Short-chain Inulin-like Fructans Reduce Endotoxin and Bacterial Translocations and Attenuate the Development of TNBS-induced Colitis in Rats

Hiroyuki Ito, M.S.[1], Hiroki Tanabe, Ph.D.[2], Hirokazu Kawakishi, Ph.D.[1], Wada Tadashi, Ph.D[3]., Tomono Yasuhiko, Ph.D[3]., Kimio Sugiyama, Ph.D.[2], Shuhachi Kiriyama, Ph.D.[4]
and Tatsuya Morita, Ph.D.[2]*

[1]Graduate School of Science and Technology, Shizuoka University, Shizuoka 422-8529, Japan

[2]Department of Applied Biological Chemistry, Faculty of Agriculture, Shizuoka University, 836 Ohya, Shizuoka 422-8529, Japan

[3]Fuji Nihon Seito Corporation, 1-4-10 Seikai, Shizuoka 424-8737, Japan.

[4]Faculty of Nutritional Sciences, University of Shizuoka, 52-1 Yada, Shizuoka 422-8526, Japan

*For correspondence at: Department of Applied Biological Chemistry, Faculty of Agriculture, Shizuoka University, 836 Ohya, Shizuoka 422-8529, Japan; Telephone/Fax: 81-54-238-5132

E-mail: actmori@agr.shizuoka.ac.jp

Running head: short-chain fructans and colitis

Abstract

Anti-inflammatory effects of short-chain inulin-like fructans (SCF) on trinitrobenzene sulfonic acid (TNBS)-induced colitis were investigated in rats, focusing specifically on endotoxin and bacterial translocations. SCF with degrees of polymerization (DP) of 4 and 8 were used. Rats were fed either control diet or diets including 60 g of DP4 or DP8/kg for 7 d, and then received intracolonic TNBS and were fed the respective diets for a further 10 d. DP4 and DP8 significantly reduced colonic injuries as assessed by damage score, but the reduction of colonic myeloperoxidase activity was manifest solely in DP8. At 3 d after colitis induction, bacterial translocation to the mesenteric lymph node was significantly lower in the DP4 and DP8 groups, but a significant reduction in the portal endotoxin concentration was achieved solely in the DP8 group. Immediately prior to colitis induction, cecal immunogloblin A and mucin concentrations were higher in the DP4 and DP8 groups, but these changes were abolished at 10d post-colitis induction. The data suggest that SCF exert prophylactic effects against TNBS colitis, presumably as a result of inhibitory effects on endotoxin and bacterial translocations.

KEY WORDS: short-chain fructans; endotoxin; bacterial translocation; mucin; colitis.

Introduction

Short-chain inulin-like fructans (SCF) have been identified as a potential treatment strategy for inflammatory bowel disease, with successful outcomes in trinitrobenzene sulfonic acid (TNBS)-induced colitis in rats [1, 2]. The mechanism involved in the anti-inflammatory action of these oligosaccharides are considered to have some general properties such as selective stimulation of lactobacilli and bifidobacteria, reduction of colonic pH, and the maintenance of mucosal integrity due to augmentation of cecal short-chain fatty acid (SCFA) production. However, the precise mechanism has not yet been fully elucidated.

Colitis, induced by intracolonic administration of TNBS in a vehicle of ethanol, is in part due to the caustic properties of this mixture, followed by exposure of the underlying lamina propria to bacterial components. The colitis is also partly due to the induction of an IL-12-driven inflammation with a Th1-mediated response to TNBS-modified proteins [3, 4, 5]. In rats, however, the inflammation pattern of the acute phase of colitis, at 1 to 2 wk after TNBS administration, has been shown to resemble non-specific colitis induced by intracolonic acetic acid administration [5, 6, 7]. Previous studies have shown that the severity of the acute phase of TNBS colitis is correlated with the concentration of systemic endotoxin as well as the extent of

bacterial translocation [8, 9]. Therefore, insofar as the acute phase of colitis is concerned, reinforcement of mucosal barrier function, including a reduction in colonic endotoxin concentration, may be an important factor in the protection against TNBS-induced colitis.

Previously, we examined cecal amounts of IgA and mucin in rats fed inulin-like fructans with different degrees of polymerization (DP) (average DP; 4, 8, 16, and 23). The results indicated that while cecal mucin was likely to respond to cecal SCFA, IgA increased when both fermentation occurred rapidly and lactate was a major fermentation product [10]. Consequently, the cecal concentration of mucin increased significantly in rats fed fructans with DP8, 16 and 23, whereas IgA was higher in rats fed those with DP4 and 8 [10]. With regard to the anti-inflammatory action against the acute phase of the TNBS-induced colitis model, both luminal mucin and IgA may play an important role in the protection against penetration of luminal bacteria and endotoxin, presumably by limiting their motility or access to the epithelial surface [11]. It is also possible that the mucus layer serves as a binding site for immunoglobulins, particularly for secretory IgA, and works in cooperation with IgA [12]. In this regard, we hypothesize that DP8 fructan, which has the potential to increase both luminal mucin and IgA, might be a good candidate for the reinforcement

of the mucosal barrier, thereby protecting against bacterial and endotoxin translocations.

In previous studies that examined the effects of prebiotics, including dietary fibers, the majority used the acute phase of TNBS colitis in their evaluations [1, 2, 13]. However, limited data are available in regards to the role of mucin and IgA in the protection against endotoxin and bacterial translocations. The purpose of the present study was to examine whether DP8 fructan reduces endotoxin and bacterial translocations and exerts a prophylactic effect on the acute phase of TNBS colitis in rats. The effects of DP8 were compared to an established positive reference in this model, DP4, which is virtually the same as fructooligosaccharides (FOS) [1, 2].

