Dietary Fermentable Fiber Reduces Intestinal Barrier Defects and Inflammation in Colitic Mice

Tran Van Hung and Takuya Suzuki

Department of Biofunctional Science and Technology, Graduate School of Biosphere Science, Hiroshima University, Higashi-Hiroshima, Japan

Abstract

**Background:** Dietary fiber (DF) and its fermentation metabolites play an important role in establishing and maintaining intestinal health.

**Objective:** This study investigated the effects of fermentable DF, guar gum (GG), and partially hydrolyzed GG (PHGG) on the epithelial tight junction (TJ) barrier and inflammation in a murine model of dextran sodium sulfate (DSS)–induced colitis.

**Methods:** In Expt. 1, male, 7-wk-old BALB/c mice weighing ~21 g were fed diets with 0%, 5%, and 10% GG for 12 d and administered distilled water with 2% DSS for 7 d beginning 5 d after the start of feeding. In Expt. 2, mice were provided diets with or without 10% PHGG and GG for 13 d and administered distilled water with 2% DSS for 8 d from 5 d after the start of feeding. In Expt. 3, mice were provided diets with or without 10% PHGG and GG for 14 d without DSS administration. Colitis score, colon TJ proteins, and fecal SCFA concentrations were analyzed.

**Results:** In Expts. 1 and 2, the clinical score in the DSS group was ~100% greater than that in the DSS+10% GG and PHGG groups on days 12 and 13 (P < 0.01). The DSS+10% GG and PHGG groups showed ~110%, 60%, 120%, and ~110% greater (P < 0.05) expression of occludin and claudin 3, 4, and 7, respectively, in the colon than did the DSS group. The DSS+10% GG and PHGG groups had greater total fecal SCFA concentrations (25.1 and 12.0 mmol/L) than did the DSS group (3.3 mmol/L) on day 9 (P < 0.01). TJ protein expression did not differ between groups in Expt. 3.

**Conclusion:** These findings suggest that microbial metabolites of PHGG and GG, and possibly SCFAs, reduce intestinal barrier defects and inflammation in colitic mice.

**Keywords:** inflammatory bowel disease, dietary fiber, guar gum, tight junction, short-chain fatty acid

Introduction

Dietary fiber (DF) constitutes a diverse group of compounds that are resistant to digestion in the human small intestine and can be categorized as water soluble [soluble dietary fiber (SDF)] and water insoluble (insoluble dietary fiber). Consuming DF produces different physiologic effects on our health based on the physiochemical properties of the ingested fiber (1–5). Guar gum (GG) is found in guar seeds, and its main component is galactomannan, which is characterized by high viscosity. Previous studies have demonstrated that incorporating GG in the diet provides different physiologic health benefits such as improved glucose tolerance (6, 7). Besides the original GG, a partially hydrolyzed guar gum (PHGG) has been developed through controlled partial enzymatic hydrolysis of GG, and is favored in food manufacturing because of its smaller molecular weight and lower viscosity. Furthermore, PHGG often is used to avoid the effects of viscosity in GG-mediated physiologic functions (1, 8, 9).

Much of ingested SDF is readily fermented by colonic bacteria to produce organic acids including SCFAs. These organic acids promote colonic epithelial cell proliferation, mucosal blood flow, and colonic motility. In particular, butyrate is considered to be a major fuel source for colonocytes (10, 11) and has the potential to exclude mutated epithelial cells through apoptosis induction. These activities contribute to the maintenance of colonic homeostasis. Furthermore, studies have demonstrated that organic acids, particularly SCFAs, participate in the regulation of the intestinal barrier (9, 12).
The intestinal barrier is essential for maintaining a beneficial relation between the host and intestinal microbes, because a number of commensals and possibly some pathogenic bacteria colonize the lumen and produce proinflammatory substances. Intercellular tight junctions (TJs) are located at the apical side of the lateral membrane of epithelial cells and constitute the major determinant of intestinal barrier integrity (13, 14), suggesting that TJs play a critical role in intestinal defense, as well as in the maintenance of intestinal homeostasis (15, 16). The TJ complex consists of multiple proteins, including both transmembrane and cytosolic proteins, such as occludin, the claudin family, and zona occludens (ZO). Junctional adhesion molecule A (JAMA), an adherens junction (AJ) protein, also plays an important role in the regulation and maintenance of the TJ barrier (13, 14). SCFAs such as acetic and propionic acids are reported to enhance TJ barrier integrity in the rat colon and intestinal cells. N-butyric acid also seems to promote TJ protein assembly through an AMPK-dependent mechanism (17).

