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Partially hydrolyzed guar gum down-regulates colonic inflammatory response in dextran sulfate sodium-induced colitis in mice☆

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Abstract

Partially hydrolyzed guar gum (PHGG), a water-soluble dietary fiber produced by a controlled partial enzymatic hydrolysis of guar gum beans, has various physiological actions. The aim of the present study was to elucidate the beneficial effects of PHGG on colonic mucosal damage and on the inflammatory response in a dextran sulfate sodium (DSS) colitis model. After 2 weeks of prefeeding of PHGG, acute colitis was induced with 8% DSS in female BALB/c mice. Colonic mucosal inflammation was evaluated clinically, biochemically and histologically. Mucosal protein contents and mRNA levels of tumor necrosis factor-α (TNF-α) were determined by immunoassay and reverse transcription polymerase chain reaction. Disease activity scores determined by weight loss, stool consistency and blood in stool in DSS-treated mice were significantly lower in the PHGG-treated mice compared with the control mice. Shortening of the colon was significantly reversed by PHGG. Histological study also showed a reduced infiltration of inflammatory cells, especially neutrophils, and mucosal cell disruption in PHGG-treated mice compared with the control mice. The increases in tissue-associated myeloperoxidase activity and thiobarbituric acid-reactive substances after DSS administration were both significantly inhibited by pretreatment with PHGG. Partially hydrolyzed guar gum also inhibited increases in intestinal TNF-α protein and mRNA expression after DSS administration, respectively. These results suggest that chronic ingestion of PHGG prevents the development of DSS-induced colitis in mice via the inhibition of mucosal inflammatory response.

Keywords: Dextran sulfate sodium; Dietary fiber; Guar gum; Neutrophil activation; Tumor necrosis factor-α

1. Introduction

Ulcerative colitis and Crohn's disease are chronic, immunologically mediated diseases. Recent evidence has shown that enteric pathogens including microflora appear to be important in the initiation and reactivation of human inflammatory bowel disease, and may be responsible for chronic inflammation in at least a subset of patients with inflammatory bowel diseases [1]. Therefore, therapeutic alteration of the luminal microenvironment by probiotic, prebiotic and molecular strategies offers great promise for the nontoxic treatment of inflammatory bowel disease. Most notably, a mixture of *Bifidobacterium* and *Lactobacillus* [2] and nonpathogenic viable *Escherichia coli* [3] has proven to prolong remission in cases of ulcerative colitis. In addition, there have been two controlled investigations of oral administration of a short-chain fatty acid (SCFA) substrate (fermentable dietary fiber) in patients with ulcerative colitis. Fernandez-Banares et al. [4] reported the efficacy and safety of dietary fiber from *Plantago ovata* seeds vs. mesalamine to maintain remission in these patients. Kanauchi et al. [5] also showed that oral administration with germinated barley foodstuff (GBF) made from the aleurone layer and scutellum fractions of malt significantly decreased clinical activity index scores of ulcerative colitis compared with the

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control group. It is generally accepted that dietary fiber induces the anaerobic bacteria to produce SCFAs, mainly acetate, propionate and butyrate, which are important nutrients for epithelial cells [6]. Moreover, SCFAs, especially butyrate, play an important role in the homeostasis of the colonic mucosa because they stimulate colonic cell proliferation, sodium absorption and increase in mucosal blood flow [7]. Recent findings also showed that butyrate suppressed inflammatory mediator generation by inhibiting nuclear factor-κB and regulated the cell-cycle-related genes by inducing histone hyperacetylation [8].

Guar gum is a water-soluble polysaccharide found in the seeds of guar, a plant indigenous to India, Pakistan and the United States. Guar gum has galactomannan as its main component. It has been shown to be effective in the treatment of hyperlipidemia [9] and postprandial glycemia of diabetes [10]. Because guar gum is extremely viscous, it is very difficult to incorporate it in food in large enough quantities to obtain a physiological effect, so a partially hydrolyzed guar gum (PHGG) is used in beverage form. Partially hydrolyzed guar gum has proved effective in softening and improving the output of feces and in increasing bulking capacities (fecal weight, frequency of defecation and fecal excretory feeling) [11,12]. Partially hydrolyzed guar gum increased production of *Bifidobacterium* in the gut [13]. Partially hydrolyzed guar gum also reduced the incidence of diarrhea in septic patients receiving total enteral nutrition and reduced symptoms of irritable bowel syndrome [14,15]. A multicenter, randomized, open trial in patients with irritable bowel syndrome has demonstrated that PHGG is as effective as a high-fiber diet in improving the core symptoms of irritable bowel syndrome, but is better tolerated by patients [14].