Methods

Materials

Inulin-like fructan, with an average degree of polymerization of 8 (DP8; range of DP, 5-13), was prepared by enzymatic synthesis using a novel fungal enzyme, β-fructosidase, from *Bacillus sp.* 217C-11 as described previously [14]. Fructooligosaccharides (DP4; average DP, 4) were purchased from Meiji Seika (Meioligo® P, Tokyo, Japan). The composition of FOS was 44% 1-kestose, 46% nystose, and 10% 1-f-β-fructofuranosyl nystose.

Animal care

Male F344 rats were purchased from Shizuoka Laboratory Animal Center (Hamamatsu, Japan). They were individually housed in screen-bottomed stainless steel cages in a temperature- (23 ± 2°C) and light- (lights on from 8:00 to 20:00) controlled room. For the purposes of adaptation, rats were fed a control diet for at least 3 d. This diet [15] was formulated from 250 g/kg casein, 652.25 g/kg cornstarch and 50g/kg corn oil. The remainder of the diet consisted of vitamins and minerals [15]. The rats were then divided into groups based on body weight and allowed free access to experimental diets and water. Body weight and food intake were recorded each morning before replenishing the diet. The study was approved by the Animal Use Committee of Shizuoka University, and the animals were maintained in accordance with the guidelines for the care and use of laboratory animals, Shizuoka University.

Time course studies on portal endotoxin concentration and bacterial translocation to the mesenteric lymph nodes (MLN) post-TNBS administration (preliminary study)

Thirty rats weighing 154 to 175 g (8 wks old) were used. All rats were fed the control diet throughout the experiment. After being fed control diet for 7 d, the rats were lightly anesthetized with diethyl ether and then treated with an intracolonic injection of 20% glycerin solution (0.2 ml/rat) using a

lubricated polypropylene catheter (diameter 1.5 mm) inserted 8 cm into the colon via the anus. Preliminary results indicated that this treatment was useful for removing the colonic contents. Usually, defecation was completed within 15 min, and the colon was kept empty for at least 60 min after treatment. At 60 min after administration of the glycerin solution, the rats were anesthetized with diethyl ether and given 30 mg of TNBS (dissolved in 0.25 ml of 50% ethanol (v/v)) via polypropylene catheter, as described above, inserted 8 cm through the anus. After instillation, the rats were kept in a vertical position for 30 s and returned to their cages. At 1 and 6 h and 1, 3 and 7 d after instillation, the rats were anesthetized with diethyl ether and underwent laparotomy under aseptic conditions. Portal blood (300 μl) was collected with a heparinized syringe. Following centrifugation at 2000 × *g* for 10 min, plasma was obtained and used for endotoxin measurement. Mesenteric lymph nodes were also collected from the ileo-cecal junction at 1, 3 and 7 d after instillation and used for bacterial translocation assessment.

Cecal fermentation, mucin and IgA in rats fed the respective diets pre-TNBS administration (experiment 1)

Twenty-four rats weighing 148 to 169 g (8 wk old) were acclimatized, then divided into 3 groups of 8 rats and allowed free access to control or experimental diet (60 g of DP4 or DP8/kg). Each of the test materials was substituted with the same amount of cornstarch as in the control diet. After being

fed the respective diets for 7 d, the rats were anesthetized with diethyl ether, and the cecum excised. The cecal contents were removed, weighed and divided into two portions. One was freeze-dried and used for mucin analysis, and the other was used for the measurement of pH, organic acids and IgA.

Protective effects of DP4 and DP8 ingestion against TNBS-induced colitis (experiment 2)

Forty-two rats weighting 149 to 177 g (8 wks old) were acclimatized, then divided into 3 groups of 14 rats and allowed free access to the same experimental diets as in experiment 1. After being fed the respective diets for 7 d, the rats were administrated 20% glycerin, followed by TNBS/50% ethanol in the same manner as in the preliminary experiment. At 3 d after instillation, 6 rats from each group were anesthetized with diethyl ether, and portal blood and MLN were collected in the same manner as in the preliminary experiment. The remaining 8 rats from each group continued to be fed the respective diets for 10 d after colitis induction. Feces were collected during the last 3 d. The fecal samples were prepared in the same manner as cecal contents and used for the analysis of mucin and IgA. At the end of the test period, the rats were anesthetized with diethyl ether, and the cecum and colon were excised. The cecal contents were handled in the same manner as experiment 1. The colon was cut open longitudinally and the colonic contents were removed. The colon was then weighed, length measured and

scored for macroscopically visible damage on a 0-10 scale by two observers unaware of the treatment, according to the criteria described by Bell et al. [16] (**Table 1**). The respective whole colon specimens were divided longitudinally into two fragments. One fragment was stored at -80°C pending measurement of myeloperoxidase (MPO) activity, and the other fragment was fixed with 10% neutral buffered formalin and embedded in paraffin. The sections embedded in paraffin were cut at 4 μm and stained with periodic acid-Schiff.

Portal endotoxin

Portal endotoxin was determined by the limulus amebocyte lysate test [17], which involves a turbidimeteric time assay at 450 nm with a toxinometer ET-2000 (Wako Pure Chemicals, Osaka, Japan). The plasma sample was diluted ten-fold with sterile water for injection (Otsuka Pharmaceutical Factory, Inc., Tokushima, Japan) and heated at 80°C for 5 min to deactivate the lipopolysaccharide binding protein. The sample was then mixed with limulus reagent (Wako Pure Chemicals, Osaka, Japan) and applied to toxinometer analysis. Endotoxin prepared from *Escherichia coli* O113:H10 (Wako Pure Chemicals, Osaka, Japan) was used as the standard.

Bacterial translocation to MLN

MLNs were minced and homogenized in 0.5 mL of sterile Brain Heart Infusion (BHI) broth (Wako Pure Chemicals, Osaka,

Japan) by a hand-operated Polytron homogenizer. The homogenate (0.1 mL) was inoculated onto BHI agar plates (Wako Pure Chemicals, Osaka, Japan), which were subsequently incubated under either aerobic or anaerobic conditions at 37°C for 72 h. Duplicate assay was conducted. After incubation, colonies were counted and the microorganisms were quantified as colony forming units per gram (log_{10}CFU/g).