Because of its essential role in intestinal homeostasis, TJ barrier dysfunction is involved in the initiation and development of a number of diseases, including inflammatory bowel diseases (IBDs). IBD is the term applied to a group of disorders characterized by intestinal inflammation, such as Crohn disease and ulcerative colitis (UC) (18, 19). Patients with IBD suffer from diarrhea, rectal bleeding, abdominal pain, and cramps for long periods, with usually recurring inflammation (20). Although the etiology of IBD remains unclear, it is suggested that impairment of the TJ barrier, as well as the subsequent penetration of luminal proinflammatory substances into the intestinal mucosa, is involved in IBD pathogenesis. Indeed, colon biopsies from patients with UC demonstrate decreased expression and distribution of occludin, claudin 1, claudin 4, tricellulin, and JAMA (15, 21–23). Meanwhile, it is interesting to note that SCFA concentrations in the colonic lumen of patients with UC are lower than those in normal subjects (24, 25), as well as in the cotton-top tamarin model of idiopathic colitis (26). Theoretically, the decreased SCFA concentration could be attributed to a lower DF intake, resulting in alterations in the colonic flora and in the relation between bacteria and colonocytes, leading to colonic inflammation (27). On the basis of these findings, supplementation with fermentable DF such as GG could be a potential preventive and therapeutic tool for IBDs (3, 4, 25, 28).

Dextran sodium sulfate (DSS)–induced colitis in mice is known to mimic many of the morphologic and pathophysiologic features observed in human IBDs, such as mucosal injury, ulceration, diarrhea, impaired barrier function, and inflammatory cytokine production (29–31). In previous studies, the effects of some DF were examined on DSS-induced colitis in mice (3, 4, 28, 32); however, their effect on the intestinal barrier has not been investigated. Furthermore, the influence of hydrolysis on the effects of highly viscous DF remains completely unknown.

The purpose of the present study was to investigate the effects of fermentable GG on intestinal barrier function and on inflammation in a murine model of DSS-induced colitis. Furthermore, PHGG, which is characterized by lower molecular weight and viscosity, also was used to highlight the involvement of microbial fermentability of fiber and its metabolites, such as SCFAs, in the prevention of colitis.

**Methods**

**Chemicals.** DSS (molecular weight 36,000–50,000) was purchased from MP Biomedicals. Rabbit anti-ZO1, –ZO2, -occludin, -claudin 3, -claudin 4, -claudin 7, and -JAMA and goat Alexa Flour 488–conjugated anti-rabbit IgG were purchased from Life Technologies. HRP-conjugated anti-rabbit IgG was purchased from Sigma. GG and PHGG were kindly provided by Taiyo Kagaku. All other chemicals were obtained from Wako Pure Chemical Industries.

**Mice.** All study protocols were preapproved by the Animal Use Committee of the Hiroshima University and all mice were maintained in accordance with the guidelines for the care and use of laboratory animals of the Hiroshima University.

Male BALB/c mice aged 7 wk and weighing ~21 g were obtained from Charles River and were housed under conditions with controlled temperature (22°C ± 2°C), relative humidity (40–60%), and lighting (light: 0800–2000) throughout the study. The mice were allowed to acclimatize to the laboratory environment with free access to an AIN-93G formula control diet (33) and distilled water for 1 wk before the start of the experiments.

**Experiment design.** In Expt. 1, mice (n = 24) were randomly allocated to 4 groups: control, DSS, DSS+5% GG, and DSS+10% GG (n = 6/group). The DSS+5% GG and DSS+10% GG groups were provided with diets containing 5% and 10% GG by weight, respectively, until the end of the experiment. GG was added to the control diet by substituting for an equal amount of starch. The control and DSS groups were provided with the control diet. Five days after the start of feeding, the DSS, DSS+5% GG, and DSS+10% GG groups received 2% (wt:vol) DSS in drinking water for 7 d, whereas the control mice received distilled water alone. The clinical score for colitis was evaluated every day after the start of DSS treatment as described below. At the end of the experiment, blood was collected from the abdominal vein under ether anesthesia to measure the LPS-binding protein (LBP), and the mice were killed by exsanguination. Plasma LBP was measured with the use of a commercially available ELISA kit (Biometec). Colons were dissected and their length was measured. Colon tissues were subjected to immunoblot and myeloperoxidase activity analyses as described below.

