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Yogurt Containing *Lactobacillus gasseri* OLL2716 Exerts Gastroprotective Action Agaisnt Acute Gastric Lesion and Antral Ulcer in Rats

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Abstract. Yogurt containing *Lactobacillus gasseri* OLL2716 (LG21 yogurt) is reported to improve *Helicobacter pylori*-induced gastric mucosal inflammation in clinical studies. However, other beneficial effects of LG21 yogurt have not been clarified. Therefore, we examined whether LG21 yogurt exhibits a gastroprotective action against acute gastric lesion or antral ulcer in rats. Moreover, the mechanism of gastroprotective action was also evaluated. After fasting, acute gastric lesions were induced by 0.6 M HCl. Gastric mucosal folds were stained by oral administration of methyl violet. Antral ulcers were induced by the combined administration of diethyldithiocarbamate and HCl in refed rats after fasting. LG21 yogurt was orally administered before HCl treatment or staining the mucosal folds. LG21 yogurt significantly and dose-dependently inhibited the formation of acute gastric lesions, and this gastroprotective action was attenuated by pretreatment with indomethacin. LG21 yogurt also significantly increased prostaglandin E_2 generation in the gastric mucosa. Stained length of gastric mucosal fold was reduced by LG21 yogurt. Antral ulcer formation was also significantly inhibited by LG21 yogurt. From the above results, it was found that the ingestion of LG21 yogurt is useful for the prevention of gastric ulcer. Moreover, endogenous prostaglandin was suggested to be one of the gastroprotective mechanisms of LG21 yogurt.

Keywords: *Lactobacillus gasseri* OLL2716, LG21 yogurt, gastric lesion, antral ulcer (rat)

Introduction

Recently, various functional foods, such as blood pressure or blood glucose lowing foods, have been developed and marketed. On the gastrointestinal function, most of the foods have been reported to be effective for constipation. However, anti-ulcer or ulcer healing foods have not been developed as much.

In 1984, Materia et al. (1) reported the efficacy of milk against stress-induced gastric ulcer in rats and the importance of prostaglandin containing in the milk. On the active substance for gastroprotective action, Malhotra (2) reported the importance of short-chain fatty acids present in milk, yogurt, and other fermented milk products by retarding gall bladder contraction.

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Recently, the yogurt containing *Lactobacillus gasseri* OLL2716 (LG21 yogurt) was reported to be effective against *Helicobacter pylori* and improves the pepsinogen I/II ratio, a marker of gastric mucosal inflammation, in *Helicobacter pylori* positive healthy volunteers (3). However, other beneficial effects of LG21 yogurt on the stomach have not been clarified. Thus, the present study was designed to evaluate the effect of LG21 yogurt on acute gastric lesions induced by HCl in rats. Moreover, the mechanism of the gastroprotective effect by LG21 yogurt was also investigated.

Although most of the human gastric ulcers are reported by Oi et al. (4) to be located in the antrum, most of gastric lesions were induced at the corpus in animals. In 1981, Satoh et al. (5) reported the antral ulcer model in rats by refeeding after fasting. Recently, the present author developed a novel and reliable antral ulcer model by the combined administration of HCl

and diethyldithiocarbamate, a superoxide dismutase inhibitor (6), in refed rats after fasting. We employed this model to evaluate the efficacy of LG21 yogurt in the present study.

Materials and Methods

Animals

Male Sprague-Dawley rats weighing about 200 g was purchased from SLC (Shizuoka) and kept for 1 week in a room whose temperature and humidity were kept at 21 ± 2°C and 55 ± 15%, respectively. Rats were fasted in meshed cages for the indicated time to prevent coprophagy before the each experiment, but allowed free access to drinking water.

The following animal studies were performed according to the "Guiding Principles for the Care and Use of Laboratory Animals" approved by The Japanese Pharmacological Society.

