Biosci. Biotechnol. Biochem., **77** (8), 1782–1784, 2013

Note

Non-Effect of an Antibiotic Treatment on Dietary Fiber-Induced Goblet Cell Proliferation in the Ileum of Rats

Shingo HINO, Hiroyoshi SAITA, and Tatsuya MORITA†

Department of Applied Biological Chemistry, Faculty of Agriculture, Shizuoka University, 836 Ohya, Suruga-ku, Shizuoka 422-8529, Japan

Received March 21, 2013; Accepted April 15, 2013; Online Publication, August 7, 2013
[doi:10.1271/bbb.130241]

We examined the effect or not of an antibiotic treatment on dietary fiber-induced goblet cell proliferation in the rat ileum. The number of goblet cells in the ileum increased when rats consumed dietary fiber. However, this effect was maintained with a concurrent treatment of antibiotics, suggesting that the fiber effect on goblet cell response would remain irrespective of a bacterial component such as endotoxin.

Key words: goblet cell; dietary fiber; endotoxin; rat

The surface of the small intestine is covered with a layer of mucus to prevent luminal insults. Mucin secreted by goblet cells is a major component of this mucus. A change in the number of goblet cells is therefore considered to influence the intestinal barrier function. Our series of studies has shown that dietary fiber (DF) up-regulated the secretion of small intestinal mucins in proportion to either the bulk-forming property or viscosity of DF ingested.[1–5] The increase in mucin content of the small intestine has appeared to be due to the proliferation of goblet cells.[2–5] However, the underlying mechanism responsible for the increase in number of goblet cells by DF ingestion is unclear.

Both bulky and viscous DFs are supposed to increase the resistance to peristaltic contractions. This is likely to lead to an increase in intra-luminal pressure.[6] Yamada *et al.*[7] have shown that high intra-luminal pressure (40 cm of H_2O) in the rat ileum stimulated the translocation of fluorescein isothiocyanate-labeled endotoxin from the intestinal lumen to the blood circulation. Another study has shown that the oral administration of endotoxin (35 μg/100 g of body weight) to germ-free rats increased the number of goblet cells in the colon.[8] Furthermore, although goblet cell proliferation by DF ingestion was accompanied by an accelerated epithelial turnover,[2,5] Goodlad *et al.* have indicated that this accelerated turnover by DF was inhibited by the absence of luminal bacteria.[9] Indeed, the proportion of goblet cells among epithelial cell types increased caudally from the duodenum (4%) to distal colon (16%) in proportion to the number of luminal bacteria.[10] These findings prompted us to hypothesize that elevated intra-luminal pressure by DF ingestion may induce the translocation of endotoxin or other bacterial components in the ileum and thereby stimulate goblet cell proliferation.

We treated rats in the present study with antibiotics to reduce the endogenous endotoxin concentration in the intestine and examined whether the effect of DF ingestion on the number of goblet cells in the rat ileum would be preserved with an antibiotic treatment. We first measured the cecal concentrations of endotoxin and organic acids in rats treated with antibiotics (experiment 1). Male Wistar rats purchased from Shizuoka Laboratory Animal Center (Japan) were individually housed in screen-bottomed stainless-steel cages in a room with controlled temperature (23 ± 2 °C) and lighting (lights on from 07:00 to 19:00) under the approval of the Animal Use Committee of Shizuoka University. The rats were acclimatized for 3 d and fed a control diet[11] formulated from 250 g/kg of casein, 652.5 g/kg of cornstarch and 50 g/kg of corn oil. The remainder of the diet consisted of choline bitartrate (2.5 g/kg), an AIN-76 vitamin mix (10 g/kg) and AIN-76 mineral mix (35 g/kg). The body weight and food intake were recorded every morning before replenishing the diet. Twelve rats, weighing 141–160 g (6 weeks of age), were assigned to 2 groups of 6 rats each and were allowed free access for 7 d to the control diet in the presence or absence of an antibiotic cocktail (50 kU/L of benzyl penicillin potassium, 2000 mg/L of neomycin sulfate, and 500 mg/L of cefoperazone sodium) in drinking water. At the end of the experiment, the rats were killed by decapitation, and the cecal contents were collected and analyzed for the concentrations of endotoxin (the limulus amebocyte lysate test[12]) and organic acids (HPLC method[11]). The sum of formate, acetate, propionate, *i*-butyrate, *n*-butyrate, succinate, lactate, *i*-valerate and *n*-valerate is defined as the total organic acids.

The antibiotic treatment lowered the food intake, but did not affect the body weight gain. The cecal concentration of endotoxin in the presence of antibiotics was reduced by more than 80%, while the cecal organic acids were almost eliminated. The results show that the antibiotic treatment for 7 d was sufficient to suppress the microbial activity and reduce the endotoxin concentration (Table 1).