Cecal pH and organic acids

After the cecal contents were homogenized, a portion of the homogenate was diluted with an equal weight of distilled water, and cecal pH was measured with a compact pH meter (Model C-1, Horiba, Tokyo, Japan). Cecal organic acids were measured by the internal standard method [18] using HPLC (LC-10A, Shimadzu, Kyoto, Japan) equipped with a Shim-pack SCR-102H column (8 mm i.d. × 30 cm long, Shimadzu) and an electroconductibity detector (CDD-6A, Shimadzu).

Secretory IgA

Cecal IgA was determined by enzyme-linked immunosorbent assay using Nunc-Immuno plates (MaxiSorb F96) and a slight modification [19] of the method described by Grewal et al [20]. Assays were conducted in duplicate.

O-linked oligosaccharide chains

Mucins were extracted by the method of Bovee-Oudenhoven et al. [21] with some modifications [22]. *O*-linked oligosaccharide chains were determined as a mucin marker. After an appropriate

dilution of the mucin fraction, *O*-linked oligosaccharide chains were measured using a fluorimetric assay [23] that discriminated *O*-linked glycoproteins (mucin) from *N*-linked glycoproteins, as described by Bovee-Oudenhoven et al. [21]. Standard solutions of *N*-acetylgalactosamine (Sigma, St. Louis, MO, USA) were used to calculate the quantity of oligosaccharide chains liberated from mucins during the procedure.

Myeloperoxidase activity

Myeloperoxidase activity was determined by the method of Bradley et al. [24]. Briefly, the colon was minced and homogenized in 50 mmol/l potassium phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethylammoniumbromide (SIGMA, St Louis, MO, USA) by a polytron homogenizer. Homogenate was subjected to three cycle of freeze-thawing and sonication, and centrifuged at 20,000 × *g* for 30 min. The supernatant was used to determine MPO activity utilizing 0.0005% hydrogen peroxide as a substrate for the MPO. A unit of MPO activity was defined as that converting 1 μmol of hydrogen peroxide to water per 1 min at 25°C.

Statistical analyses

Data were analyzed by one-way analysis of variance (ANOVA), and significant differences among means were separated by the Tukey-Kramer test. When variances were not homogeneous by the Bartlett test [25], data were transformed logarithmically and

then analyzed by ANOVA followed by multiple comparisons, or analyzed by the Steel-Dwass test. Normally, results were expressed as means with SEM, otherwise as median and range for non-parametric data, and all statements of significant differences reflected the 5% level of probability. The Tukey-Kramer test and the Bartlett test were performed using StatView 5.0 computer software (SAS Institute, Cary, N.C., U.S.A.), and the Steel-Dwass test and linear regression analyses were performed using Excel Statistics program (version 6.0; Esumi, Tokyo, Japan).

Results

Portal endotoxin and bacterial translocation post-TNBS administration (preliminary experiment)

Before TNBS administration, the average body weight of rats was 185 ± 2. After TNBS administration, food intake and body weight decreased sharply for the first 3d and then gradually recovered in the following days (data not shown). Portal endotoxin was detected beginning at 1 h after TNBS administration, reached maximum at d 3, and then decreased at d 7. Bacterial counts in MLN reached maximum at d 3, and this level persisted at d 7 (**Figure 1**).

Cecal fermentation, mucin and IgA pre-TNBS administration

(experiment 1)

Food intake and body weight gain were significantly lower in rats fed the DP4 and DP8 diets than in those fed the control diet, however, no significant differences were detected between the DP4 and DP8 diet-fed groups. The weights of cecal tissue and cecal contents in rats fed the DP4 and DP8 diets were significantly higher than in those fed the control diet. Cecal pH declined significantly in the DP4 and DP8 diet-fed groups (**Table 2**). The cecal concentrations of propionate, butyrate and lactate differed among the groups. Propionate and butyrate concentrations were higher in rats fed the DP4 and DP8 diets, respectively. Cecal concentration of mucin was highest in the DP8, intermediate in the DP4, and lowest in the control diet-fed groups. Cecal concentration of IgA in rats fed the DP4 and DP8 diets was significantly higher than in those fed the control diets, however, there were no differences detected between the DP4 and DP8 diet-fed groups (**Table 2**). Linear regression analysis showed that the cecal concentrations of mucin and IgA were significantly correlated with the cecal concentrations of butyrate (r=0.57, P=0.004) and lactate (r=0.57, P=0.003), respectively. No other correlations among the cecal concentrations of organic acid, mucin and IgA were significant (data not shown) in the present study.

Protective effects of DP4 and DP8 ingestion against TNBS-induced colitis (experiment 2)

Prior to TNBS administration, daily food intake and body weight were significantly lower in rats fed the DP4 and DP8 diets than in those fed the control diet, however, no differences were detected between the DP4 and DP8 diet-fed groups (**Figure 2-a, b**). Post-TNBS administration, food intake and body weight in all dietary groups decreased drastically for the first 3 d, and then gradually recovered in the following days. From d5 to d10 after TNBS administration, food intake in rats fed the DP4 and DP8 diets was significantly greater than in those fed the control diet, and this difference was reflected in the recovery rate of body weight gain among the groups (**Figure 2-a, b**).

At d 3 post-TNBS administration, portal endotoxin was detected in all rats (6/ 6 rats) fed control diet and showed an average concentration of 6.9 ± 0.9 pg/ml. However, the detection ratio in rats fed DP4 and DP8 diets was reduced to 4/6 and 1/6, respectively. Further, portal endotoxin concentration in rats fed DP8 diet was significantly lower than in those fed the control and DP4 diets (**Figure 3-a**). Bacterial translocation into the MLN in rats fed DP4 and DP8 diets was significantly reduced by 70% as compared to that in rats fed control diet. (**Figure 3-b**).