In Expt. 2, mice (n = 24) were randomly allocated to 4 groups: control, DSS, DSS+10% PHGG, and DSS+10% GG (n = 6/group). The PHGG and DSS+10% GG groups were provided with diets containing 10% PHGG and 10% GG, respectively, until the end of the experiment. The control and DSS groups were provided with the control diet. Five days after the start of feeding, the DSS, DSS+10% PHGG, and DSS+10% GG groups received 2% (wt:vol) DSS in drinking water for 8 d, whereas the control mice received distilled water alone. The clinical score for colitis was evaluated every day after the start of DSS treatment as described below. Fresh fecal samples were collected from the mice 1 d before and 4 d after the start of DSS treatment, and were immediately stored at ~80°C for organic acid analysis as described below. At the end of the experiment, blood and colon tissues were collected as described for Expt. 1. The colon tissues were subjected to myeloperoxidase activity, immunoblot, immunofluorescence, and qRT-PCR analyses as described below.

In Expt. 3, mice (n = 18) were randomly allocated to 3 groups: control, 10% PHGG, and 10% GG (n = 6/group). The 10% PHGG and 10% GG groups were provided with the control diet containing 10% PHGG and 10% GG, respectively, until the end of the experiment. The control group was provided with the control diet. Fourteen days after the start of feeding, blood and colon tissues were collected as described for Expts. 1 and 2. The colons were subjected to immunoblot analysis as described below.

**Evaluation of the clinical score for colitis.** To assess the severity of colitis, the clinical score was determined on the basis of a standard scoring system, described previously (31, 34). In brief, the clinical score was calculated as the sum of scores for diarrhea, bloody stools, and weight loss (Supplemental Table 1).

**Measurement of myeloperoxidase activity.** The myeloperoxidase activity in colonic tissues was determined by a standard enzymatic...
than that in the DSS group. Body weight gain in the DSS group was lower than that in the DSS+10% GG groups at and after 4 d (P < 0.01; Figure 1B). The clinical scores for the DSS+10% GG groups were higher than those for the DSS group on and after day 4 (P < 0.05).

It is known that shortening of the colon in mice is correlated with histologic changes, and colon length is often used as a morphologic marker for degree of inflammation (34). DSS administration decreased colon length in mice, and feeding GG partially prevented this decrease in a dose-dependent manner. The colon length in the DSS group was shorter than that in the control and DSS+10% GG groups (P < 0.01; Figure 1C).

Development of DSS-induced colitis is characterized by neutrophil accumulation in the colonic mucosa with high myeloperoxidase expression (19, 31, 34). The level of colonic myeloperoxidase activity was markedly increased by DSS administration and the activity in the DSS group was higher than that in the control group (P = 0.03; Figure 1D). Feeding GG suppressed the increased myeloperoxidase activity in a dose-dependent manner, and myeloperoxidase activity in the DSS+10% GG group was lower than that in the DSS group, indicating that feeding GG reduced neutrophil infiltration into the colonic mucosa.

LBP is a major transporter of proinflammatory LPS in the plasma (38, 39). Because barrier impairment in the colon allows penetration of bacterial LPS from the lumen into the circulatory system, plasma LBP concentration often is used as an indicator of intestinal barrier integrity. Plasma LBP in the DSS group was higher than that in other groups (P < 0.01; Figure 1E). No differences were observed in plasma LBP concentrations between the control, DSS+5% GG, and DSS+10% GG groups.

Effect of GG on barrier function (Expt. 1). TJ's are located at the intercellular junctions of epithelial cells and play a critical role in intestinal barrier integrity (13–15, 22). The concentrations of ZO1, ZO2, occludin, claudin 3, claudin 4, and claudin 7 in the colon were lower in the DSS group than those in the other groups (P < 0.01 at all time points), as shown in Figure 2. The expression of ZO1, occludin, JAMA, claudin 3, claudin 4, and claudin 7 proteins in the DSS+10% GG group, but not those in the DSS+5% GG group, were higher than those in the DSS group (P < 0.01), indicating that feeding the 10% GG diet protected the TJ barrier.

