Nutritional Sciences 7(4), November, 2004, pp.196-200

Effects of Fructans and Isomaltooligosaccharide on Large Bowel Mass and Plasma and Fecal Immunoglobulin A in Rat*

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There are increasing evidences that prebiotics can modulate various properties of the immune system. This study was conducted to investigate effects of three kinds of fructans (chicory inulin, chicory inulin oligosaccharide and fructooligosaccharide) and a glucose oligomer (isomaltooligosaccharide) in large bowel mass and immunoglobulin A (IgA) in rats. Forty five Sprague-Dawley male rats weighing about 190g were randomly sorted to receive one of the five treatments, which were control diet, control diet+6% isomaltooligosaccharide (IMOS), control diet+6% fructooligosaccharide (FOS), control diet+6% chicory inulin oligosaccharide (CIOS), or control diet + 6% chicory inulin (CI). Rats were pair-fed and received the experimental diets for 5 weeks. Cecal and colonic wall weights were significantly higher in fructan (FOS, CIOS, CI)-fed groups compared with control and IMOS groups, and the length of colon was elevated in FOS and CIOS groups compared with control group. Fecal concentrations of acetic acid and total short-chain fatty acids (SCFAs) were significantly elevated in fructan-fed groups. Plasma and cecal levels and fecal excretion of immunoglobulin A (IgA) in rats were not significantly different among groups. However, fructooligosaccharide tended to increase IgA level in cecum. Cecal IgA level was significantly negatively correlated with pH of cecal content (r=-0.337), positively correlated with acetic acid level (r=0.310). Fecal IgA excretion was positively correlated with total SCFA (r=0.311) and propionic acid (r=0.400) level in feces. These results indicate that fructooligosaccharide and chicory inulin oligosaccharide exerted trophic effects in large bowel wall, increased production of SCFAs and decreased pH, which were conditions positively associated with cecal and colonic IgA secretion.

Key words: IgA, Colonic wall, Fructan, Oligosaccharide, Short-chain fatty acid

INTRODUCTION

Most-non-energy-related effects of carbohydrates can be related to short-chain fatty acid (SCFA) production or other effects of bacterial fermentation in the colon. Short-chain fatty acids appear to be a preferred fuel for use by the large bowel mucosa.[1] The colonic epithelium represents the major barrier to the invasion of bacteria into the portal blood, so the maintenance of this barrier is very important. SCFAs may have some anti-inflammatory effects, and these effects can be achieved even with parenteral administration of SCFAs.[2]

Fructan is a term used for any carbohydrate with fructosyl-fructose links constituting the majority of the glycosidic bonds, and it includes chicory inulin, chicory inulin oligosaccharide (oligofructose) and fructooligosaccharide. Chicory inulin and oilgosaccharides are some of the most popular functional food components in world, and they are defined as "prebiotics", which are considered non-digestible food ingredients. They beneficially affect the host, selectively stimulating the growth and/or activity of one or limited number of bacteria such as bifidobacteria in the colon.[3,4] Colonic fermentation of fructans produces SCFAs, lactic acid and gases as products of digestion. Their energy content is only 40-50% compared with digestible carbohydrates, giving them a caloric value of 1.0-2.0 kcal/g.[5] Consumption of fructooligosaccharide increased the concentration of bifidobacteria and also the ratio of bifidobacteria to total anaerobic flora in cecum and colon of weanling mice.[6] Isomaltooligosaccharides (IMO) are glucose oligomers with alpha-1,6-glucosidic linkages, and they have received attention due to their ability to stimulate the health-promoting activity of colonic bacteria as well as mild sweet taste and low cariogenic properties.

There are increasing evidences that prebiotics can modulate various properties of the immune system, also of the gut-associated lymphoid tissues (GALT). Oligofructose increased lymphocyte and/or leucocytes numbers in GALT and peripheral blood.[7] Oligofructose-enriched inulin supplement[8] and polydextrose[9] increased the secretion of immunoglobulin A (IgA) in cecum. Feeding lactulose was associated with increases of IgA or IgA^+

* This work was supported by the RRC program of MOST and KOSEF.
Accepted : October 13, 2004
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secretion cells in GALT.[10] However, it is unknown if prebiotics modulate the immune response directly or indirectly, by affecting the composition of the intestinal flora and thus affecting the GALT, or if they have these effects by SCFA production.