At autopsy, the colonic mucosa in rats fed the control diet was severely inflamed, as indicated by the macroscopic damage score, relative colon weight and colonic MPO activity. Compared with the control group, a significant reduction in the

macroscopic damage score and relative colon weight was observed in the DP4 and DP8 groups, while MPO activity was significantly decreased solely in the DP8 group (**Figure 4-a, b, c**).

Even after TNBS administration, the weights of cecal tissue and cecal contents in rats fed the DP4 and DP8 diets were significantly higher than in those fed the control diet. A weak but significant decline of cecal pH was observed in the DP4 and DP8 diet-fed groups (**Table 3**). However, in contrast to the findings obtained with rats before TNBS administration, cecal concentrations of organic acids, mucin and IgA were comparable among the groups. Fecal IgA concentration also did not differ among the groups, but fecal mucin in rats fed the DP8 diet was significantly greater than in those fed the control diet (**Table 3**). The histological appearance of inflammatory lesions showed that as well as an intensive infiltration of granulocytes into the mucosal tissue, loss of goblet cells was manifest in rats fed the control diet. Compared with those fed control diet, the mucosal integrity was apparent from the morphological features of goblet cells in rats fed DP4 and DP8 diets (**Figure 5-a, b, c**).

Discussion

As expected from the previous study [10], prior to induction of colitis, cecal concentration of mucin was highest in the DP8,

intermediate in the DP4, and lowest in the control diet-fed groups, whereas those of IgA in rats fed either DP4 or DP8 diet were elevated to an equal extent compared to those fed the control diet (**Table 2**). Pretreatment with DP4 or DP8, initiated 7 d prior to TNBS administration, reduced colonic inflammation at 10d post-TNBS administration, as assessed by the macroscopic damage score, relative colon weight and colonic MPO activity (a marker of neutrophil infiltration) (**Figure 4**). Furthermore, bacterial translocation to the MLN at 3d post-colitis induction was significantly reduced in rats fed either the DP4 or DP8 diet as compared to those fed the control diet, whereas a significant reduction in the portal endotoxin concentration was observed only in rats fed the DP8 diet (**Figure 3**). To our knowledge, this is the first time that the ingestion of SCF reduced the systemic endotoxin concentration in TNBS-colitis rats.

It has been shown that TNBS itself deteriorates mucosal barrier function by interacting with surface-active phospholipids of the colonic mucosa [3]. Shortly after intracolonic administration, TNBS reduces surface hydrophobicity and increases tissue susceptibility to bacteria and endotoxin, leading to an inflammatory cascade, including the release of inflammatory cytokines such as IL-1β, IL-6, and TNF-α [6, 9]. The present results, in this model, are largely in accordance with the findings of Gardiner et al. [8], reporting that the severity of

acute phase inflammation in TNBS colitis was correlated with the degree of bacterial translocation and the concentration of systemic endotoxin. In fact, the ingestion of DP8, which dramatically reduced the portal endotoxin concentration, as well as bacterial translocation to the MLN (**Figure 3**), showed the strongest inhibitory effects on the induction of colonic MPO activities (**Figure 4-C**). The precise mechanism by which DP8 ingestion reduced portal endotoxin concentration is not fully elucidated. However, Enss et al. [26] showed that mucins attached to the epithelial surface, as well as mixed to luminal contents, possessed a binding capacity for *E. coli* that may act as an endotoxin reservoir. Because the cecal mucin concentration immediately prior to TNBS administration was highest in rats fed DP8 diet (**Table 2**), it is possible to assume that the increased concentration of cecal mucin could be responsible for decreasing the permeability to endotoxin. Another possible explanation may exist in the lactic acid-bacteria inducing property of SCF [27, 28]. Bifidobacteria have been shown to reduce intestinal endotoxin levels [29, 30], while it has been suggested that lactobacilli possess the potential ability to bind endotoxin [31]. It is also reasonable to assume that higher concentrations of the cecal IgA in rats fed the DP4 and DP8 diets may cooperate with luminal mucin and contribute to protect the bacterial translocation to MLN (12).

Interestingly, at 10 d post-TNBS administration, the beneficial effects of DP4 and DP8 on cecal SCFA, lactate, pH, mucin and IgA were totally abolished, and there were no differences in these variables among the groups. There is a number of evidence that induction of colitis by this method is associated with a significant increase in the number of aerobic Gram-negative bacilli in the large bowel (32, 33). This might affect the fermentation pattern of SCF and lead the decreased concentrations of cecal SCFA, mucin, and IgA. Our results differ from those of Cherbut et al. [1], showing lower pH, and higher lactate and butyrate in rats fed FOS (virtually the same as DP4) at 7 d post-TNBS administration. The reason for this remains unclear, but could be partly explained by differences in the DP4 treatment method (i.e., dietary inclusion in the present study or intragastric infusion) [1]. Nevertheless, the lack of beneficial effects on cecal variables post-TNBS administration suggests that the anti-inflammatory effects of DP4 and 8 might be exerted through a shield-like effect against endotoxin and bacterial translocations at the very early stage of TNBS colitis, leading to reduced colonic damage at 10 d post-TNBS administration. Consequently, at least under the present experimental condition, the anti-inflammatory effects of SCF are likely to be prophylactic.

Generally, two mechanisms have been considered in explaining

the beneficial effects of SCF on colonic inflammation: changes in the intestinal microflora, which stimulate selective growth of lactic acid-bacteria, and an increase in colonic SCFA concentration [1, 2, 34]. In this regard, Cherbut et al. [1] suggested that the capacity of FOS to increase lactobacilli counts was the main mechanism explaining its anti-inflammatory effect, rather than the increment in SCFA production. Indeed, lactic acid-bacteria evoke a local immune stimulus to increase the levels of luminal secretory IgA [10, 35] and anti-inflammatory cytokine, like IL-10 [36]. Furthermore, the selective growth of lactic acid-bacteria could reduce the number of Gram-negative bacilli that may serve as an endotoxin reservoir, as discussed above. While, the present results suggest that the protective effects against endotoxin influx by DP8 ingestion are likely to be largely dependent on the SCFA-stimulated increases in cecal mucin secretion (particularly butyrate) [32, 37, 38]. In fact, cecal mucin concentration in rats fed the control, DP4 and DP8 diets was significantly correlated with cecal butyrate concentration (**Table 2**). Therefore, it is possible to consider that an increase in colonic butyrate may also be necessary for the anti-inflammatory effects of SCF.