The aim of the present study was to elucidate the effects of PHGG on colonic mucosal damage and on the inflammatory response in a dextran sulfate sodium (DSS) colitis model. Although the pathogenesis of DSS-induced colitis is unclear, its induction may result from the toxic effects of DSS on colonic epithelial cells, alterations of luminal bacterial flora [16] or increases in oxidative and nitrosative stress [17]. In addition, the cytokine expression and histological findings in acute DSS-induced colitis are very similar to those observed in human inflammatory bowel disease [16,18]. Using this experimental model, GBF showed preventive and therapeutic effects with notable amelioration of severe bloody diarrhea and an attenuation of colonic mucosal damage [19]. In the present study, special attention was paid to the effect of PHGG on DSS-induced intestinal inflammatory response, including neutrophil accumulation and tumor necrosis factor-α (TNF-α).

2. Materials and methods

2.1. Chemicals

All chemicals were prepared immediately before use. Thiobarbituric acid (TBA) and 3,3′,5,5′-tetramethylbenzidine were obtained from Wako (Osaka, Japan). 1,1,3,3-Tetramethoxypropane was obtained from Tokyo Kasei (Tokyo, Japan). An enzyme-linked immunosorbent assay kit for mouse TNF-α was obtained from BioSource International (Camarillo, CA). All other chemicals used were of reagent grade.

2.2. Partially hydrolyzed guar gum used

The commercial PHGG preparation (Sunfiber^R) used in this study was a gift from Taiyo Kagaku (Tokyo, Japan). The PHGG was prepared by treatment of guar gum with β-endogalacto-mannase from a strain of *Aspergillus niger*, and its average molecular mass measured by HPLC was 20,000 Da. The total dietary fiber content of the PHGG was 85% measured by the method of Association of Official Agricultural Chemists.

2.3. Experimental procedures

Nine-week-old female BALB/c mice weighing 18–20 g were purchased from Shimizu Experimental Animals (Osaka, Japan). The mice were housed individually in cages in a room kept at 18–24°C and 40% to 70% relative humidity, with a 12-h light/dark cycle. They were allowed free access to their food and drinking water. First, the mice were fed rodent diet CE-2[1] (Nihon Clea, Tokyo, Japan) for 1 week during their acclimatization period. They were then divided into two groups, a PHGG-diet test group and a control group. After 2 weeks of prefeeding, mice in the two groups were given 8.0% DSS (molecular weight, 8000; Lot No. DS-605, Seikagaku, Tokyo, Japan) in the drinking water to induce colitis. Intake of the DSS solution was monitored throughout the experiments and was found to be unchanged among the experimental groups (data not shown).

2.4. Evaluation of colitis severity

The parameters recorded in the experiments were the disease activity index (DAI), colon length and histology. Disease activity index was determined by scoring changes in weight, occult blood positivity and gross bleeding, and stool consistency, as described previously (Table 1) [20]. Occult bleeding was tested using a commercial kit based on the detection of the peroxidase activity of heme in stool (Occult Blood Slide 5 Shionogi; Shionogi, Osaka, Japan). The DAI score has been shown to correlate well with histological measures of inflammatory and crypt damage. We used five grades of weight loss (0, no loss or weight gain; 1, 1% to 5% loss; 2, 5% to 10% loss; 3, 10% to 20% loss; 4, loss of more than 20%), three grades of stool consistency (0, normal; 2, loose; and 4, diarrhea) and three grades of occult blood (0, normal; 2, occult blood positive;

[1] CE-2 (/100 g): protein, 25.2 g; fat, 4.6 g; fiber, 4.4 g; water, 8.7 g; ash, 6.5 g; vitamins retinol, 0.8 mg; B1, 1.5 mg; B2, 1.3 mg; B6, 1.2 mg; B12, 2.5 mg; C, 12.5 mg; E, 6.8 mg; pantothenic acid, 3.8 mg; niacin, 18.3 mg; folic acid, 0.2 mg; choline, 0.2 g; biotin, 43.5 μg; inositol, 495.5 mg.