The present study was conducted to investigate effects of fructans and isomaltooligosaccharide, a glucose isomer, on large bowel mass and IgA in rats.

MATERIALS AND METHODS

1. Animals and Diets

Male Sprague-Dawley rats (Daehan Biolink Co., Chungbuk, Korea) weighing about 100 g were acclimated to the facility for one week with a commercial pellet diet (Samyang Feed Co., Korea). When their weights increased to 190 g, they were randomly divided into five groups with nine animals each one, and then fed with the experimental diets (Table 1) for 5 weeks. They were kept individually in wire-mesh cages, in a room maintained at 20±2 ℃ and 50±5% relative humidity.

Experimental diets were AIN[76]-based diets,[11] which fructan or isomaltooilgosaccharide was supplemented at 6%. The diets were named as control diet, isomaltooligosaccharide (IMOS; Daesang Co., Korea) diet, fructooligosaccharide (FOS; Cheil-Jedang CO., Korea) diet, chicory inulin oligosaccharide (CIOS) diet, and chicory inulin (CI; Sigma Co., USA) diet. Chicory inulin oligosaccharide, with 5~7 degree of polymerization, was produced from chicory inulin by endoinulinase made of Xanthomonas oryzae No. 5.[12] Rats were fed ad-libitum for a week and then, pair-fed to food intake of FOS group which consumed the least, for four weeks. The rats were weighed every other day and at the last four days, feces were collected and stored frozen at -70 ℃.

Table 1. Composition of experimental diets (g/kg)

Ingredients	Group[1]				
	Control	IMOS	FOS	CIOS	CI
Casein	200	200	200	200	200
Corn starch	404.5	404.5	404.5	404.5	404.5
Sucrose	168	52.6	58.3	108	108
Lard	40	40	40	40	40
Soybean oil	75	75	75	75	75
AIN-76 mineral mix[2]	40	40	40	40	40
AIN-76 vitamin mix[2]	10	10	10	10	10
Cholesterol	10	10	10	10	10
Sodium cholate	2.5	2.5	2.5	2.5	2.5
Cellulose	50	50	50	50	50
Isomaltooilgosaccharide	0	60(153.8)[3]	0	0	0
Fructooligosaccharide	0	0	60(146.3)[3]	0	0
Chicory inulin oligosaccharide	0	0	0	60	0
Chicory inulin	0	0	0	0	60

1) IMOS; isomaltooligosaccharide, FOS, fructooligosaccharide; CIOS, chicory inulin oligosaccharide; CI, chicory inulin.
2) AIN-76 mineral mix and AIN-76 vitamin mix from Teklad CO. (USA).
3) The value in parenthesis was the amount of oligosaccharide syrup added actually to diets to come to 6% level.

2. Sample Collection

The rats were anesthetized by inhaling diethyl ether, and blood was withdrawn from the abdominal aorta with a syringe containing heparin solution. Plasma was separated immediately by centrifugation at 3,000×g for 20 min and frozen at -70 ℃. Cecum and colon were excised and weighed.

3. Determination of Short-Chain Fatty Acids

Cecal content or feces were diluted 4 times with distilled water, and pH was measured using a pH electrode (ORION pH meter, model 420A) embedded completely in the sample solution. Approximately 0.4 g of fresh cecal content or fresh feces in 0.8 mL metaphosphoric acid solution (250 g/L) was added with 3 mL of distilled water, mixed well, and then centrifuged at 10,000 rpm for 20 min at 4 ℃. The supernatant was filtered through a 0.45 ㎛ Millipore filter. 1 ㎕ of the filtrated sample was injected into a BP21 capillary column (25 m×0.32 ㎜×0.25 ㎛ film thickness). Chromatographic separations of SCFAs were performed using a gas chromatograph (Varian Star 3600X) equipped with a flame ionization detector, and a split injector. The temperatures of the injector and detector were 270 ℃ and 280 ℃, respectively. The carrier gas was N_2 at 10 mL/min flow rate and the split flow ratio was 40 mL/min.