In conclusion, both the SCF of DP8 and DP4 exerted a prophylactic effect on the acute phase of TNBS-induced colitis

in rats, possibly through reduction of bacterial and endotoxin translocations to the MLN. Compared with DP4, the greater inhibitory effect of DP8 on endotoxin influx from the intestine might be linked to the greater anti-inflammatory effects of DP8, presumably as a result of a greater concentration of cecal mucin.

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1

Table 1
Criteria for assessment of macroscopic damage score

Score	Criteria
0	No damage
1	Hyperemia, no ulcers
2	Linear ulcer with no significant inflammation
3	Linear ulcer with inflammation at one site
4	Two or more sites of ulceration/inflammation
5	Two or more sites of ulceration and inflammation or one site of ulceration/inflammation extending >1 cm along the length of the colon
6-10	If damage covers >2 cm along the length of colon, the score is increased by 1 for each additional centimeter of involvement

Table 2
Food intake and body weight gain, and cecal concentrations of organic acids, mucin and IgA in rats fed the respective diet for 7d (experiment 1)

	Control	DP4[1]	DP8
Food intake, g/7 d	118.8 ± 2.4[b]	100.2 ± 2.4[a]	99.1 ± 2.9[a]
Body weight gain, g/7 d	29.9 ± 1.5[b]	24.1 ± 1.7[ab]	21.2 ± 1.8[a]
Cecum			
Tissue, g	0.4 ± 0.0[a]	1.0 ± 0.1[b]	1.0 ± 0.0[b]
Contents, g	1.7 ± 0.0[a]	3.5 ± 0.3[b]	3.0 ± 0.1[b]
pH	7.4 ± 0.1[b]	6.0 ± 0.1[a]	5.9 ± 0.1[a]
Organic acids, µmol/g			
Acetate	38 (31-53)	20 (9-89)	30 (16-80)
Propionate	14 (11-15)[a]	22 (14-82)[b]	21 (11-59)[ab]
n-Butyrate	3 (2-3)[a]	6 (2-25)[ab]	15 (9-27)[b]
Total SCFA[2]	54 (48-67)	49 (26-196)	61 (45-164)
Lactate	0 (0-18)[a]	38 (13-102)[b]	24 (0-127)[b]
Succinate	16 ± 5	16 ± 5	26 ± 6
Mucin, µmol/g	0.4 ± 0.0[a]	0.7 ± 0.1[b]	1.2 ± 0.2[c]
IgA, µg/g	132.8 ± 23.8[a]	793.5 ± 169.3[b]	542.3 ± 99.7[b]

Data are expressed as mean ± SE or median (range), n=8. Values not sharing a common superscript letter are significantly different when analyzed by the Tukey-Kramer test (parametric data) or the Steel-Dwass test (non-parametric data).
[1]Degree of polymerization. [2]Sum of acetate, propionate and n-butyrate.

Table 3
Cecal concentrations of organic acids, mucin and IgA, and fecal concentrations of mucin and IgA in rats fed the experimental diets at 10d after TNBS administration (experiment 2)

	Control	DP4[1]	DP8
Cecum			
Tissue, g	0.5 ± 0.0[a]	0.9 ± 0.0[b]	0.9 ± 0.0[b]
Contents, g	1.9 ± 0.1[a]	4.3 ± 0.3[b]	4.9 ± 0.2[b]
pH	7.8 ± 0.1[b]	7.4 ± 0.1[a]	7.3 ± 0.1[a]
Organic acids, µmol/g			
Acetate	60 ± 8	52 ± 9	57 ± 3
Propionate	46 (11-58)[ab]	26 (20-75)[b]	19 (15-25)[a]
n-Butyrate	8 (2-15)	9 (7-20)	7 (5-9)
Total SCFA[2]	105 ± 17	102 ± 14	83 ± 5
Lactate	0	0	0
Succinate	13 ± 4	23 ± 4	20 ± 7
Mucin, µmol/g	0.4 ± 0.1	0.5 ± 0.0	0.5 ± 0.0
IgA, µg/g	103 ± 9	55 ± 4	61 ± 6
Feces			
Mucin, µmol/g	1.2 ± 0.1[a]	1.6 ± 0.2[ab]	2.0 ± 0.2[b]
IgA, µg/g	79 ± 23	64 ± 18	47 ± 11

The data are expressed as mean ± SE or median (range), n=8. Values not sharing a common superscript letter are significantly different when analyzed by the Tukey-Kramer test (parametric data) or the Steel-Dwass test (non-parametric data). [1]Degree of polymerization. [2]Sum of acetate, propionate and n-butyrate.

Figure legend

Figure 1.
Changes in portal endotoxin concentration (a) and bacterial translocation to MLN (b) in rats post-TNBS administration
Data are expressed as mean ± SE (n=5).
N.D.: not detected.

Figure 2.
Changes in body weight (a) and daily food intake (b) in rats fed the respective diets pre- and post-TNBS administration
Data are expressed as mean ± SE (n=8).
* $P<0.05$ vs. DP4. †$P<0.05$ vs. DP8.

Figure 3.
Portal endotoxin concentration (a) and bacterial translocation to MLN (b) in rats fed the respective diets at 3 d post-TNBS administration
Data are expressed as mean ± SE, values with different superscript letters are significantly different when analyzed by one-way ANOVA, followed by Tukey Kramer.

Figure 4.
Macroscopic damage score (a), relative colon weight (b) and MPO activity (c) in rats fed the respective diets at 10 d post-TNBS administration
Data are expressed as mean ± SE (n=8), values with different superscript letters are significantly different when analyzed by one-way ANOVA, followed by Tukey Kramer.