Effects of PHGG and GG administration on weight gain, clinical score, colon length, colon myeloperoxidase activity, and plasma LBP (Expt. 2). GG is an SDF characterized by high viscosity and high fermentability (8, 9). To examine the physiochemical properties participating in the GG-mediated amelioration of experimental colitis, Expt. 2 used a PHGG that is characterized by low viscosity and high fermentability. The DSS group showed lower gains in body weight, as observed in Expt. 1, with the value in the DSS group being lower than that in the control group at and after 5 d (Figure 3A). Feeding PHGG and GG suppressed DSS-induced body weight loss in a similar manner, and the body weight gains in the DSS+10% PHGG and DSS+10% GG groups were higher than those in the DSS group at and after 7 d (P < 0.05 at all time points).

Suppression of the clinical score for colitis upon feeding PHGG and GG was observed to follow a similar trend to that of body weight gain. The clinical score for the DSS+10% PHGG group at and after 6 d and that for the DSS+10% GG group at and after 5 d were lower than that in the DSS group (P < 0.01; Figure 3B).

Colon length was lower in the DSS group than that in the other groups (Figure 3C). Feeding PHGG and GG improved the
DSS-induced decrease in colon length, and colon lengths in the DSS+10% PHGG and DSS+10% GG groups were higher than that in the DSS group ($P < 0.01$).

The myeloperoxidase activity level in the DSS group was higher than that in the control group ($P < 0.01$), whereas that in the DSS+10% GG group was lower than that in the DSS group ($P < 0.01$), as shown in Figure 3D. The level of myeloperoxidase activity in the DSS+10% PHGG group did not differ significantly from that in the DSS+10% GG and DSS groups.

Feeding both PHGG and GG suppressed the increase in plasma LBP concentration that was induced by DSS administration (Figure 3E), and the plasma LBP concentrations in the DSS+10% PHGG ($P < 0.01$) and DSS+10% GG ($P = 0.02$) groups were lower than those in the DSS group, indicating protection of the colon barrier.

**Effects of PHGG and GG administration on barrier function (Expt. 2).** The expression levels of ZO1, ZO2, occludin, JAMA, claudin 3, claudin 4, and claudin 7 were lower in the DSS group than they were in the control group ($P < 0.01$), whereas that in the DSS+10% GG group was lower than that in the DSS group ($P < 0.01$), as shown in Figure 4. Feeding PHGG and GG restored the decreased expression of TJ and AJ proteins that was induced by DSS administration, and the expression of JAMA (an AJ protein) and TJ proteins in the DSS+10% PHGG and DSS+10% GG groups was higher than that in the DSS group ($P < 0.01$).

**Effects of PHGG and GG administration on the intercellular localization of TJ proteins in the colon (Expt. 2).** The intercellular localization and expression of the TJ proteins ZO1, occludin, claudin 3, and claudin 7 in the colons were visualized by immunofluorescence (Figure 5). TJ proteins were observed in the epithelial cells of colons, demonstrating different patterns of localization and expression.
expression and localization throughout the crypts in control mice. ZO1 was assembled at the lateral membrane close to the apical side throughout the crypts. Occludin and claudin 3 were highly expressed in the intercellular junction of the epithelial cells located on the luminal surface and in the upper crypts. Claudin 7 was observed in the junctional region of epithelial cells throughout the crypts. In addition, claudin 3 and claudin 7 also were observed in the basolateral membrane of the epithelial cells. DSS administration severely impaired the expression and localization of these TJ proteins. Furthermore, impaired crypt structures also were observed in the colons of mice in the DSS group. The colons of mice in the DSS+10% PHGG and DSS+10% GG groups showed relatively intact expression and localization of TJ proteins in comparison with those in the DSS group. Feeding PHGG and GG also partially prevented the impairment of colonic crypt structures. These results were consistent with those of the immunoblot analysis of TJ proteins in Figure 4.