4. Immunoglobulin A Assay

The amounts of IgA in plasma, cecal contents and feces were measured by the enzyme-linked immunoabsorbent assay method. Samples were mixed with 1 ml of bovine serum albumin (BSA) solution (10 g/L) in Tris buffer (pH 8.0) and incubated for 10 min at room temperature. Samples were centrifuged (3,000×g, 20 min, 4 ℃) and supernatant was collected and stored at -20 ℃ until assay. Maxisorb 96-well microtiter plates (NUNC, Denmark) were coated with 100 μL of goat anti-rat IgA antibody (Bethyl Lab Inc., TX, USA) prepared after 1:100 dilution with a solution (50 g/L) of Tween 20 in Tris buffer and incubation for 1 h at 37 ℃. The plates received blocking with 100 μL of BSA (40 g/L) in the Tween solution for 1 h and after washing, 100 μL titers of the samples were applied. After new incubation for 1 h, 100 μL of horseradish peroxidase-conjugated goat anti-rat IgA (Bethyl Lab Inc.) diluted 1:50,000 with the Tween-BSA solution were added, and the plates were then incubated for 1 h. A peroxidase substrate, 100 μL of tetramethylbenzidine was then added,

and the plates were incubated for 30 min in the dark. The enzyme reaction was stopped with 100 μL of 2 mol/L H_2SO_4. The relative amount of IgA was quantified by measuring the absorbance at 450 nm.

5. Statistical Analysis

Results were expressed as means±standard error, using SPSS release 11.0 software package for statistics. Differences among groups were tested using one-way ANOVA followed by Duncan's multiple range test at $p<0.05$. Correlations between variables were shown by Pearson's correlation coefficients.

RESULTS

1. Large Bowel Wall Weight and Content Weight

Cecal and colonic wall weights, as shown in Table 2, were significantly higher in fructan (FOS, CIOS, CI)-fed groups compared with control and isomaltooligosaccharide groups. Cecal contents of FOS and CIOS groups were significantly higher than others. Colonic contents of fructan-fed groups were not significantly different from the control, while length of colon was elevated in FOS and CIOS groups compared with the control. Results indicate that fructooligosaccharide and chicory inulin oligosaccharide exerted trophic effects in large bowel wall.

Table 2. Cecal and colonic wall weights, content weights, cecal pH and colonic length in rats

Group[1)]	Cecum		Colon		
	Wall weight (g)	Content (g)	Wall weight (g)	Content (g)	Length (cm)
Control	0.945±0.055^{d}	4.62±0.362^{b}	1.26±0.043^{b}	1.29±0.225^{a}	17.48±0.565^{bc}
IMOS	0.930±0.054^{d}	4.37±0.220^{b}	1.27±0.035^{b}	0.574±0.100^{b}	17.27±0.485^{c}
FOS	1.91±0.127^{a}	7.67±0.586^{a}	1.80±0.079^{a}	1.35±0.354^{a}	19.91±0.518^{a}
CIOS	1.57±0.131^{b}	7.18±0.617^{a}	1.66±0.096^{a}	1.74±0.242^{a}	19.87±0.645^{a}
CI	1.26±0.075^{c}	5.04±0.761^{b}	1.66±0.073^{a}	1.46±0.174^{a}	18.90±0.414^{ab}

Values are mean±SE for n=9, and those in the same column with different superscript letters are significantly different, $p<0.05$.
1) IMOS; isomaltooligosaccharide, FOS, fructooligosaccharide; CIOS, chicory inulin oligosaccharide; CI, chicory inulin.

2. Immunoglobulin A in Plasma, Cecum and Feces

As shown in Table 3, plasma and cecal levels and fecal excretion of IgA in rats were not significantly different among groups. However, FOS group tended to have an increased level of IgA in cecum, and all fructans seemed to increase fecal excretion of IgA.

