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Cellobiose Prevents the Development of Dextran Sulfate Sodium (DSS)-Induced Experimental Colitis

Takashi Nishimura, Akira Andoh*, Takayoshi Hashimoto, Ayako Kobori, Tomoyuki Tsujikawa and Yoshihide Fujiyama

Department of Medicine, Shiga University of Medical Science, Seta Tukinowa, Otsu 520-2192, Japan

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Summary **Cellobiose is produced from cellulose using specific bacterial enzymes, and is hydrolyzed into glucose by the enzymes cellobiosidase and cellulase. In this study, we examined the effects of cellobiose on colonic mucosal damage in a dextran sulfate sodium (DSS) colitis model. BALB/c mice were divided into two groups. In the first group, the mice were fed 3.5% DSS mixed with normal chow. In the second group, the mice were fed 3.5% DSS plus 6.0 or 9.0% (weight/weight) cellobiose mixed with normal chow. The development of colitis was assessed on day 21. Mucosal cytokine expression was analyzed by RT-PCR. Body weight loss was significantly attenuated in the 9.0% cellobiose-fed DSS mice as compared to the DSS mice. Colonic weight/length ratio, a maker of tissue edema, was significantly higher in the DSS mice than in the 9.0% cellobiose-fed DSS mice. The disease activity index and histological colitis score were also significantly higher in the DSS mice than in the 9.0% cellobiose-fed DSS mice. Mucosal mRNA expression for IL-1β, TNF-α, IL-17 and IP-10 were markedly reduced in the 9.0% cellobiose-fed DSS mice. In conclusion, a preventive effect of cellobiose against DSS colitis suggests its clinical use for inflammatory bowel diseases patients.**

***Key Words*: IBD, Prebiotics, Probiotics, Butyrate, Fiber**

Introduction

Inflammatory bowel diseases (IBD), ulcerative colitis (UC) and Crohn's disease (CD) are chronic intestinal disorders of unknown etiology. As a characteristic feature of IBD, the mucosal immune system shows an aberrant response towards luminal antigens such as commensal bacteria in genetically susceptible individuals [*1*, *2*]. The correlation between a dysregulated bacterial ecosystem and mucosal inflammation has been reported in a variety of clinical and basic literature ion IBD [*3–5*].

It is well known that non-absorbable and/or non-digestible oligosaccharides escape digestion in the upper gastrointestinal tract, and when transferred to the colon, they are fermented by commensal bacteria, catabolized to short chain fatty acids and hydrogen, methane and carbon dioxide [*6*, *7*]. Short-chain fatty acids (SCFAs) are important nutrients for epithelial cells, and among the SCFAs, butyrate most effectively protects against intestinal mucosal injury and promotes mucosal healing.

Cellobiose (*o*-β-D-glucopyranosyl-(1→4)-α-D-glucopyranose) is produced from cellulose using specific bacterial enzymes, and is hydrolyzed into two molecules of glucose by the enzymes cellobiosidase and cellulase [*6*]. However, the physiological functions of cellobiose are not well understood due to its difficulty in mass production. Recently, it has been developed from the viewpoint of biomass utilization, and cellobiose is now available for food and feed [*6*]. In this study, we examined the effects of cellobiose on colonic mucosal damage and on the intestinal environment in a dextran sulfate sodium (DSS) colitis model.

*To whom correspondence should be addressd.
Tel: +81-077-548-2217 Fax: +81-077-548-2219
E-mail: andoh@belle.shiga-med.ac.jp

Table 1. PCR primers used in this study

Genes		Sequence	PCR products (bp)
IL-1β	F	5'-CAG GAT GAG GAC ATG AGC ACC-3'	983
	R	5'-CTC TGC AGA CTC AAA CTC CAC-3'	
TNF-α	F	5'-ATG AGC ACA GAA AGC ATG ATC-3'	306
	R	5'-TAC AGG CTT GTC ACT CGA ATT-3'	
IL-17	F	5'-TCT CAT CCA GCA AGA GAT CC-3'	249
	R	5'-AGT TTG GGA CCC CTT TAC AC-3'	
IP-10	F	5'-GTG CTG CCG TCA TTT TCT GC-3'	230
	R	5'-CTT AGA TTC CGG ATT CAG AC-3'	
β-actin	F	5'-GTG GGC CGC TCT AGG CAC CA-3'	245
	R	5'-CGG TTG GCC TTA GGG TTC AGG GG-3	