Induction of acute gastric lesions

Acute gastric lesions were induced by the oral administration of 0.6 M HCl in a volume of 5 ml/kg in fasted rats for 18 h. One hour after HCl treatment, the rat was sacrificed after cervical dislocation. The stomach was excised and cut along the greater curvature. Length of lesions was measured and total length of the lesions was treated as the lesion index (mm).

Effects of LG21 yogurt and non-fermented milk on acute gastric lesions

Acute gastric lesions were induced by the oral administration of 0.6 M HCl as mentioned above. LG21 yogurt was administered orally 30 min before HCl treatment at doses of 1.25 – 5 ml/kg. In the control rats, distilled water was administered orally instead of LG21 yogurt.

In another experiment, the effect of non-fermented-milk at a dose of 5 ml/kg was investigated. In the control rats, distilled water was administered orally in a volume of 5 ml/kg instead of non-fermented-milk.

Effects of indomethacin on the gastroprotective action by LG21 yogurt

Acute gastric lesions were induced by the oral administration of 0.6 M HCl as mentioned above. LG21 yogurt was administered orally 30 min before HCl treatment at doses of 1.25 – 5 ml/kg. Indomethacin at a dose of 10 mg/kg (1 ml/kg) was administered subcutaneously 1 h before LG21 yogurt treatment to evaluate the involvement of prostaglandin on the gastroprotective action by LG21 yogurt.

In the indomethacin non-treated group, 1% gum arabic solution was administered subcutaneously instead of indomethacin.

Effect of LG21 yogurt on prostaglandin E_2 generation in the gastric mucosa

Prostaglandin E_2 generation in the gastric mucosa after the LG21 yogurt treatment was determined according to the method reported by Lee and Feldman (7, 8). LG21 yogurt was administered orally at doses of 2.5 ml/kg, which was a significantly effective dose against HCl-induced gastric lesions; and in the control group, distilled water was administered orally instead of LG21 yogurt. After 30 min, the stomach was excised after cervical dislocation and the corpus was cut from the other portions, the antrum and the forestomach. The gastric mucosa was washed in ice-cold 50 mM Tris-HCl (pH 8.4) buffer and minced with scissors. The corpus was washed and re-suspended in 1 ml of buffer and subjected to voltex mixing at room temperature for 1 min to stimulate prostaglandin E_2 generation. After mixing, the tissue samples were centrifuged at 10000× *g* for 15 s. The supernatant was separated from the tissue and prostaglandin E_2 levels in the supernatant were determined by means of an enzyme immunoassay (Prostaglandin E_2 Biotrak Enzymeimmunoassay (EIA) system; Amersham Bioscience, Little Chalfont, Buckinghamshire, UK). Wet weight of gastric mucosa was measured. Prostaglandin E_2 generation was expressed as ng of prostaglandin E_2/min per g tissue.

Staining of gastric mucosal fold

Gastric mucosal folds were stained by the oral administration of methyl violet solution according to the method by Mersereau et al. (9) with slight modification in fasted rats for 18 h. In brief, 1% methyl violet solution was administered orally in a volume of 5 ml/kg. LG21 yogurt was administered orally in a volume of 2.5 ml/kg 30 min before methyl violet treatment. In the control group, distilled water was administered orally in a volume of 2.5 ml/kg instead of LG21 yogurt. One hour after methyl violet treatment, rats were sacrificed after cervical dislocation. The stomach was excised and cut along the greater curvature. The length of the mucosal fold stained by methyl violet was measured. The total length of the stained mucosal folds was calculated.

Induction of antral ulcer

According to the method by Uchida et al. (6), antral ulcers were induced in rats refed for 1 h after 48-h fasting. Diethyldithiocarbamate (250 mg/kg) was administered subcutaneously just after refeeding, and 1 M HCl (5 ml/kg, p.o.) was treated 1 h after refeeding. Rats were sacrificed 4 days after ulcer induction. The stomach was excised and cut along the greater curvature.