Table 3. plasma and cecal levels and fecal excretion of immunoglobulin A in rats

Group[1)]	Plasma (μg/mL)	Cecal contents (μg/cecum)	Feces (μg/day)
Control	33.78±1.75	2066.71±202.44	142.14±17.52
IMOS	38.97±2.15	1921.27±252.45	120.43±13.88
FOS	33.73±2.44	2680.47±290.44	168.56±34.26
CIOS	35.77±2.41	2145.14±261.38	165.20±23.22
CI	33.41±3.30	2102.97±283.84	176.56±16.80

Values are mean±SE for n=9.
1) IMOS; isomaltooligosaccharide, FOS, fructooligosaccharide; CIOS, chicory inulin oligosaccharide; CI, chicory inulin.

Cecal pH was in the order of FOS < CIOS, CI < Control, IMOS, and fecal pH was in the order of FOS < CI < CIOS < Control, IMOS (Table 4). Cecum concentrations of SCFAs were not significantly different among groups except by butyric acid. Butyric acid concentrations in fructan-fed and isomaltooligosaccharide-fed groups were lower than control group. Dilution of cecal contents due to increased osmotic pressure in cecum could be the reason for nonsignificant SCFA concentration among groups or even higher concentration in the control group. In feces, concentrations of acetic acid and total SCFA were significantly elevated in fructan-fed groups compared with those of the control and isomaltooligosaccharide groups. Propionic acid was significantly higher in CI group, and butyric acid was higher in CIOS group than other groups. Isomaltooligosaccharide showed no effect not only on decreasing cecal and fecal pH but also on production of SCFAs.

Table 4. Concentrations of short chain fatty acids in cecal contents and feces of rats

Group[1)]	pH	Acetic acid	Propionic acid	n-Butyric acid	Total SCFA
Cecum			μmol/g content		
Control	6.25±0.045^{a}	20.84±2.93	13.43±1.52	1.998±0.752a	36.3±4.92
IMOS	6.33±0.067^{a}	22.95±3.02	12.02±0.99	0.873±0.176b	35.8±3.68
FOS	5.19±0.096^{c}	19.62±3.63	11.82±2.03	0.355±0.086b	31.8±5.32
CIOS	5.42±0.137^{bc}	19.86±2.91	17.19±2.69	0.768±0.256b	37.8±5.69
CI	5.54±0.131^{b}	21.94±2.36	16.44±1.87	0.425±0.120b	38.8±4.02
Feces			μmol/g feces		
Control	6.98±0.020^{a}	11.06±1.61^{c}	7.83±1.60^{b}	0.441±0.097^{b}	19.33±3.23^{c}
IMOS	7.13±0.079^{a}	15.08±1.27^{bc}	7.04±1.25^{b}	0.519±0.196^{b}	22.65±1.99^{bc}
FOS	6.20±0.057^{d}	30.75±5.13^{a}	6.70±1.05^{b}	0.742±0.155^{b}	38.19±5.64^{a}
CIOS	6.71±0.043^{b}	25.65±3.07^{ab}	6.82±0.75^{b}	1.806±0.330^{a}	34.28±2.70^{ab}
CI	6.49±0.517^{c}	22.02±3.60^{ab}	12.20±2.32^{a}	0.915±0.156^{b}	35.13±5.66^{a}

Values are mean±SE for n=9, and those in the same column with different superscript letters are significantly different, $p<0.05$.
1) IMOS; isomaltooligosaccharide, FOS, fructooligosaccharide; CIOS, chicory inulin oligosaccharide; CI, chicory inulin.

As shown in Table 5, cecal IgA level was significantly negatively correlated with pH of cecal content ($r=-0.337$, $P<0.05$) and positively correlated with acetic acid level

(r=0.310, P<0.05) and total SCFA (r=0.284, P<0.10). Fecal IgA excretion was positively correlated with concentrations of total SCFA (r=0.311, P<0.05) and propionic acid (r=0.400, P<0.01), and negatively correlated with fecal pH (r=-0.254, P<0.10).