**Effects of PHGG and GG administration on proinflammatory and anti-inflammatory cytokine expression in the colon (Expt. 2).** Feeding PHGG and GG for 5 d before DSS administration increased the fecal concentrations of some organic acids. Fecal concentrations of acetic, propionic, and n-butyric acids in the DSS+10% PHGG and DSS+10% GG groups were higher than those in the control and DSS groups on day 0 (before the start of DSS administration, \( P < 0.05 \); Figure 7A and B). Lactic and succinic acid concentrations in the DSS+10% GG group were higher than those in the control and DSS groups (\( P < 0.01 \)), even though the increases observed by feeding PHGG were not statistically significant (\( P = 0.06 \)). Total SCFAs in the DSS+10% PHGG and DSS+10% GG groups were higher than those in the other groups, and the value in the DSS+10% GG was even higher than that in the DSS+10% PHGG group on day 0 (Figure 7C). DSS administration for 4 d decreased the fecal concentrations of propionic, n-butyric, isobutyric, isovaleric acids, and those of total SCFAs, and these values in the DSS group were lower than those in the control group (Figure 7D and E). Feeding PHGG and GG restored the decreased concentrations of propionic, n-butyric, isobutyric, isovaleric acids and those of total SCFAs, and these values in the DSS group were lower than those in the control group (Figure 7D and E). Feeding PHGG and GG restored the decreased concentrations of propionic, n-butyric, and isobutyric acids, and these concentrations in the DSS+10% PHGG and DSS+10% GG groups were higher than those in the DSS group on day 4 (\( P < 0.01 \)). The concentrations of acetic, propionic, n-butyric, lactic, and succinic acids in the DSS+10% GG group were even higher than those in the DSS+10% PHGG group. Along with these changes in individual SCFA concentrations, the decrease in the total concentration of SCFAs induced by DSS administration was restored upon feeding PHGG and GG on day 4, and the values in the DSS+10% PHGG and DSS+10% GG groups were higher than those in the DSS group (\( P < 0.01 \); Figure 7F).
Effect of PHGG and GG on colon length and barrier function in normal mice (Expt. 3). The effects of PHGG and GG on colon length and TJ protein expression were investigated in normal mice. The colon lengths of mice in the 10% GG group were higher than those in the control group \( (P < 0.01) \), whereas those in the 10% PHGG group did not differ significantly from those in the control and 10% GG groups \( (5.4 \pm 0.17, 5.9 \pm 0.09, \text{and} \ 5.9 \pm 0.14 \text{ cm in the control, 10% PHGG, and 10% GG groups, respectively}) \).

No differences were observed in the expression levels of JAMA and any of the TJ proteins evaluated between the control, 10% PHGG, and 10% GG groups (Supplemental Figure 1). Claudin 7 expression in the 10% GG and 10% PHGG groups tended to be higher than that in the control group \( (P = 0.06) \).

**Discussion**

A growing body of evidence suggests that impairment of the intestinal barrier has a critical role in the initiation and development of IBDs, including UC \((2, 21, 23, 29)\). The present results demonstrated that fermentable types of DF such as GG and PHGG had ameliorative effects on intestinal barrier defects and inflammation in a murine model of colitis. Although the preventive effects of other types of DF on colitis have been reported, their roles in regulating the intestinal barrier remain unclear. Our results suggest that fermentable DF-mediated protection of the intestinal barrier can provide a preventive approach to IBDs.

The results of Expt. 1 clearly showed that feeding fermentable GG suppressed the development of DSS-induced colitis and...
intestinal barrier defects. Although the pathogenesis of IBDs remains unclear, protection of the intestinal barrier as indicated by decreased plasma LBP and restored TJ protein expression seems to be involved at least partly in the GG-mediated prevention of colitis. Previous studies have shown that TJ strand count and meshwork are reduced in colon biopsies from UC patients (29). The decreased expression and redistribution of TJ proteins such as claudins and occludin also have been observed in the colons of patients with UC (22, 23). These findings suggest that the microbial fermentability of GG plays a role in the protection of the intestinal barrier. However, GG also is characterized by high viscosity, and this property of SDF sometimes provides a beneficial effect against diseases such as diabetes and obesity. To examine whether the viscosity of GG contributes to the protection of the colon from DSS-induced colitis, PHGG produced by the controlled partial enzymatic hydrolysis of GG, which is characterized by low viscosity and high fermentability (1, 8, 11), was used in Expt. 2. The results revealed that dietary supplementation with GG and PHGG suppressed the intestinal barrier defects and clinical symptoms of colitis in a similar manner, suggesting that high fermentability rather than high viscosity of GG is involved in the prevention of colitis. Importantly, feeding PHGG and GG also protected the intestinal barrier in a similar manner, as indicated by plasma LBP concentrations and the colonic expression of TJ proteins. Otherwise, feeding PHGG and GG did not have any great impact on TJ proteins and JAMA (an A protein) expression in normal mice.

