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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.