Table 5. Correlations of short chain fatty acids and pH with IgA in cecal and fecal contents

		Total SCFA	Butyric	Propionic	Acetic	pH
Cecal IgA	r	0.284	-0.110	0.240	0.310*	-0.337*
	(P)	(0.062)	(0.475)	(0.116)	(0.040)	(0.025)
Fecal IgA	r	0.311*	0.048	0.400**	0.231	-0.254
	(P)	(0.040)	(0.758)	(0.007)	0.131	0.096

N=45. **p<0.01 *p<0.05

DISCUSSION

The relative proportions of the three main SCFAs (acetic, propionic, and butyric) can be changed, due to the selective fermentation of indigestible carbohydrates at the large bowel. This change affects hyperplasia of the mucosa and increases wall thickness in the cecum and colon.[13] Unlike other undigestible sugars which are hydrolyzed by a wide variety of gut bacteria, short-chain fructooligosaccharides are only fermented *in vitro* by a limited number of microorganisms, including most species of bifidobacteria. These bifidobacteria have relatively high amounts of β-fructosidase, which is selective for the β-(1, 2) glycosidic bonds present in short-chain FOS.[14]

As the intestine is the first line of defense from the environment, and must integrate complex interactions among diet, external pathogens, and local immunological and non-immunological processes, the production of immune responses is critical to protect against potential pathogens. The colonic epithelium represents a major barrier to the invasion of bacteria into the portal blood and maintenance of this barrier is very important.[1] The gut-associated lymphoid tissue (GALT) constitutes an important line of defense and this is the largest immune organ of the body. In the colon, there are only small isolated lymphoid nodules, and the lamina propria is also endowed with lymphocytes belonging to the B-cell lineage. There are mainly memory cells and plasmocytes of which 70 to 90% are IgA producing cells.[14] The secretory Ig system is the best effector mechanism of mucosal immunity.[15]

Recently, several studies were carried out to investigate roles of prebiotics in immune responses in colon cancer model.[16-18] Short-chain fructooligosaccharides dramatically reduced the incidence of colon tumors and concomitantly developed gut-associated lymphoid tissue in Min mice.[17] Oligofructose have shown increased lymphocyte and/or leucocytes numbers in GALT and peripheral blood.[7] Feeding lactulose was associated with increases in IgA or IgA^+ secretion cells in GALT.[10] Polydextrose increased the secretion of IgA in the cecum and secretion of IgA increased even more with the combination of polydextrose and lactitol.[9]

Mechanisms by which prebiotics modulate the immune response are presently unknown. Schley and Field[19] proposed mechanisms underlying the immunomodulating effects of dietary fibers that change the gut microflora: direct contact of lactic acid bacteria or bacterial products (cell wall or cytoplasmic components) with immune cells in the intestine, production of SCFAs from fiber fermentation, and modulation of mucin production. It was reported that butyric acid suppress both constitutive and cytokine-induced expression of the transcription factor NF-kB in the colonic cell line HT-29.[20]

Oligofructose-enriched inulin supplement enhanced the production of interleukin-10 in Peyer's patches as well as the production of secretory IgA in the cecum.[8] This indicates that prebiotics supplement acted primarily at the gut-associated lymphoid tissue level.

In our study, we did not observe a significant difference in plasma level, cecal and fecal level of IgA after feeding oligosaccharides and chicory inulin. However, we observed the tendency of increased cecal IgA in short-chain fructooligosaccharide-fed rats. This difference could be attributed by the difference in chain lengths. Fructans used in this study have different degrees of polymerization (DP): fructooligosaccharide has 3~4 DP, chicory inulin, about 35 DP, and chicory inulin oligosaccharide, 5~7 DP. Perrien *et al.*[21] fed rats with different fibers and found that, among them, only resistant starch and short-chain fructooligosacchardes produced large amounts of butyric acid, with trophic effect in large intestine and subsequently fewer aberrant crypt foci. We also observed trophic effect in cecum and colon of rats fed with fructans, but no effect in those fed with isomaltooliogosaccharide.

Cecal IgA level was significantly negatively correlated with pH of cecal content and fecal IgA excretion was positively correlated with total SCFA and propionic acid concentration. This study may confirm that cecal and colonic IgA secretion was associated with SCFAs produced by selected microorganisms in large bowel, which in turn, affected local and systemic immune responses.

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