Materials and Methods

Induction of colitis

Six- to eight-week-old male BALB/c mice were purchased from Charles River Japan (Kanagawa, Japan). They were acclimatized for one week before the experiment, and were housed individually in a room maintained at 22°C under a 12-h day/night cycle throughout the experiments. According to previous reports describing preventive effects of prebiotics on DSS-colitis [*8*, *9*], we used mild DSS-colitis model. The mice were divided into two groups. In the first group, the mice were fed 3.5% [weight/weight (wt/wt)] DSS (molecular weight 5000; Wako Pure Chemical Industries, Ltd, Osaka, Japan) mixed with normal chow (MF; Oriental Yeast Co., Ltd, Tokyo, Japan) and water *ad libitum*. In the second group, mice were fed 3.5% DSS plus 6.0 or 9.0% (wt/wt) cellobiose (Nippon Paper Chemicals Co., LTD, Tokyo, Japan) mixed with normal chow. This study protocol was approved by the Animal Care and Use Committee of the Shiga University of Medical Science (Otsu, Japan).

Assessment of inflammation in DSS-induced colitis

A daily clinical assessment of the DSS-induced colitis was performed, including a measurement of food intake and body weight, an evaluation of stool consistency, and the presence of blood in the stools by the guaiac paper test. The stool consistency was assessed using the following four point-scale: 0, normal; 1, soft; 2, very soft but formed; and 3, liquid. The intensity of the guaiac paper test was scored by the following scale: 0, negative; 1, faintly blue; 2, moderately blue; 3, dark blue; and 4, blood visible. A validated clinical disease activity index ranging from 0 to 4 was calculated using the following parameters: stool consistency, presence of fecal blood, and changes in body weight [*10*]. The mice were sacrificed on day 14, and the length and weight of the colon were measured.

Histology

A histological examination was performed on three samples of the distal colon from each animal. The samples were fixed in 10% buffered formalin, dehydrated in ethanol, and then embedded in paraffin. Four micron-thick sections were then prepared and stained with hematoxylin and eosin. All histologic evaluations were performed in a blinded fashion using a validated scoring system [*11*].

Reverse transcription-polymerase chain reaction (RT-PCR)

Cytokine mRNA expression in the mucosa was evaluated by RT-PCR. Total cellular RNA was isolated by the acid guanidium thiocyanate-phenol-chloroform (AGPC) method [*12*]. For each sample, the first-strand cDNA was synthesized using 0.5 µg of total cellular RNA with an oligo(dT) primer and Superscript reverse transcriptase (GIBCO BRL, Rockville, MD). One µl of the cDNA sample was amplified in 25 µl of a reaction mixture containing 10 × Taq Buffer (Perkin Elmer Cetus Corp., Norwalk, CT), 1.5 mM $MgCl_2$, 0.1 µM of each 5' and 3' primers, and 1 U of *TaqGold* polymerase (Perkin-Elmer Cetus) (Table 1). The PCR was performed in a thermal cycler (GeneAmp Model 2400; Perkin-Elmer Cetus) for 25 cycles (94°C for 30 s, 55°C for 30 s and 72°C for 40 s), followed by an 8 min. extension at 72°C. Five µl of the PCR products were subjected to electrophoresis on 1.5% agarose gels, and were stained with 0.5 µg/mL ethidium bromide. A 100-bp DNA ladder (GIBCO BRL) was used as the marker. Primers specific for the mouse cytokines have been described in previous reports [*13*, *14*].

Statistical analysis

Statistical analysis were performed using one-way ANOVA with Scheffe's post hoc test or the Kruskal-Wallis test when appropriate. A two-way ANOVA for repeated measures was used to test for group and time effects on the clinical data (e.g., disease activity index). *p values less than 0.05 were considered to be statistically significant.