We next examined the effect of DF ingestion on the number of goblet cells in the ileum at the time of reduced bacterial activity (experiment 2). Polystyrene foam (PSF, JSP Co., Japan) with a settling volume of 13 mL/g was used as a DF source, and was added to the control diet at the expense of an equal amount of

† To whom correspondence should be addressed. Tel/Fax: +81-54-238-5132; E-mail: atmorit@ipc.shizuoka.ac.jp
Abbreviations: DF, dietary fiber; PSF, polystyrene foam

cornstarch (the 80 g/kg diet). Twenty-four rats, weighing 141–162 g (6 weeks of age), were assigned to 2 groups of 12 rats each and fed the control diet in the presence or absence of antibiotics for 7 d (the former period). The rats were then subdivided into 2 groups and further fed the control diet or PSF-added diet for 7 d (the latter period). There were thus 4 groups of 6 rats; the control and PSF groups in the presence (+) or absence (−) of the antibiotic treatment. The rats were finally killed by decapitation, and the small intestine was excised. The mid-portions of the ileum segments were placed in 10% buffered formalin, and the sections were stained by periodic acid-Schiff. The terminal ileum contents (a 5-cm segment from the ileocecal valve) were collected by flushing with 5 mL of endotoxin-free water to determine the endotoxin concentration. The analysis of cecal variables was performed as described for experiment 1.

In the former period, a significant reduction in food intake was manifest in the presence of antibiotics (82 ± 3 g *vs.* 107 ± 5 g), although the body weight gain did not differ, presumably due to the different cecum size as expected from the results of experiment 1. Upon entering the latter period, the food intake in the antibiotic-treated PSF group was sharply restored, while the growth rate was almost parallel with the other groups after a transient body weight loss (Fig. 1). Two-way ANOVA showed that both factors (PSF and antibiotics) and their interaction were all significant in the number of goblet cells per villus in the ileum. In the absence of antibiotics, PSF ingestion significantly increased the goblet cell number per villus, although this effect was apparently cancelled in the presence of antibiotics (Table 2). However, both factors of PSF and antibiotics independently affected the villus height, the villus height in the antibiotic-treated PSF group being the lowest among all the groups. Consequently, despite the presence of antibiotics, the goblet cell number per villus height (100 μm) was significantly higher in the PSF group than in the control group. Two-way ANOVA showed that only the PSF factor, and not the antibiotics factor was significant. The analysis of luminal bacterial activities showed significant reductions in the ileum endotoxin and cecal organic acid concentrations, as in the case of experiment 1.

We focused on the ileum endotoxin concentration in the present study, because increased luminal pressure has enhanced endotoxin permeability in the ileum by stimulating serotonin release into the lumen[6] that may be related to goblet cell proliferation. The diets used in this study contained much less endotoxin (control, 0.25 ± 0.07; PSF, 0.17 ± 0.09 μg/g) than the ileum endotoxin concentration (Table 2). Endotoxin contamination in the diets is consequently unlikely to hinder our intentions in the present study. However, in contrast to our hypothesis that an increase in the number of ileum goblet cells by DF ingestion might be associated with such a luminal bacterium component as endotoxin, the DF effect was unaffected by the antibiotic treatment in the rat ileum, even with a low endotoxin concentration. Only one study is currently available to indirectly support the present findings, McCullough *et al.*[13] showing that an elemental diet containing 30% fiber (a 1:9 mixture of psyllium and wheat bran) increased the number of goblet cells in the small intestine of germ-free rats. Although they did not refer to the rat growth and food intake in their study, it is obvious that rats fed the 30% fiber diet could not meet the calorie requirement for normal growth by compensatory eating of the diet. It is clear that a further study on germ-free rats under proper dietary conditions will be needed to completely discount the relevance of microbial components in the goblet cell response of rats fed DF.

Table 1. Food Intake, Body Weight Gain and Cecal Variables for Rats Fed the Control Diet in the Presence or Absence of Antibiotics in Drinking Water for 7 d (experiment 1)[1]

	− Antibiotics	+ Antibiotics
Food intake (g/7 d)	107 ± 5	82 ± 3*
Body weight gain (g/7 d)	38 ± 3	32 ± 3
Cecum		
Contents (g)	1.7 ± 0.1	11.1 ± 0.6*
Tissue weight (g)	0.5 ± 0.0	1.1 ± 0.0*
Endotoxin (μg/g of contents)	23 ± 2.7	3.6 ± 0.4*
Total organic acids (μmol/g of contents)[2]	68.6 (52.3–76.6)	0.3 (0.1–0.6)†

[1]Data are expressed as the mean ± SE, $n = 6$ and analyzed by Student's *t*-test. *, $p < 0.05$.
[2]Data are expressed as the median (range), $n = 6$ and analyzed by Welch's *t*-test. †, $p < 0.05$.