The length and width of the antral ulcer was measured and the product of length × width was treated as the ulcer index (mm^2).

Effects of LG21 yogurt and non-fermented milk on antral ulcer

Antral ulcers were induced in rats refed for 1 h after 48-h fasting as mentioned above. LG21 yogurt was administered orally 30 min before HCl-administration at doses of 1.25 and 2.5 ml/kg. In the control group, distilled water was administered instead of LG21 yogurt.

In another experiment, the effect of non-fermented-milk was investigated at a dose of 5 ml/kg. In the control rats, distilled water was administered orally in a volume of 5 ml/kg instead of non-fermented milk.

Preparation of samples

Drugs used were HCl, ethanol, methyl violet (Wako Pure Chemical, Osaka) and indomethacin (Sigma. St. Louis, MO, USA). LG21 yogurt (Meiji Probio Yogurt LG21) was the product of Meiji Dairies Corporation (Tokyo). Non-fermented milk was composed of the raw materials used for the LG21 yogurt except for lactic acid bacteria, *Lactobacillus burgaricus*, *Staphylococcus thermophylus*, and *Lactobacillus gasseri* OLL2716 contained in the LG21 yogurt. Indomethacin was suspended with 1% gum arabic solution.

Statistical analyses

Data were represented as the mean ± S.E.M. for used animals. Statistical analyses were performed by using a two-tailed Dunnett's multiple comparison test or Student's *t*-test, and values of $P<0.05$ were regarded as significant.

Results

Effects of LG21 yogurt and non-fermented milk on acute gastric lesions

Oral administration of HCl induced acute gastric lesions along on the gastric mucosal folds. In the control group, the lesion index was 55.8 ± 8.3 mm (Fig. 1). LG21 yogurt dose-dependently inhibited the acute gastric lesions and significant differences were observed at doses of 2.5 and 5 ml/kg (Fig. 1).

The lesion index in the non-fermented milk-treated group was 72.3 ± 10.2 mm, being not significantly different from the control group (49.0 ± 6.2 mm) (Fig. 2).

Effects of indomethacin on the gastroprotective action by LG21 yogurt

In the indomethacin-non-treated control group, the lesion index was 118 ± 5.7 mm (Fig. 3). LG21 yogurt dose-dependently inhibited the acute gastric lesions and significant differences were observed at doses of 2.5 and 5 ml/kg. In the control group pre-treated with indometahcin, the lesion index was 126 ± 7.0 mm, being not significant from that in indomethacin-non-treated control group (Fig. 3). The gastroprotective action by LG21 yogurt was attenuated, and no significant difference was observed even at a dose of 5 ml/kg, which was significantly effective in the case of indometahcin non-treatment. A significant difference was observed

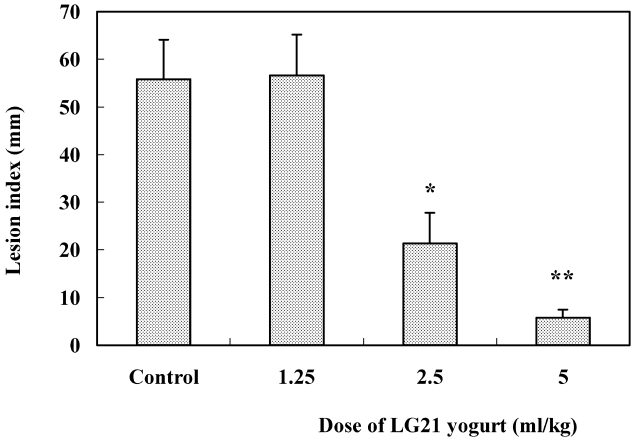

Fig. 1. Effect of LG21 yogurt on HCl-induced acute gastric lesion in rats. Acute gastric lesions were induced by the oral administration of 0.6 M HCl. One hour after HCl treatment, rats were sacrificed. LG21 yogurt was administered orally 30 min before HCl-treatment. In the control group, distilled water was administered orally instead of LG21 yogurt. Values represent the mean ± S.E.M. of 5 rats. *, **Significant difference from the control group at $P<0.05$, 0.01.