Table 1
Scoring system of DAI

Score	Weight loss (%)	Stool consistency	Occult/gross bleeding
0	None	Normal	Normal
1	1–5		
2	6–10	Loose stools	Positive occult blood test
3	11–20		
4	>20	Diarrhea	Gross bleeding

The DAI is a mean of individual scores of weight loss, stool consistency and bleeding [20]. Normal stool=formed pellets; loose stool=pasty and semiformed stools that do not stick to the anus; diarrhea=liquid stools that stick to the anus.

and 4, gross bleeding), and the combined scores were then divided by 3 to obtain the final DAI. After determining the DAI, mice were killed by cervical dislocation, and the colon was resected between the ileocecal junction and the proximal rectum, close to its passage under the pelvisternum. The colon was placed on a nonabsorbent surface and measured with a ruler. The entire colon was divided into three segments (proximal, middle and distal), and part of each segment was fixed in 10% neutral buffered formalin. After fixation, the species were embedded in paraffin, divided into 7-μm sections and stained with hematoxylin and eosin.

2.5. Measurements of TBA-reactive substances and myeloperoxidase activity

The concentration of TBA-reactive substances was measured in the colon mucosa using the method of Ohkawa et al. [21] as an index of lipid peroxidation. After the experiments, the colon mucosa was scraped off using two glass slides, then homogenized with 1.5 ml of 10 mmol/L potassium phosphate buffer (pH 7.8) containing 30 mmol/L KCl in a Teflon Potter-Elvehjem homogenizer. The level of TBA-reactive substances in the mucosal homogenates was expressed as nanomoles of malondialdehyde per milligram of protein using 1,1,3,3-tetramethoxypropane as the standard. Total protein in the tissue homogenates was measured by the method of Lowry et al. [22].

Tissue-associated myeloperoxidase (MPO) activity was determined by a modification of the method of Grisham et al. [23] as an index of neutrophil accumulation. Two milliliters of mucosal homogenates were centrifuged at 20,000×*g* for 15 min at 4°C to pellet the insoluble cellular debris. The pellet was then rehomogenized in an equivalent volume of 0.05 mol potassium phosphate buffer (pH 5.4) containing 0.5% hexadecyltrimethylammonium bromide. The samples were centrifuged at 20,000×*g* for 15 min at 4°C, and the supernatants were saved. Myeloperoxidase activity was assessed by measuring the H_2O_2-dependent oxidation of 3,3′ ,5,5′ -tetramethylbenzidine. One unit of enzyme activity was defined as the amount of MPO present that caused a change in absorbance of 1.0/min at 655 nm and 25°C.

2.6. Determination of intestinal content and mRNA expression of TNF-α

We determined the concentration of TNF-α in the supernatant of mucosal homogenates using an enzyme-linked immunosorbent assay kit specific for mouse TNF-α with a sandwich method. The assay was performed according to the manufacturer's instructions. After color development, optimal densities were measured at 450 nm with a microplate reader (MPR A4i; Tosoh, Tokyo, Japan). The minimum detection level of TNF-α was 4 pg/ml, and the assay has no cross-reactivity with other cytokines. The concentration of TNF-α was expressed as nanograms per milligram of protein.

For the quantification of TNF-α mRNA, we removed samples for mRNA isolation from the colons of three mice in each group. Total RNA was isolated with the acid guanidinium phenol chloroform method using an Isogen kit (Nippon Gene, Tokyo, Japan). RNA concentrations were determined by their absorbance at 260 nm in relation to the absorbance at 280 nm. RNA was stored at −70°C until reverse transcription was performed. According to the previously described protocol [24], total RNA was extracted and used for reverse transcription polymerase chain reaction (RT-PCR) amplification. The primers had the following sequences: for TNF-α, sense 5′-ATGAGCACAGAAAGCATGATC-3′ and antisense 5′-TACAGGCTTGTCACTCGAATT-3′; and for β-actin, sense 5′-TGTGATGGTG-GGAATGGGTCAG-3′ and antisense 5′-TTTGATGTCACGCACGATTTCC-3′. The mixture was subjected to PCR amplification for 30 cycles (1 min at 94°C, 1 min at 54°C and 1 min at 72°C). The PCR reaction products were separated electrophoretically in a 2.5% agarose gel and stained by ethidium bromide.