Results

As shown in Fig. 1, at day 15 after the initiation of DSS-induced colitis, the body weight was significantly lower in the DSS-treated mice than in the DSS plus 9% cellobiose-treated mice. The colonic weight/length ratio, a maker of tissue edema, was significantly higher in the DSS-treated mice than in the DSS plus cellobiose-treated mice (*p<0.05, Fig. 2). On day 21, the disease activity index was significantly higher in the DSS-treated mice than in the DSS plus cellobiose-treated mice (*p<0.05, Fig. 2). These observations indicate that cellobiose suppressed the development of DSS colitis.

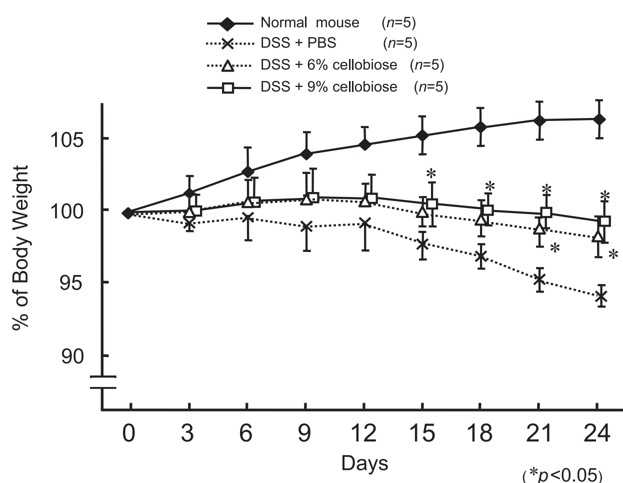

Fig. 1. Changes in body weight. The mice were fed 3.5% DSS, 3.5% DSS plus 6.0% cellobiose (wt/wt) or 3.5% DSS plus 9.0% cellobiose (Nippon Paper Chemicals Co., LTD, Tokyo, Japan). The weight of each individual mouse was then followed daily. The data represent means +/− SEM (n = 5 mice/group). *p<0.05: DSS plus 9.0% cellobiose group versus DSS group.

DSS-colitis is characterized by histological findings such as edema, the infiltration of inflammatory cells into both the mucosa and submucosa, the destruction of epithelial cells and mucosal thickening. The histological score was significantly higher in the DSS-treated mice than in the DSS plus curcumin-treated mice (*p<0.05, Fig. 2). As shown in Fig. 3, the histological analysis indicated that the histological severity of the colitis was more severe in the DSS-treated mice as compared to the DSS plus cellobiose-treated mice.

Mucosal cytokine mRNA expression was evaluated by semi-quantitative RT-PCR, and a representative picture is shown in Fig. 4. The DSS mice showed an increased expression of IL-1β, TNF-α, IL-17 and IP-10. Treatment with cellobiose markedly reduced the expression of these cytokine mRNAs. Similar results were observed in 5 different experiments.

Discussion

Although the detailed pathogenesis of IBD is not known, our understanding of the cellular and molecular mechanisms associated with IBD has increased dramatically. Several drugss (corticosteroids, 5-aminosalicylate compounds and immune suppressing agents) and new therapeutics (e.g. leukocytapheresis and anti-TNF-α antibody) have proven effective for the treatment of IBD. These treatments were based on the attenuation of local inflammation in the mucosa.

It has been recently reported that an improvement or reinforcement in colonic mucosal barrier functions via modifying the luminal environment may play an important

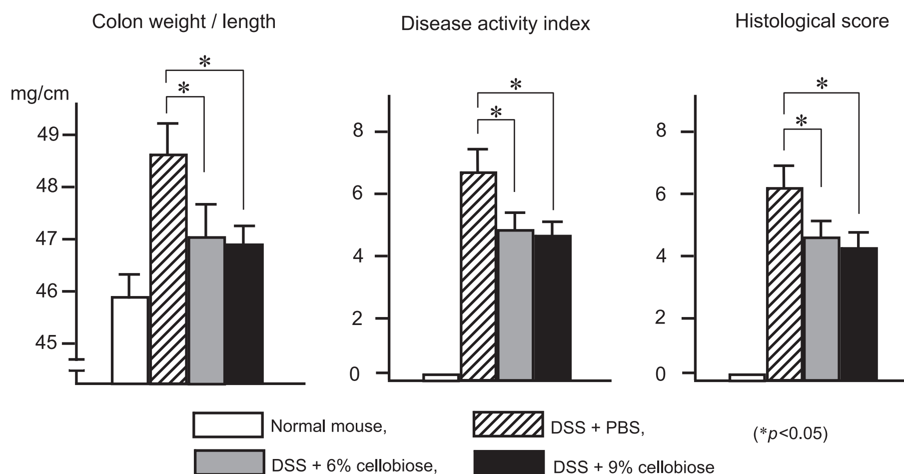

Fig. 2. Effects of cellobiose on the colonic weight/length, disease activity index (B), and histological score (C) on day 21. The scoring criteria were described in the Materials and Methods. The data represent means +/− SEM (n = 5 mice/group). *p<0.05.