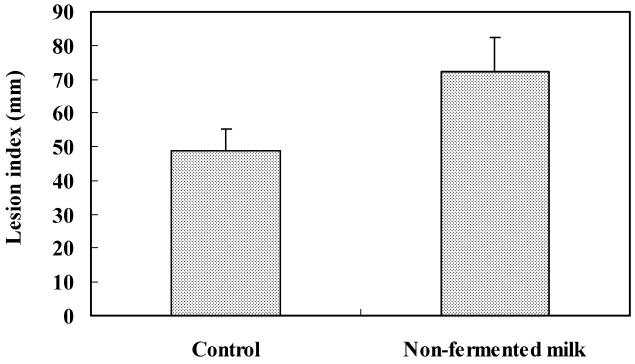

Fig. 2. Effect of non-fermented milk on HCl-induced acute gastric lesion in rats. Acute gastric lesions were induced by the oral administration of 0.6 M HCl. One hour after HCl treatment, the rat was sacrificed. Non-fermented milk was administered orally 30 min before HCl-treatment. In the control group, distilled water was administered orally instead of non-fermented milk. Values represent the mean ± S.E.M. of 5 rats.

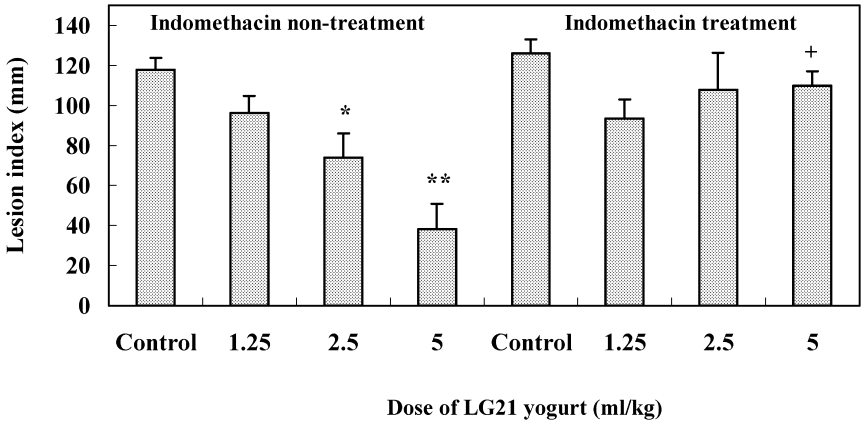

Fig. 3. Effect of indomethacin on the gastroprotective effect by LG21 yogurt against HCl-induced acute gastric lesions in rats. Acute gastric lesions were induced by the oral administration of 0.6 M HCl. One hour after HCl treatment, the rat was sacrificed. LG21 yogurt was administered orally 30 min before HCl-treatment. In the control group, distilled water was administered orally instead of LG21 yogurt. Indomethacin (10 mg/kg) was administered subcutaneously 1 h before HCl-treatment. Values represent the mean ± S.E.M. of 5 rats. *, **Significant difference from the control group at $P<0.05$ or $P<0.01$. +Significant difference from LG21 yogurt (5 ml/kg)-treated group in indomethacin non-treated rats at $P<0.05$.

between the indomethacin non-treated group and indomethacin-treated group at a dose of 5 ml/kg of LG21 yogurt ($P<0.05$).

Effect of LG21 yogurt on prostaglandin E_2 generation in the gastric mucosa

In the control group, prostaglandin E_2 generation in the gastric mucosa was 2.85 ± 0.08 ng/min per g tissue. LG21 yogurt significantly increased the prostaglandin E_2 generation ($P<0.01$) and its value was 4.28 ± 0.07 ng/min per g tissue.