2.7. Micobiological assay

Feces of the subjects were collected in plastic air-free bags and kept at 4°C. Bacteriological analysis of the feces

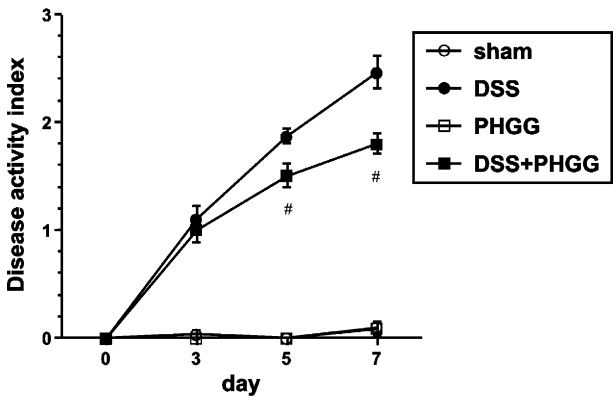

Fig. 1. Effect of PHGG on the DAI during the development of DSS-induced colitis in mice. After 2 weeks of prefeeding, mice were given 8.0% DSS solution in the drinking water to induce colitis. Each value indicates the mean±S.E. for 11–15 mice. $^{\#}P<.001$ when compared to the control mice receiving 8.0% DSS solution.

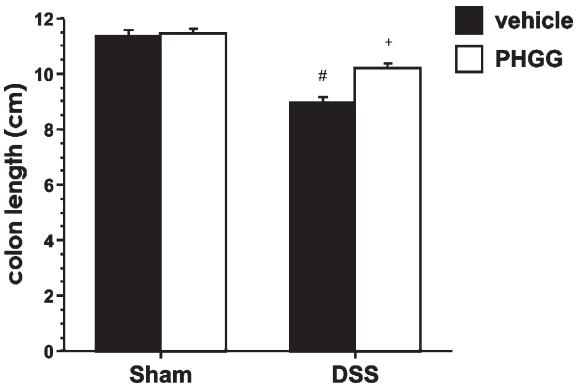

Fig. 2. Effect of PHGG on the total colon length after DSS administration. Each value indicates the mean±S.E. for 10 mice. $^{\#}P<.001$ when compared to the mice receiving vehicle solution and $^{+}P<.001$ when compared to the control mice receiving 8.0% DSS solution.

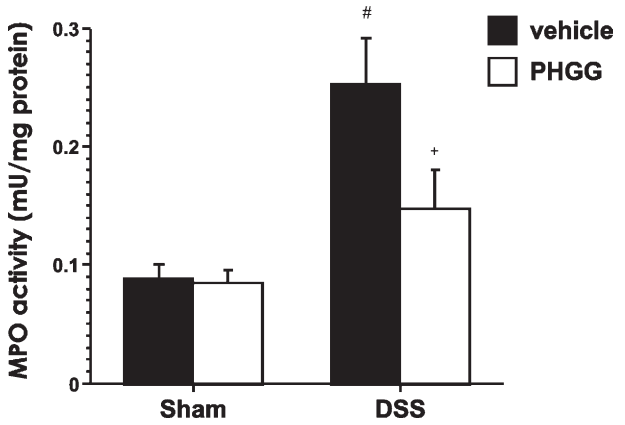

Fig. 4. Effect of PHGG on neutrophil accumulation expressed as MPO activity in the colonic mucosa of mice administered 8% DSS. Each value indicates the mean±S.E. for six to eight mice. $^{\#}P<.01$ when compared to the mice receiving vehicle solution and $^{+}P<.05$ when compared to the control mice receiving 8.0% DSS solution.

was carried out according to the method described by Ikeda et al. [25]. One gram of feces was weighed and transferred to 9 g of prereduced phosphate-buffered saline containing 0.1% purified agar and 0.5% cysteine–HCl. Serial 10-fold dilution was made. Selective and nonselective media for total aerobes were inoculated with 10^{-1}, 10^{-3}, 10^{-5} and 10^{-7} dilution. Nonselective media for total bacterial counts were inoculated with 10^{-6}, 10^{-7} and 10^{-8} dilutions. Plates for anaerobic culture were incubated for 72 h in anaerobic jars by the steel wool method in which reduced steel wool is placed in a jar and the atmosphere is replaced by CO_2. Plates for aerobic culturing were incubated at 37°C for 24–48 h. After incubation, different colony types were counted and identified to the genus level by Gram-strain characteristics and cell morphology. The bacterial cell counts per gram of wet feces were expressed by their numbers in $\log_{10}$. The lower limit of detection was 2×10^2 microorganisms per gram of wet stool.