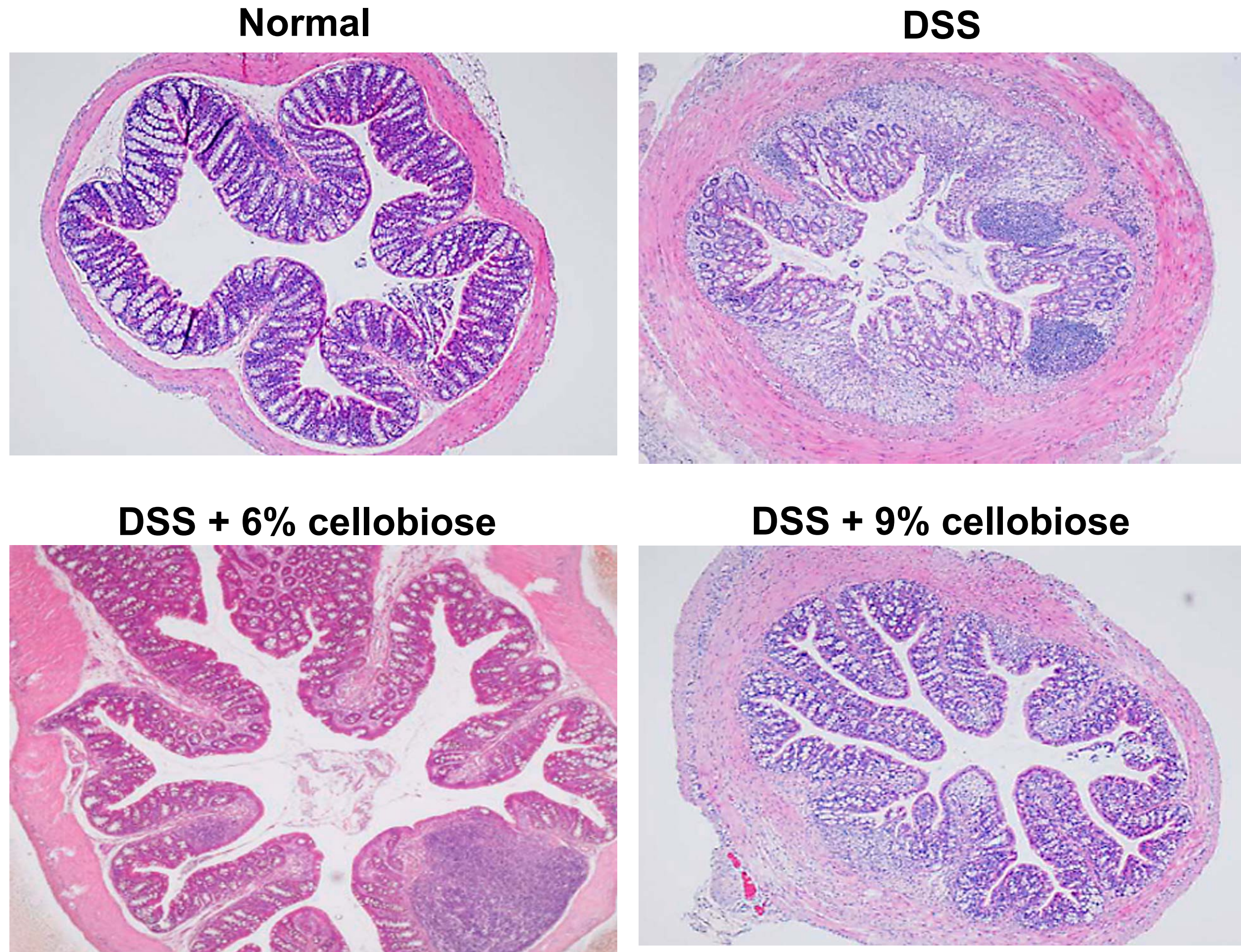

Fig. 3. Histological analyses for cellobiose effects on DSS-colitis. The colons were excised at 21 days after DSS treatment, and stained with hematoxylin and eosin (HE). Magnification ×100.