Staining of gastric mucosal fold

In the control group, the length of mucosal fold stained by methyl violet was 161 ± 12.6 mm (Figs. 4 and 5). By the treatment with LG21 yogurt, the length of the stained mucosal fold was significantly reduced ($P<0.05$) (Figs. 4 and 5).

Effects of LG21 yogurt and non-fermented milk on antral ulcer

In the control group, ulcer was induced at the antrum and the ulcer index was 75.0 ± 21.3 mm^2 (Figs. 6 and 7). In the LG21 yogurt-treated group, ulcer indexes at doses of 1.25, 2.5, and 5 ml/kg were 41.8 ± 16.3, 13.0 ± 6.3, and 9.0 ± 7.9 mm^2, respectively. Significant differences were observed at both doses of 2.5 and 5 ml/kg ($P<0.05$) (Figs. 6 and 7).

Ulcer index in the non-fermented milk-treated group was 37.6 ± 12.6 mm^2, being not significantly different from the control group (61.5 ± 19.6 mm^2) even at a dose of 5 ml/kg, which was significantly effective in the case of LG21 yogurt (Fig. 8).

Discussion

Milk has been ingested as dairy foods and recommended as therapy for patients with peptic ulcer. Dial et al. reported that milk has a potent anti-ulcer activity against 0.6 M HCl-induced acute gastric lesions (10) and that surface-active lipids contained in milk, such as phospholipids, may account for a significant portion of milk's antiulcer activity by maintaining the hydrophobicity of the luminal surface of the gastric mucosa in the presence of a damaging agent (11). In 1984, Materia et al. (1) found that prostaglandins may be responsible for the presumed beneficial effects of milk in the prevention and treatment of peptic ulcer disease by using rat cold-restraint stress ulcer model. Moreover, Koo (12) reported that milk could be beneficial for the healing of gastric mucosal lesions, and this may be due to the membrane stabilization and to the cellular restitution by calcium in milk. On the contrary, Ippoliti et al. (13) reported that there is question for the frequent ingestion of milk by patients with peptic ulcer because milk contains both protein and calcium that stimulate gastric acid secretion. However, milk has been generally regarded to be effective for ulcer healing.

In the present study, LG21 yogurt dose-dependently inhibited acute gastric lesions induced by HCl. However, non-fermented milk, which is composed of the same raw materials except for lactic acid bacteria, did not inhibit but slightly aggravated HCl-induced acute

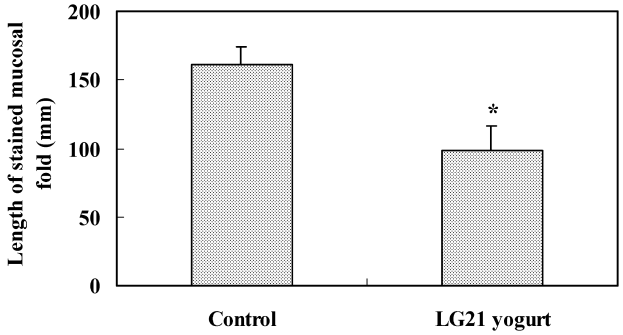

Fig. 4. Effect of LG21 yogurt on the length of gastric mucosal fold stained by methyl violet in rats. Methyl violet solution of 1% was administered orally. LG21 yogurt was administered orally 30 min before methyl violet treatment. In the control group, distilled water was administered orally instead of methyl violet. One hour after methyl violet treatment, the rat was sacrificed. Values represent the mean ± S.E.M. of 4 rats. *Significant difference from the control group at $P<0.05$.