2.8. Statistics

The results are presented as the mean±S.E.M. The data were compared by two-way analysis of variance, and differences were considered significant if the P value was .05 based on Scheffe's multiple comparison test. All analyses were performed using the StatView 5.0-J program (Abacus Concepts, Berkeley, CA) on a Macintosh computer. Results were considered significant at a P value of less than .05.

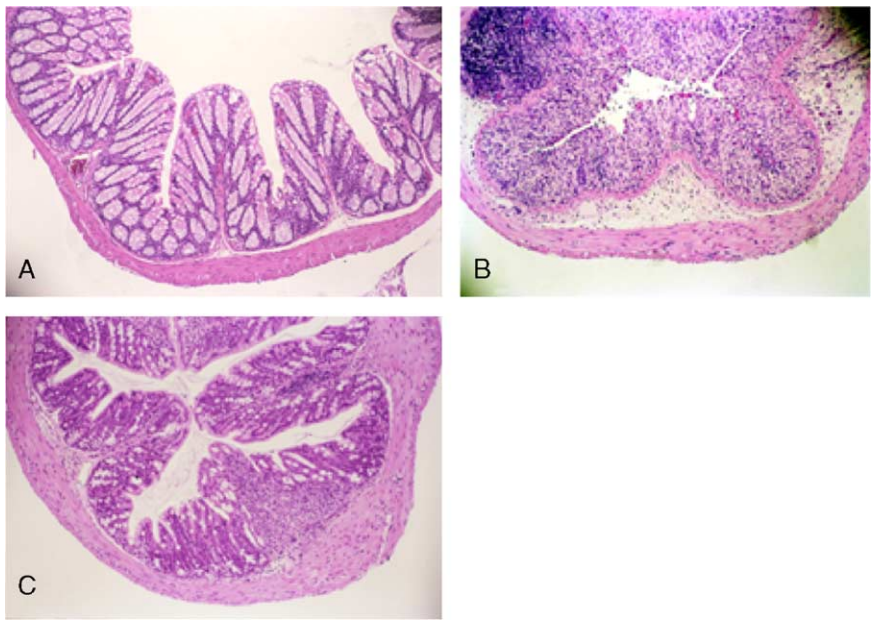

Fig. 3. Appearance of the colon in a sham-treated mouse (A), a DSS-treated mouse (B) and a mouse receiving DSS plus PHGG (C). Loss and shortening of crypts, mucosal erosions, inflammatory cell infiltration and goblet cell depletion are seen in B. In C, smaller erosions are associated with less inflammatory cell infiltration. Hematoxylin and eosin staining. Magnification, ×40.

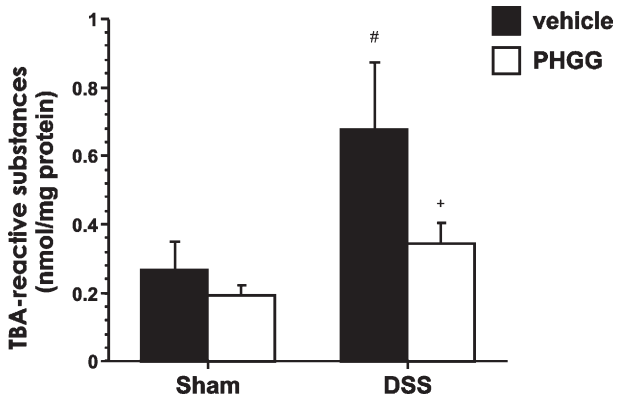

Fig. 5. Effect of PHGG on the increase in lipid peroxide concentration expressed as TBA-reactive substances in colonic mucosa of mice administered 8% DSS. Each value indicates the mean±S.E. for six to eight mice. $^{\#}P<.01$ when compared to the mice receiving vehicle solution and $^{+}P<.05$ when compared to the control mice receiving 8.0% DSS solution.

2.9. Ethical considerations

All animal experiments were carried out in accordance with the *Guide for the care and use of laboratory animals* and with the approval of the Animal Care Committee of Kyoto Prefectural University of Medicine (Kyoto, Japan).

3. Results

3.1. Effects of PHGG on DAI scores, colon length, and histology

Mice exposed to 8% DSS developed symptoms of acute colitis, with diarrhea being observed first followed by rectal bleeding and severe weight loss. To determine a relevant dose for the study with PHGG, we first performed a dose–response study using mice after 2 weeks of prefeeding of a diet containing PHGG (5%, 10% and 15% of the diet). All three groups significantly reversed shortening of the colon induced by the DSS administration; however, there were no significant differences in its efficacy among the groups. Therefore, in the following experiments, we used PHGG at the dose of 5% of the diet and examined its pharmacological action.