Figure 5.
Histological appearance of colonic lesions in rats fed the respective diets at 10 d post-TNBS administration
a, control; b, DP4; c, DP8

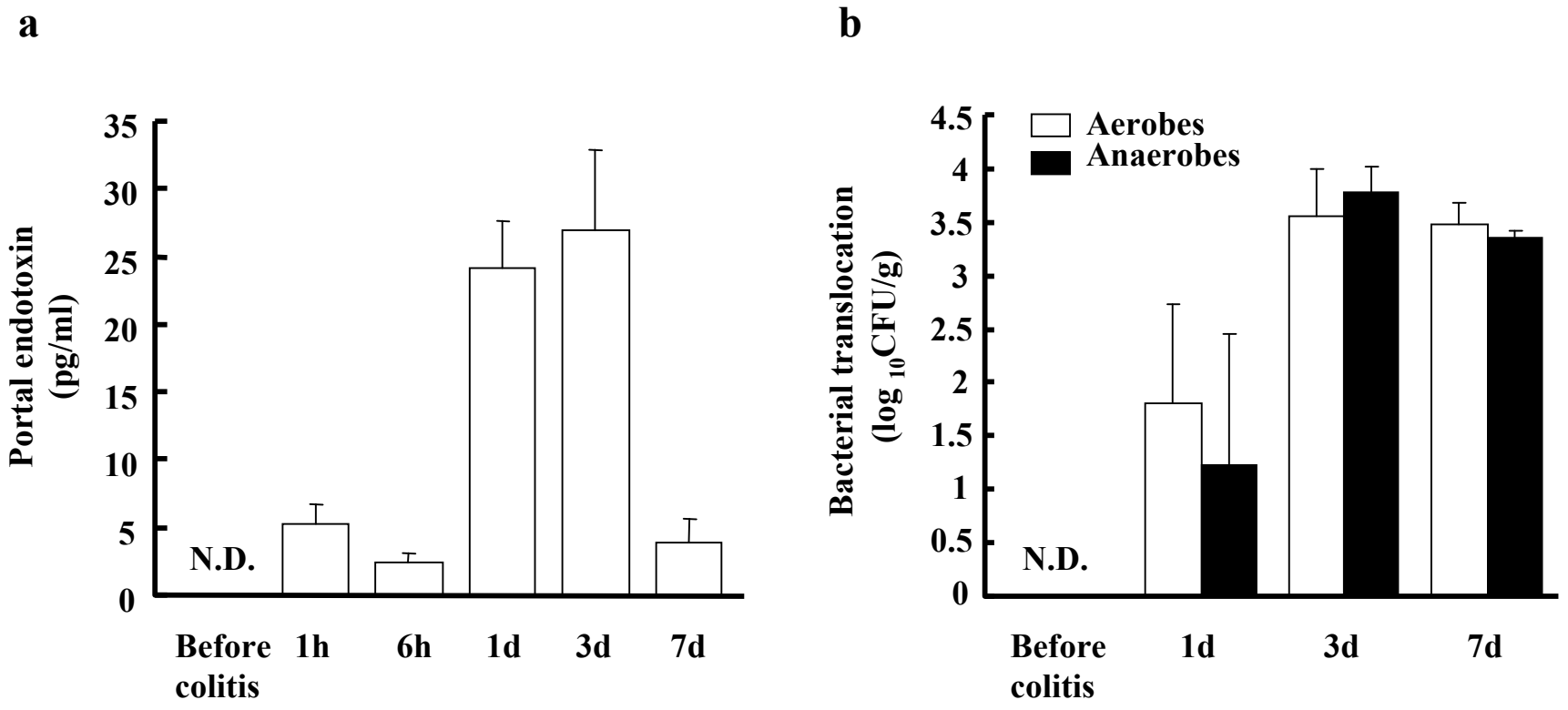
a
Portal endotoxin (pg/ml)
35
30
25
20
15
10
5
0
N.D.
Before colitis
1h
6h
1d
3d
7d
b
Bacterial translocation (log 10CFU/g)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Aerobes
Anaerobes
N.D.
Before colitis
1d
3d
7d