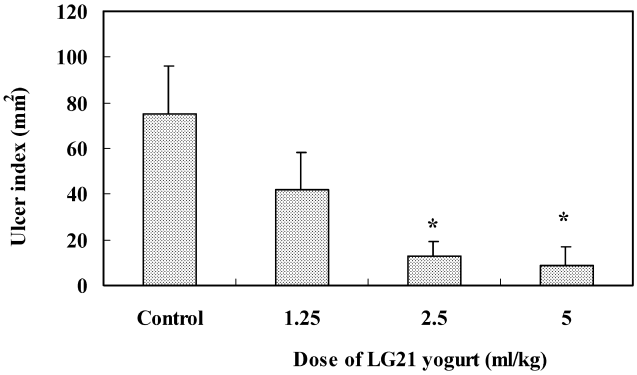

Fig. 6. Effect of LG21 yogurt on the antral ulcer in rats. Antral ulcers were induced in rats refed for 1 h after 48-h fasting. Diethyldithiocarbamate (250 mg/kg) was administered subcutaneously just after refeeding, and 1 M HCl (5 ml/kg, p.o.) was treated 1 h after refeeding. LG21 yogurt was administered orally 30 min before HCl-treatment. In the control group, distilled water was administered orally instead of LG21 yogurt. Rats were sacrificed 4 days after ulcer induction. Values represent the mean ± S.E.M. of 5 rats. *Significant difference from the control group at $P<0.05$.

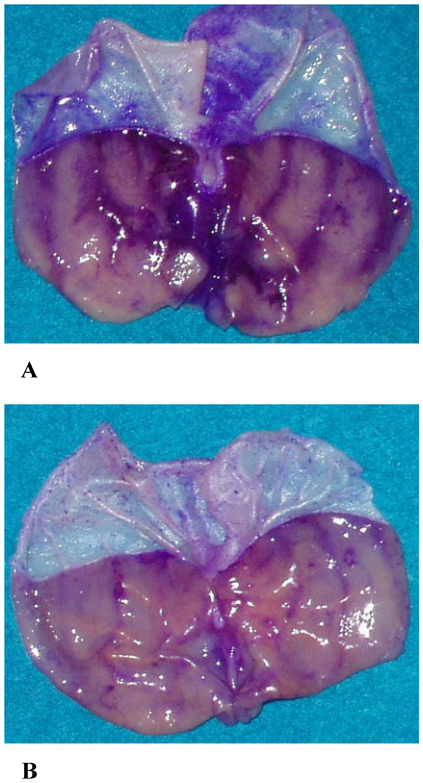

Fig. 5. Macroscopical findings of the gastric mucosal folds stained by methyl violet in rats. Methyl violet solution (1%) was administered orally. LG21 yogurt was administered orally 30 min before methyl violet treatment. In the control group, distilled water was administered orally instead of LG21 yogurt. One hour after methyl violet treatment, the rat was sacrificed. A: Control rat: Gastric mucosal folds were stained clearly. B: LG21 yogurt-treated rat: Stained gastric mucosal folds were reduced and lighter than those of the control rat.