Disease activity index scores, determined by weight loss, stool consistency and blood in stool, in DSS-treated mice were lower in the PHGG-treated mice compared with the control mice (significant from days 5 and 7; Fig. 1). Shortening of the colon was observed upon removal in the control mice. Decrease in total colon length was significantly reversed by treatment with PHGG (Fig. 2). The protective effect of PHGG was also confirmed by histological study. Fig. 3 shows typical histological appearances in a PHGG-treated group and in control mice. Administration of 8% DSS alone for 7 days resulted in large areas of epithelial crypt loss, prominent neutrophilic infiltration throughout the mucosa, ulceration and mucosal bleeding. In contrast, treatment with PHGG resulted in smaller erosions with few neutrophils.

3.2. Effects of PHGG on MPO activity and TBA-reactive substances

Neutrophil accumulation was also evaluated by the measurement of MPO activity in the colonic mucosal homogenates. Tissue-associated MPO activity in the colonic mucosa increased from a basal concentration of 0.09±0.01 to 0.25±0.14 mU/mg protein 7 days after DSS administration (Fig. 4). The increase in MPO activity in the colonic mucosa after DSS administration was significantly inhibited by treatment with PHGG. Thiobarbituric acid-reactive substances in the colonic mucosa, an index of lipid peroxidation, increased significantly from a basal concentration of 0.270±0.079 to 0.677±0.194 nmol/mg protein 7 days after DSS administration (Fig. 5). The increase in TBA-reactive substances in the colonic mucosa was inhibited by PHGG (Fig. 5).

3.3. Effect of PHGG on mucosal contents and mRNA levels of TNF-α

To test whether PHGG treatment modulates the inflammatory response through regulation of cytokine production, we analyzed intestinal levels of TNF-α. A substantial

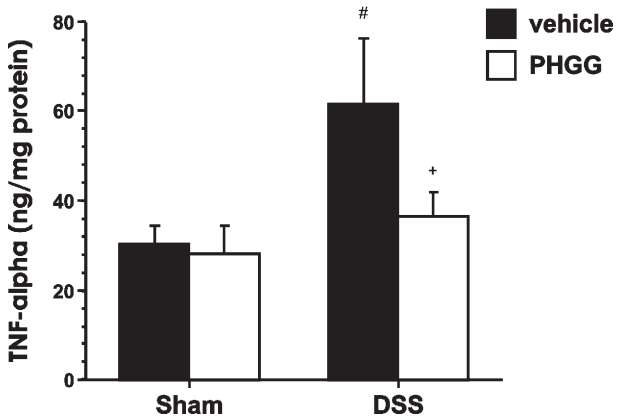

Fig. 6. Effect of PHGG on TNF-α contents in colonic mucosa of mice administered 8% DSS. The concentration of TNF-α in the supernatant of mucosal homogenates was determined by an ELISA kit specific for mouse TNF-α. Each value indicates the mean±S.E. for six to eight mice. $^{\#}P<.01$ when compared to the mice receiving vehicle solution and $^{+}P<.05$ when compared to the control mice receiving 8.0% DSS solution.

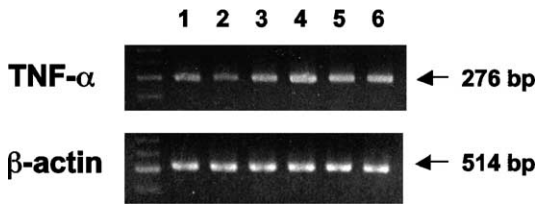

Fig. 7. Effects of PHGG on mRNA expression for TNF-α in colonic tissues of mice given 8% DSS. A representative 2% agarose gel of RT-PCR products is shown, also including β-actin mRNA, for colon tissues from mice after 2 weeks of prefeeding (the control diet, lane 1; PHGG diet, 2), sham mice (the control diet, 3; PHGG diet, 5) and DSS-administered mice (the control diet, 4; PHGG diet, 6).

