin or non-steroidal anti-inflammatory drugs (NSAIDs), and clopidogrel[6–8] are considered essential for a large number of people worldwide, but is often accompanied by GI aggravation and discomfort. Proton pump inhibitors (PPI) are currently the drug of choice to prevent GI injury in patients undergoing treatment for rheumatoid arthritis, cardiovascular disease, or who have drug-eluting stents (DES) and receive antiplatelet therapy[9]. The effects of PPI have been mostly evaluated endoscopically, not based on electron microscopic observation. In this context, our study may contribute to a more precise understanding of intestinal mucosa protection. Furthermore, it shows that dietary supplementation with LFS could be a viable option in clinical practice as there exist few reliable conventional means of protect-

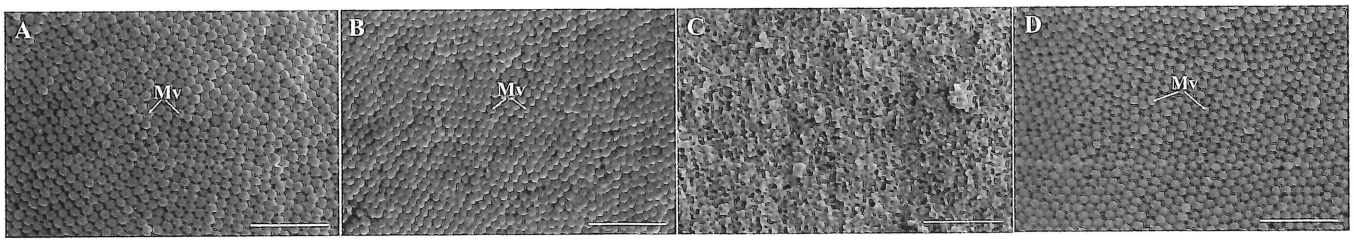

Fig. 2. Representative scanning electron micrographs (SEM) of the jejunal microvilli of BALB/c mice showing the mucosal membrane surfaces of the four groups studied.

(A) Normal microvilli in Group A-1. The jejunal microvilli show a regularly-aligned structure resembling that of a corncob. (B) Microvilli given 0.5ml of distilled water in Group A-2. The jejunal microvilli show a normal appearance. (C) Microvilli given 0.5ml of 0.1% detergent in Group A-3. The jejunal microvilli show an extremely damaged appearance. (D) Microvilli given 0.5ml of 0.1% detergent following one week supplementation with LFS in Group B-4. The jejunal microvilli show a normal appearance. The bars in each figure indicate 1 μm. Mv: microvilli.

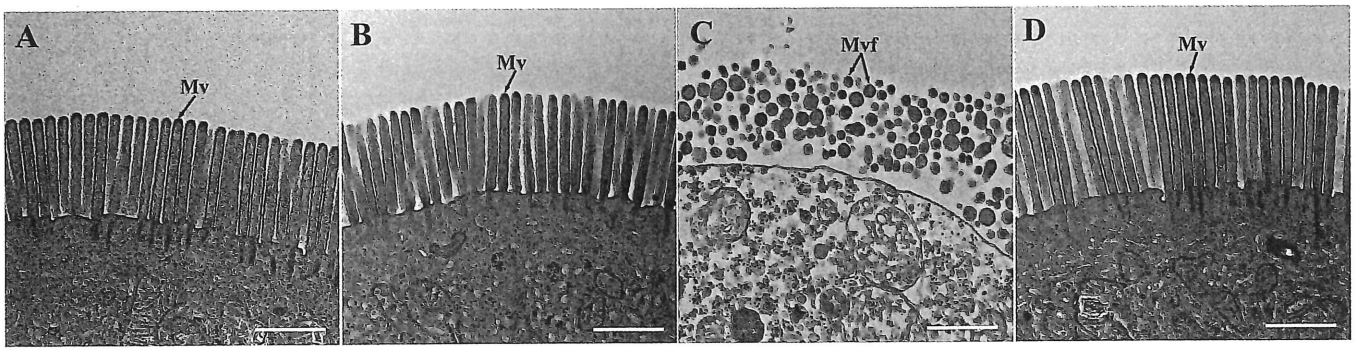

Fig. 3. Representative transmission electron micrographs (TEM) of the jejunal microvilli of BALB/c mice showing cross-sections of the microvilli of the four groups studied.

(A) Normal microvilli in Group A-1. The jejunal microvilli show a normal TEM appearance. (B) Microvilli given 0.5ml of distilled water in Group A-2. The jejunal microvilli show a normal appearance. (C) Microvilli given 0.5ml of 0.1% detergent in Group A-3. The jejunal microvilli show advanced fragmentation. (D) Microvilli given 0.5ml of 0.1% detergent following one week supplementation with LFS in Group B-4. The jejunal microvilli show a normal appearance. The bars in each figure indicate 1 μm. Mv: microvilli, Mvf: microvilli fragmentation.

ing against mucosal damage and GI irritation.

There is one particular limitation of this study. Although damage caused by aspirin and other agents is usually localized to the stomach[8], our experiment used the nearby jejunal membrane for investigation. EM observation using stomach mucosa is technically difficult to investigate precisely because the mucosa is easily damaged by many intrinsic and extrinsic factors.