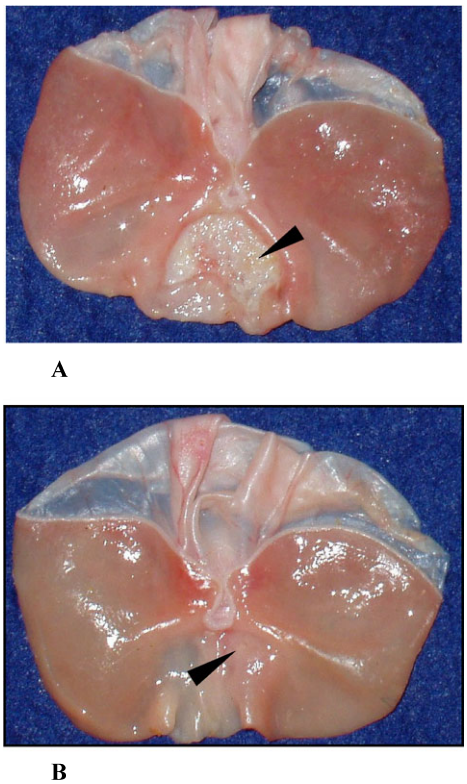

Fig. 7. Macroscopical findings of the antral ulcer in rats. Antral ulcers were induced in rats refed for 1 h after 48-h fasting. Diethyldithiocarbamate (250 mg/kg) was administered subcutaneously just after refeeding, and 1 M HCl (5 ml/kg, p.o.) was treated 1 h after refeeding. LG21 yogurt was administered orally 30 min before HCl-treatment. In a control rat, distilled water was administered orally instead of LG21 yogurt. Rats were sacrificed 4 days after ulcer induction. A: Control rat: A large and clear ulcer (arrow) was induced in the antrum. B: LG21 yogurt-treated rat: Size of the antral ulcer was markedly smaller (arrow) than that of the control rat.

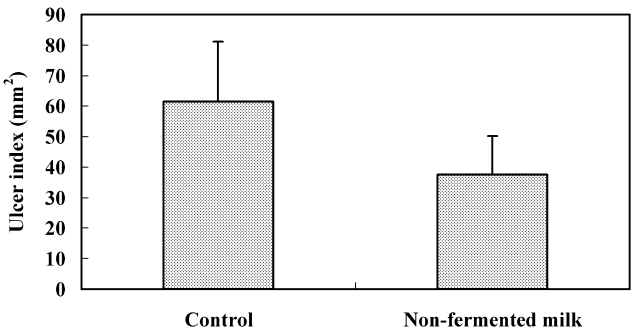

Fig. 8. Effect of non-fermented milk on the antral ulcer in rats. Antral ulcers were induced in rats refed for 1 h after 48-h fasting. Diethyldithiocarbamate (250 mg/kg) was administered subcutaneously just after refeeding, and 1 M HCl (5 ml/kg, p.o.) was treated 1 h after refeeding. Non-fermented milk was administered orally 30 min before HCl-treatment. In the control group, distilled water was administered orally instead of non-fermented milk. Rats were sacrificed 4 days after ulcer induction. Values represent the mean ± S.E.M. of 5 or 6 rats.

gastric lesions at a dose of 5 ml/kg, although a significant difference was not observed, suggesting non-fermented milk does not play an important role in the gastroprotective action by LG21 yogurt. However, Dial et al. reported that milk has a potent anti-ulcer activity against 0.6 M HCl-induced acute gastric lesions (10). This difference may be caused by a difference between milk and non-fermented milk. However, at a dose of 20 ml/kg, milk alone significantly inhibited HCl-induced acute gastric lesions (data not shown).

There have been few reports about the fermented milk such as yogurt, except for that the relative dietary preponderance of short-chain fatty acids has a protective action (14). In the present study, LG21 yogurt showed a dose-dependent gastroroprotective effect against 0.6 M HCl-induced acute gastric lesions, and this gastroprotective action was attenuated by the pre-treatment with indomethacin, suggesting the involvement of prostaglandin in the gastroprotective mechanism by LG21 yogurt. The generation of prostaglandin E_2 in the gastric mucosa was increased indeed by the oral administration of LG21 yogurt. Prostaglandins, such as prostaglandin E_2 or prostaglandin I_2, have been known to inhibit acute gastric lesions through the increase of gastric mucosal blood flow or bicarbonate secretion. Materia et al. (1) reported the importance of prostaglandin contained in the milk against stress-induced gastric ulcer in rats. However, considering that non-fermented milk did not inhibit acute gastric lesion formation in the present study, prostaglandin contained in the milk was suggested not to be concerned in the gastroprotective effect by LG21.