Table 2
Effects of oral administration with PHGG on intestinal microflora before and after colitis induction

	Before DSS administration		After DSS administration	
	Vehicle	PHGG	Vehicle	PHGG
Total	9.42±0.08	9.60±0.19	10.66±0.09	10.68±0.03
Total aerobes	8.96±0.10	8.90±0.08	8.82±0.13	8.90±0.14
Enterobacteriaceae	5.64±0.15 (100)	5.78±0.18 (100)	7.58±0.29 (100)	7.52±0.28 (100)
Streptococcus	6.38±0.13 (100)	6.24±0.14 (100)	8.06±0.16 (100)	7.94±0.29 (100)
Staphylococcus	6.06±0.06 (100)	6.06±0.09 (100)	6.68±0.38 (100)	6.46±0.28 (100)
Lactobacillus	8.96±0.10 (100)	8.90±0.08 (100)	8.62±0.19 (100)	8.76±0.14 (100)
Total anaerobes	9.16±0.12	9.46±0.25	10.66±0.09#	10.68±0.04†
Bacteroidaceae	9.16±0.12 (100)	9.46±0.25 (100)	10.60±0.04# (100)	10.66±0.04† (100)
Bifidobacterium	4.6 (20)	– (0)	– (0)	3.8 (20)
Anaerobic GPC	– (0)	7.3 (20)	9.8 (40)	– (0)
Clostridium	– (0)	– (0)	– (0)	– (0)
Lecithinase (+)	– (0)	– (0)	– (0)	– (0)
Lecithinase (−)	8.2 (20)	– (0)	9.9 (20)	9.8 (20)

Data are expressed as the mean of log bacterial counts per gram of wet feces (frequency of occurrence %). –, below 2 log 2/g wet feces. $^{\#}P<.05$ and $^{\dagger}P<.05$ vs. the vehicle- and the PHGG-treated mice before DSS administration, respectively.

increase in mucosal TNF-α content was found in the control mice on day 5 after DSS administration (Fig. 6). The level of this cytokine was significantly lower in the PHGG-treated mice in comparison with the level in control animals. To further confirm the inhibitory effect of PHGG on TNF-α production, we analyzed intestinal expression of TNF-α mRNA on day 5 after DSS administration. As shown in Fig. 7, we found TNF-α gene expression in control animals to be faint. In contrast, the expression of TNF-α mRNA was up-regulated in the intestine after DSS administration (Fig. 7, lane 4). The increased expression of TNF-α mRNA was inhibited by PHGG (Fig. 7, lane 6).

3.4. Effect of PHGG on bacterial counts in feces

Log viable bacterial counts in feces of each subject are shown in Table 2. There were no significant differences in the concentrations of total aerobes among groups. The concentrations of total anaerobes, mainly Bacteroidaceae, were significantly increased by the DSS administration, whereas PHGG treatment did not affect the counts of aerobes or anaerobes compared with the vehicle group.

4. Discussion

The present study demonstrates that chronic treatment with PHGG, a water-soluble dietary fiber, attenuates DSS-induced colonic injury and inflammation in mice. In our study, intestinal injury was assessed by a variety of methods including DAI, length of the colon and histology. By each assessment, PHGG treatment significantly inhibited colonic injury. In addition, we showed that MPO activity, TBA-reactive substances and expression of TNF-α gene were enhanced in DSS-induced intestinal inflammation, and that these increases were reversed by PHGG treatment.

Authors of recent reports have hypothesized that neutrophil-mediated inflammation is involved in the development of DSS-induced colonic mucosal injury. Three lines of evidence support this hypothesis: (1) colonic mucosal endothelial intercellular adhesion molecule 1 (ICAM-1) expression is enhanced at an early stage in the inflammatory cascade of DSS-induced colitis [26], (2) selective depletion of neutrophils by monoclonal antibody RP-3 suppresses colitis in rats [27] and (3) immunoneutralization of ICAM-1 on endothelial cells significantly attenuated colonic mucosal injury and neutrophil accumulation in rats [28]. The present study has shown that MPO activity, an index of tissue-associated neutrophil accumulation, significantly increases in the colonic mucosa after DSS administration, and this increase is significantly inhibited by treatment with PHGG. These results indicate that the inhibition of neutrophil accumulation by PHGG may be one of the protective factors helping to decrease DSS-induced colonic mucosal injury. The inhibition of neutrophil accumulation by PHGG is also supported by the previous data showing that increased MPO activity was significantly inhibited by a diet fiber derived from *P. ovata* seeds in a model of trinitrobenzenesulfonic acid (TNBS)-induced rat colitis [29]. However, the mechanism of action by which these dietary fibers may be involved in the infiltration of neutrophils remains to be established and is currently undergoing active investigation.