Our study demonstrated that PHGG and GG administration increased the production of fecal organic acids both before and during DSS administration. This finding led us to propose that these organic acids are important mediators in the protective effects of GG and PHGG. A number of studies have highlighted the beneficial effects of organic acids, particularly SCFAs such as acetic, propionic, and n-butyric acids produced through microbial fermentation on human health, although their roles in intestinal barrier regulation and colitis prevention are poorly understood. Suzuki et al. (12) demonstrated that SCFAs, especially acetic and propionic acids, enhance the intestinal TJ barrier integrity in rat colons and cultured intestinal cells. Furthermore, n-butyric acid seems to possess the potential to recover and promote intestinal TJ barrier integrity (10, 11). These findings suggest that organic acids, including SCFAs, produced from the microbial fermentation of GG and PHGG in the colon protect the intestinal TJ barrier, resulting in the suppression of inflammatory immune activation. On the other hand, some organic acids seem to influence the function of immune cells directly. Tedelind et al. have shown that the SCFAs

**FIGURE 6** Gene expression of the inflammatory cytokines Tnfa (A), Cxcl2 (B), Il17a (C), Il16 (D), Ifng (E), Il1b (F), Il10 (G), Il23p19 (H), and Icam1 (I) in the colons of male BALB/c mice fed control, 10% PHGG, and 10% GG diets with distilled water or DSS (Expt. 2). Values are means ± SEMs, n = 6. Means without a common letter are different, P < 0.05. AU, arbitrary unit; Cxcl2, chemokine C-X-C motif ligand 2; DSS, dextran sodium sulfate; GG, guar gum; Icam1, intercellular adhesion molecule 1; Ifng, interferon γ; PHGG, partially hydrolyzed guar gum; Tnfa, tumor necrosis factor α.
acetic, propionic, and n-butyric acid suppress the expression of different inflammatory cytokines in inflamed human Colo320DM cells and in mouse colons (40). Accordingly, the SCFAs produced from GG and PHGG may influence not only TJ expression in epithelial cells but also cytokine expression in immune cells, leading to the prevention of experimental colitis.

Studies have identified 2 cell-surface G-protein coupled receptors (GPRs) for SCFAs, GPR 41 and GPR 43. These 2 SCFA receptors are expressed in the colonic epithelial cells, as well as in immune cells, particularly polymorphonuclear leuko-

_**FIGURE 7** Concentration of organic acids in the feces of male BALB/c mice fed control, 10% PHGG, and 10% GG diets with distilled water or DSS (Expt. 2). Feces were collected on day 0 and day 4 of DSS administration. Fecal concentrations of acetic, propionic, n-butyric, lactic, and succinic acids are shown for day 0 (A) and day 4 (D); fecal concentrations of isobutyric, n-valeric, and iso-valeric acids are shown for day 0 (B) and day 4 (E); and total fecal SCFA concentrations (sum of acetic, propionic, and n-butyric acids) are shown for day 0 (C) and day 4 (F). Values are means ± SEMs, n = 6. Means without a common letter are different, P < 0.05. DSS, dextran sodium sulfate; GG, guar gum; PHGG, partially hydrolyzed guar gum.

Taken together, supplementation with GG and PHGG ameliorates TJ barrier defects and inflammation in colitic mice without any impact on the colon TJ proteins in normal mice. Our findings suggest that GG and PHGG could provide preventive and therapeutic approaches against IBDs. Although the precise mechanisms underlying these GG- and PHGG-mediated effects are still unclear, increased SCFA production might contribute, at least in part, to the protection of the epithelial TJ barrier and to the suppression of inflammatory immune responses in the colon.
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TVH and TS designed the research, analyzed the data, and wrote the paper; TVH conducted the research and performed the statistical analysis; and TS had primary responsibility for the final content. Both authors read and approved the final manuscript.

References