In conclusion, this study clearly demonstrated that LFS has a marked protective effect on jejunal mucosal damage and may also strengthen the relevance of LFS, our work in phytotherapy[10], and Chinese herbal medicine (called Kampo in Japanese)[11] to conventional medicine. As LFS is not yet a clinically-approved drug, furher experimental and clinical trials are necessary using larger test groups and multiple GI sites.

DISCLOSURE

The authors have declared no conflicts of interest.

REFERENCES

1) Mescher, A. L. (2013). Small Intestine. Junqueira's Basic Histology (Ed. Weitz, M., Kearns, B.), McGraw-Hill Companies inc., New York, pp.309-316.

2) Horiuchi, K., Naito, I., Nakano, K., Nakatani, S., Nishida, K., Ohtsuka, A. (2005). Three-dimensional ultrastructure of the brush border glycocalyx in the mouse small intestine: a high resolution scanning electron microscopy study. Arch. Histol. Cytol. 68: 51-56.

3) Shin, R., Suzuki, M., Mizutani, T., Susa, N. (2009). Improvement of experimentally induced hepatic and renal disorders in rats using lactic acid bacteria-fermented soybean extract (Biofermentics™). Evid. Based. Complement. Altemet. Med. 6: 357-363.

4) Helenius, A., Simons, K. (1975). Solubilization of membranes by detergents. Biochim. Biophys. Acta. 415: 29-79.

5) Singer, S.J., Nicolson, G.L. (1972). The fluid mosaic model of the structure of cell membranes. Science. 175: 720-731.

6) Djaldetti, M., Fishman, P. (1981). The effect of aspirin on mouse small-intestinal mucosa. Arch. Pathol. Lab. Med. 105: 144-147.

7) Lai, K.C., Lam, S.K., Chu, K.M., Wong, B.C., Hui, W.M., Hu, W.H., Lau, G.K., Wong, W.M., Yuen, M.F., Chan, A.O., Lai, C.L., Wong, J. (2002). Lansoprazole for the prevention of recurrences of ulcer complications from long-term

low-dose aspirin use. N. Engl. J. Med. 346: 2033-2038.
8) Schoen. R. T., Vender, R. J. (1989). Mechanisms of non-steroidal anti-inflammatory drug-induced gastric damage. Am. J. Medicine. 86: 449-458.
9) Bhatt, D.L., Scheiman, J., Abraham, N.S.,Antman, E.M., Chan, F.K., Furberg, C.D., Johnson, D.A., Mahaffey, K.W., Quigley, E.M., Harrington, R.A., Bates, E.R., Bridges, C.R., Eisenberg, M.J., Ferrari, V.A., Hlatky, M.A., Kaul, S. Lindner, J.R., Moliterno, D.J., Mukherjee, D., Schofield, R.S., Rosenson, R.S., Stein, J.H., Weitz, H.H., Wesley, D.J.(2008).ACCF/ACG/AHA 2008 expert consensus document on reducing the gastrointestinal risks of antiplatelet therapy and NSAID use. AM. J. Gastroenterol. 103: 2890-2907.
10) Okubo, Y., Hossain, M., Horie, S., Momose, T., Isobe, M., Sekiguchi, M. (1997). Effect of herbal medicine (sho-seiryu-to and ryo-kan-kyo-mi-shin-ge-nin-to) on human eosinophil biological function, Phytotherapy. Res. 11: 485-489.
11) Takeda, H., Sadakane, C., Hattori, T., Katsurada, T., Ohkawara, T., Nagai, K., Asaka, M. (2008). Rikkunshito, an hebal medicine, suppresses cisplatin-induced anorexia in rats via 5-HT2 recepter antagonism. Gastroenterology. 134: 2004-2013.

和文抄録

界面活性剤を含む洗剤（ジョイ、P&G ファー・イースト・インク、神戸）によるマウス空腸の微絨毛障害に対する乳酸菌混合発酵産物（LFS）の保護効果について電子顕微鏡を用いて解析した。8 週齢のマウスを 4 群（各 5 匹）に分けて実験を行った：MF 粉末飼料飼育を基本として①未処置の対照群；②蒸留水 0.5ml 経口投与群；③ 0.1％洗剤 0.5ml 経口投与群；④ 7 週齢から 8 週齢まで 1 ％ LFS を含む MF 粉末飼料飼育後に 0.1％洗剤 0.5ml 経口投与群。経口投与 10 分後、各群の空腸を採取し走査型および透過型電子顕微鏡観察のための常法処理を行った。③ LFS 未飼育群の空腸微絨毛は、①対照群に比べて著しく破壊された像として観察された。一方で④ LFS 飼育群の空腸微絨毛は、①対照群および②蒸留水経口投与群と同様に障害なく正常な像として観察された。以上の結果から、LFS は洗剤によるマウス空腸の微絨毛障害の保護効果を有することが明らかになった。このことから、LFS は非ステロイド性抗炎症薬のような化学薬品に対する腸粘膜の保護効果を有することが示唆された。