Fig.1

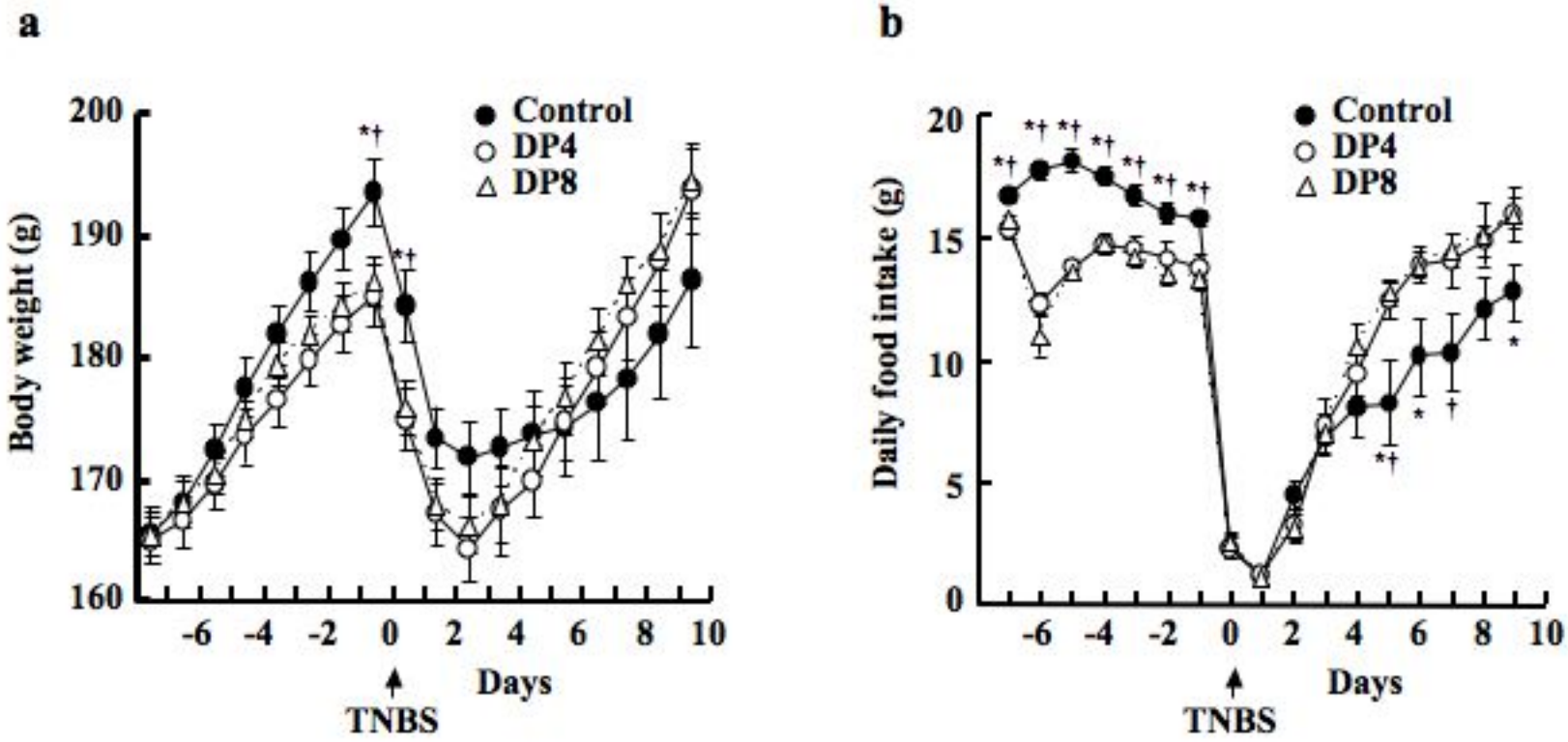
a
Body weight (g)
200
190
180
170
160
Control
DP4
DP8
*†
-6 -4 -2 0 2 4 6 8 10
Days
TNBS
b
Daily food intake (g)
20
15
10
5
0
Control
DP4
DP8
-6 -4 -2 0 2 4 6 8 10
Days
TNBS

Fig.2

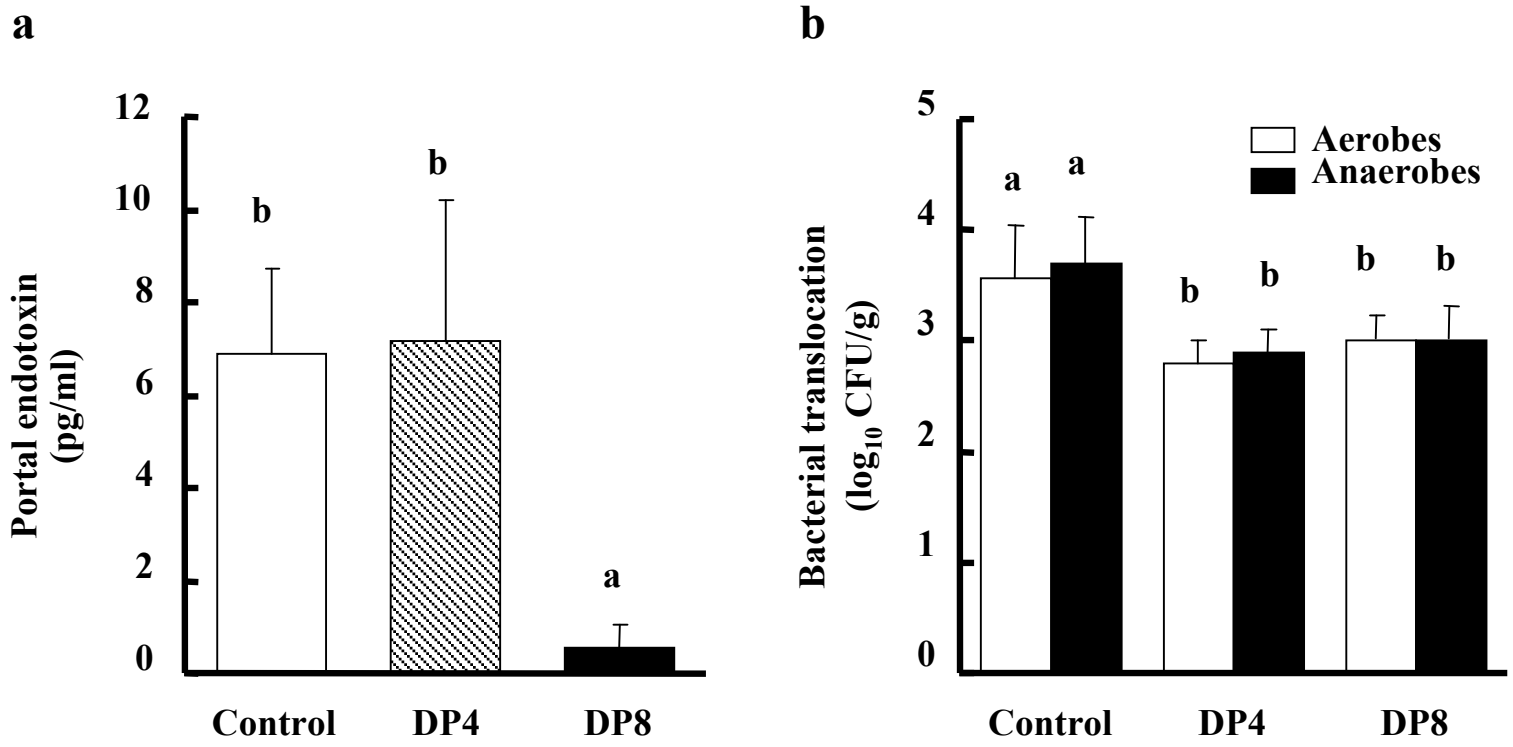
a
Portal endotoxin (pg/ml)
12
10
8
6
4
2
0
b
b
a
Control
DP4
DP8
b
Bacterial translocation (log_{10} CFU/g)
5
4
3
2
1
0
Aerobes
Anaerobes
a
a
b
b
b
b
Control
DP4
DP8