Endogenous prostaglandin has been known to increase by the treatment with a mild irritant, such as 0.2 M HCl or 20% ethanol. This increase in prostaglandin contents in the gastric mucosa plays an important role in the gastroroprotective effect against lesions caused by administration of 0.6 M HCl or absolute ethanol (15). LG21 yogurt contains lactic acid and its concentration is about 1%. However, this concentration of lactic acid did not inhibit the lesion formation in the present model (data not shown). Therefore, other components except for lactic acid were supposed to increase prostaglandin contents in the gastric mucosa. Further experiments would be needed to identify the active substance contained in LG21 yogurt.

Tekeuchi et al. (16) found that 16,16-dimethyl-prostaglandin E_2 significantly prevented the localized staining pattern seen in intact rats, suggesting distention of gastric mucosa. In this study, the length of the gastric mucosal fold stained by methyl violet was significantly reduced by the administration of LG21 yogurt. This may be caused by endogenous prostaglandin generated by LG21 yogurt treatment.

There are some reports on the anti-ulcer effect by *Lactobacilli*. Elliott et al. (17) reported that suppression of colonization of gram-negative bacteria with antibiotics resulted in marked acceleration of ulcer healing and that induction of *Lactobacillus* colonization showed marked acceleration of ulcer healing induced by acetic acid in rats. Uejima et al. (18) founded that *Lactobacillus* inhibited non-steroidal anti-inflammatory drug-induced ileal ulcer in rats by repressing the growth of ulcer-inducing bacteria, the Gram-negative facultative anaerobic rods *Esherichia coli*, *Klebsiella*, and *Proteus*, although the ulcer models were different than the models used in the present study or Elliott's model. On the other hand, Nagaoka et al. (19) reported that polysaccharide of *Lactobacillus* cell wall had a gastroprotective effect and the content of rhamnose was important. Indeed, in this study, non-fermented milk did not inhibit the lesion formation. Therefore, the polysaccharide of *Lactobacillus* may be involved the gastroprotective action. LG21 yogurt used in this study contains *Lactobacillus gasseri* OLL2716, *Lactobacillus blugaricus*, and *Streptococcus thermophylus*. Recently, *Lactobacillus gasseri* OLL2716 was found to inhibit the generation of the inflammatory cytokine interleukin-8 (IL-8) in an in vitro study (20). There have been many reports on the pathogenic role of IL-8 in gastric lesions or ulcers. Therefore, LG21 yogurt may inhibit acute gastric lesions or ulcers through the inhibition of IL-8 generation as one of the gastroprotective mechanisms. However, to clarify the involvement of IL-8 in the gastroprotective action by LG21 yogurt, further investigations would be needed.

Oi et al. (4) reported that most of the gastric ulcers are located in the antrum, although most experimental gastric ulcers were induced in the corpus in animals. Satoh et al. (5) reported the antral ulcer model in rats by refeeding after fasting. Maeda-Hagiwara and Watanabe (21) also reported the antral ulcer model by the combined administration of indomethacin with 2-deoxy-D-glucose, a centrally acting gastric acid secretagogue. The present author recently reported a novel method to produce extensive gastric antral ulcer by the combined administration of HCl and diethyldithiocarbamate in refed rats. As yet, we reported the involvement of etiologic factors, such as oxygen-derived radicals, especially superoxide anion and endogenous acid secretion (6). Moreover, diethyldithiocarbamate was found to decrease gastric mucosal blood flow in the antrum. In this study, the pretreatment with LG21 yogurt significantly inhibited the antral ulcer formation in a dose-dependent manner. On the inhibitory mechanism, the increase of prostagalandin may be involved because a prostaglandin, such as prostagalandin E_2 or prostaglandin I_2, has been known to increase gastric mucosal blood flow and accelerate the healing of ulcer. Recently the present author reported the relationship between nitric oxide and prostglandins (22). Therefore it is of interest to test the effect of nitric oxide synthase inhibitor on the gastroprotective effect by LG21 yogurt.

In conclusion, LG21 yogurt inhibited the acute gastric lesions through prostaglandin and also the antral ulcer. These findings may indicate that LG21 yogurt is a useful food for the prevention of gastric ulcer.

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