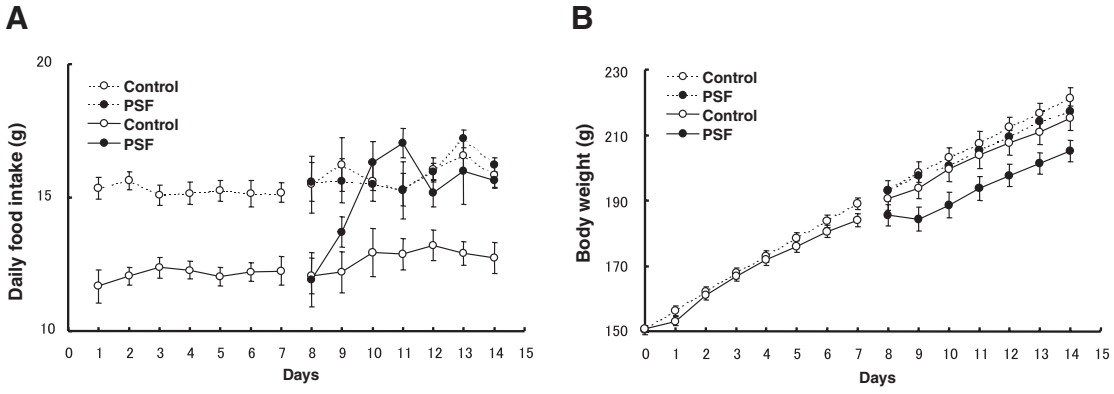

Fig. 1. Daily Food Intake (A) and Body Weight (B) for Rats Fed the Control Diet or PSF Diet for 14 d in the Presence or Absence of Antibiotics in Drinking Water (experiment 2).
Data are expressed as the mean ± SE; $n = 12$ (d 0–d 7) or $n = 6$ (d 8–d 14). ○, control diet; ●, PSF diet; —, in the presence of antibiotics; - - -, in the absence of antibiotics.

Table 2. Food Intake, Body Weight Gain, Number of Goblet Cells and Villus Height in the Ileum, Ileum Endotoxin Concentration, and Cecal Variables in Rats Fed the Control or PSF Diet in the Presence (+) or Absence (−) of Antibiotics in Drinking Water for the Latter 7 d (experiment 2)[1]

	− Antibiotics		+ Antibiotics		Two-way ANOVA, p value		
	Control	PSF	Control	PSF	PSF	Antibiotics	Interaction
Food intake (g/latter 7 d)	111 ± 5	111 ± 2	89 ± 4	106 ± 3	<0.05	<0.01	<0.05
Body weight gain (g/latter 7 d)	31 ± 1	29 ± 1	29 ± 2	23 ± 0	<0.01	<0.01	0.20
Ileum							
Goblet cells (n/villus left side)	8.9 ± 0.3	13 ± 0.4	8.9 ± 0.3	9.2 ± 0.2	<0.01	<0.01	<0.01
Villus height (μm)	386 ± 4	354 ± 6	337 ± 13	296 ± 8	<0.01	<0.01	0.61
Goblet cells (n/villus (100 μm))	4.6 ± 0.2	7.1 ± 0.2	5.3 ± 0.2	6.2 ± 0.2	<0.01	0.65	<0.01
Endotoxin (ng/g of ileal effluent)[2]	4.8 (2.8–7.4)	5.0 (3.3–18)	1.1 (0.8–3.2)*,†	0.7 (0.2–1.4)*,†			
Cecum							
Contents (g)	1.9 ± 0.1	1.7 ± 0.1	18 ± 1.6	7.5 ± 0.4	<0.01	<0.01	<0.01
Tissue weight (g)	0.5 ± 0.0	0.6 ± 0.0	1.6 ± 0.1	1.1 ± 0.0	0.22	<0.01	<0.01
Endotoxin (μg/g of contents) [2]	17.6 (13.1–20.1)	14.7 (7.8–22.2)	0.2 (0–0.3)*,†	0.1 (0–0.1)*,†			
Total organic acids (μmol/g of contents)[2]	63.3 (53.8–82.7)	33.6 (30.6–46.0)*	0.3 (0–0.7)*,†	1.2 (0.9–1.3)*,†			

[1]Data are expressed as the mean ± SE, $n = 6$ and analyzed by using two-way ANOVA. PSF, polystyrene foam.
[2]Data are expressed as the median (range), $n = 6$ and analyzed by Kruskal-Wallis one-way ANOVA, followed by Kolmogotov-Smirnov two-sample tests.
*, Different from control group without antibiotics, $p < 0.05$.
†, Different from PSF group without antibiotics, $p < 0.05$.

The number of goblet cells in the ileum increased in rats consuming dietary fiber in the present study. However, this fiber effect was well preserved in rats concurrently treated with antibiotics, suggesting that the fiber effect on goblet cell response could be exerted irrespective of such a bacterial component as endotoxin.

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