An important observation of the present study is that TBA-reactive substances, an index of lipid peroxidation, are significantly increased in the colonic mucosa after DSS administration, and this increase is significantly inhibited by treatment with PHGG. Although the TBA test is not specific for lipid peroxides, it is one of the oldest and most frequently used methods for measuring the peroxidation of fatty acid, cell membranes and food products, and it can be applied to crude biological samples. By measuring the TBA-reactive substances, we have reported that lipid peroxidation mediated by oxygen free radicals is involved in the gastrointestinal mucosal injuries induced in animal models by various types of stress [30,31], ischemia reperfusion [32] or inflammation [33,34]. Polyunsaturated fatty acids of cell membranes are degraded by lipid peroxidation with

subsequent disruption of membrane integrity, suggesting that lipid peroxidation mediated by oxygen radicals is an important cause of damage and destruction of cell membranes [35,36]. Our data support another recent finding in our laboratory, in which colonic TBA-reactive substances were markedly reduced by treatment with Mn-superoxide dismutase [37] and a synthetic vitamin E analogue [38] in the same DSS colitis mouse model; this suggests that induction of lipid peroxidation is an early critical event in this experimental inflammatory bowel disease. However, neither PHGG nor SCFA have been reported to have antioxidant activities in vitro, especially the inhibition of lipid peroxidation. The inhibitory effect of PHGG on DSS-induced lipid peroxidation in the mice colonic mucosa may result from its anti-inflammatory activity, rather than from its direct antioxidant activity.

Importantly, TNF-α has lately attracted attention due to its key role in the pathogenesis of inflammatory bowel disease, and recent clinical trials have reported marked improvement in patients with Crohn's disease treated with high-affinity chimeric and humanized monoclonal antibody directed against TNF-α [39], which supports the concept of interference with the function of TNF-α in inflammatory bowel disease. Previous reports of DSS colitis in mice have emphasized the involvement of T-helper 1 responses with the production of TNF-α in this model of colitis; treatment with anti-TNF-α antibody markedly decreased severity [40]. We also previously reported that the inhibition of TNF-α expression by pioglitazone, a peroxisome proliferator-activated receptor-γ ligand, was accompanied by significant suppression of intestinal inflammation in the same model [41]. Therefore, we tested the effects of PHGG on inflammatory responses after the induction of colitis. In the present study, we confirmed the anti-inflammatory properties of PHGG in vivo by demonstrating the reduction of mucosal TNF-α protein contents as well as down-regulation of the gene expression in inflamed mucosa. Our data are in agreement with a recently reported study in which a fiber-supplemented diet (*P. ovata* seeds) inhibited mucosal contents of TNF-α in vivo in a rat TNBS-induced colitis [29]. Whereas a fiber-supplemented diet increases luminal contents of SCFAs, especially butyrate, the precise mechanism by which SCFAs inhibit TNF-α gene expression is not completely resolved. Recent studies have reported that SCFAs decrease TNF-α production by isolated lamina propria mononuclear cells via the inhibition of nuclear factor-κB activation and IκBα degradation [42]. Further studies will be required to fully clarify the mechanism of PHGG-induced reduction of inflammation.

In a DSS-induced colitis model, previous studies have shown that concentrations of *Bacteroides* and clostridium species are increased in the acute phase of disease [16,43]. It has been suggested that after induction of mucosal damage by DSS, intestinal bacteria penetrate the injured mucosa and perpetuate mucosal inflammation [44]. Recent evidence suggests that feeding dietary fiber able to modify the intestinal flora composition can prevent the development of inflammatory response in the gut [45]. Our results are in part compatible with these studies in showing that concentrations of anaerobic *Bacteroides* species are increased after the DSS administration. However, this increase was not affected by the treatment with PHGG. This observation suggests that the decreased inflammation did not result from the growth inhibition of *Bacteroides* species. On the contrary, the intake of PHGG caused a considerable increase in frequency of occurrence of lactobacilli in the human volunteer study [13]. Because these bacterial species were rarely cultured in our method, further studies will be necessary to confirm the effect of PHGG on bacterial flora.

Our results showed that DSS-induced intestinal inflammation is characterized by neutrophil accumulation and increased expression of TNF-α. Inhibition of TNF-α by PHGG-supplemented diet was accompanied by significant suppression of intestinal inflammation in vivo. These data suggest that oral administration of PHGG may be an additional therapeutic strategy for inflammatory bowel disease.

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