Fig.3

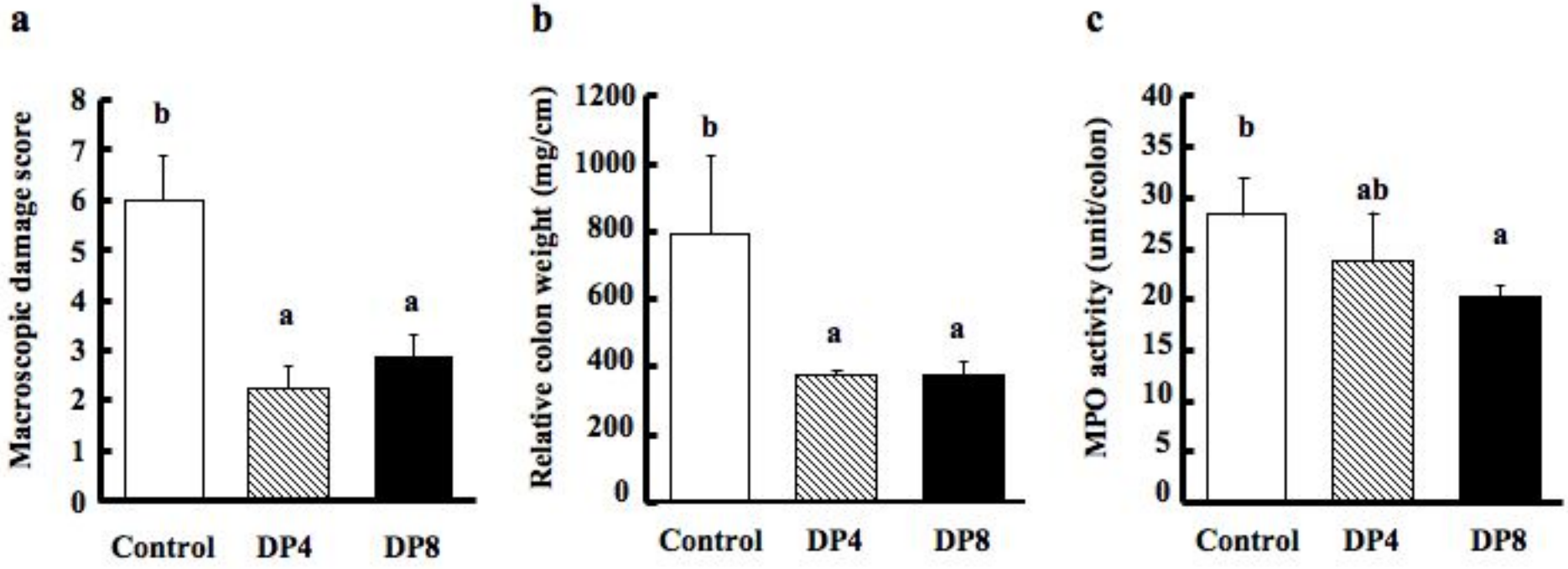
a
Macroscopic damage score
0
1
2
3
4
5
6
7
8
b
a
a
Control
DP4
DP8
b
Relative colon weight (mg/cm)
0
200
400
600
800
1000
1200
b
a
a
Control
DP4
DP8
c
MPO activity (unit/colon)
0
5
10
15
20
25
30
35
40
b
ab
a
Control
DP4
DP8

Fig.4

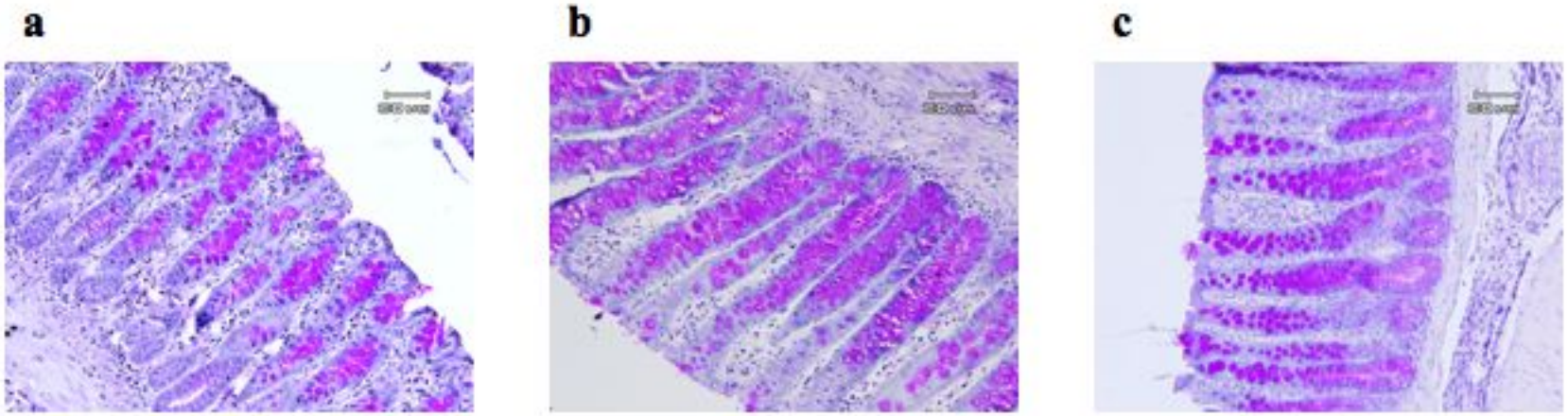

Fig.5