role in the treatment of UC. One of these therapeutic strategies is prebiotics, which escape digestion in the upper intestinal tract and are utilized as substrates for the microflora. It is generally accepted that prebiotics induce the production of short chain fatty acids (SCFAs), such as acetate, propionate and butyrate, as the major byproduct nutrients for epithelial cells by the intestinal flora [*15–18*]. The typical concentration of each SCFA is ~10 mM [*19*]. Of the most abundant SCFAs, butyrate has been shown to have significant protective effects on the colonic epithelium both *in vivo* and *in vitro*. Butyrate serves as the primary energy source for the normal colonic epithelium [*17*], and stimulates the growth of the colonic mucosa [*17*]. However, in tumor cell lines, it inhibits growth and induces apoptosis [*20*]. Previously, the effectiveness for butyrate enemas in the treatment of active ulcerative colitis (UC) has been reported [*21*, *22*]. The precise molecular mechanisms underlying this response have not been identified, but it is believed to be partially based on the inhibitory actions of butyrate on the production of pro-inflammatory mediators in the intestine. For example, butyrate inhibits both IL-8 and complement components from colonic epithelial cells [*23*], and blocks IP-10 secretion from colonic myofibroblasts [*24*].

Cellulose is the main component of the lignocellulosic biomass. It is preferable to use the lignocellulosic biomass instead of edible sugar, because it is cheap and exists abundantly on Earth. Cellobiose is one of the main components of oligosaccharides derived from cellulose, and recent studies suggest that cellobiose, fermented by the intestinal microflora, acts as a prebiotic [*16–18*]. Dextran sulfate sodium (DSS)-induced colitis is commonly used to evaluate the efficacy of new drugs for inflammatory bowel disease. Oral cellobiose administration improved both clinical and pathological signs of colitis with a decrease in mucosal proinflammatory cytokines mRNA expression. Previous studies have shown that cytokines evaluated in this study play important roles in the pathogenesis of experimental colitis and human IBD [*25–27*]. So, it was speculated that

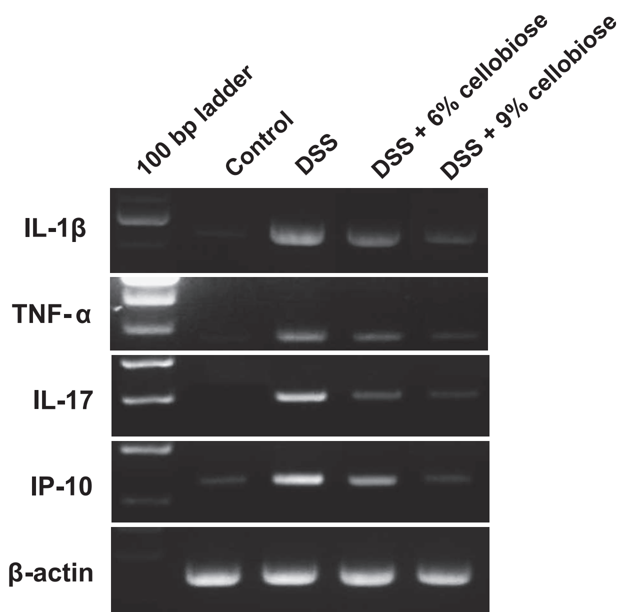

Fig. 4. RT-PCR analysis of mucosal cytokine expression on day 21. A representative picture from 5 different experiments is presented.

cellobiose acts at least in part through the inhibition of pro-inflammatory cytokine expression via the anti-inflammatory actions of SCFAs, especially butyrate. To clarify how cellobiose modulates intestinal inflammation, alterations in the microbiota and fecal concentrations of SCFAs should be investigated in cellobiose-treated mice.

In conclusion, a protective effect of cellobiose against DSS colitis suggests its clinical use for IBD patients.

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