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Comparative Effects of Cellulose and Soluble Fibers (Pectin, Konjac Glucomannan, Inulin) on Fecal Water Toxicity toward Caco-2 Cells, Fecal Bacteria Enzymes, Bile Acid, and Short-Chain Fatty Acids

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The aim of this study was to compare the effects of cellulose and three soluble dietary fibers, pectin, konjac glucomannan (KGM), and inulin, on the cytotoxicity and DNA damage of fecal water-treated Caco-2 cells, a human colon adenocarcinoma cell line, and to investigate the fecal components that potentially modulate the fecal toxicity, that is, bacterial enzymes, bile acids, and short-chain fatty acids. Six-week-old BALB/cJ mice were randomly allocated to consume an AIN-93 diet that contained no dietary fiber (fiber-free) or 5% (w/w) cellulose, pectin, KGM, and inulin for 3 weeks. Feces were collected during days 18−21. Fecal waters were co-incubated with Caco-2 cells to determine the cytotoxicity and DNA damage. In addition, the fecal bacterial enzymes, bile acids, and short-chain fatty acids were determined. Results indicated that all fiber diets similarly increased the survival rate (%) of fecal water-treated Caco-2 cells as compared with the fiber-free diet. The inhibition of fecal water-induced DNA damage in Caco-2 cells was greater for the pectin and inulin diets than for the cellulose and KGM diets. In contrast, cellulose exerted the greatest inhibitory effect on the fecal β-glucuronidase activity. Cellulose and all soluble dietary fibers reduced the secondary bile acid concentrations in the fecal water, but only soluble fibers increased the fecal concentrations of short-chain fatty acids, as compared with no fiber. Therefore, this study suggests that all dietary fibers substantially reduced the fecal water toxicity, which is associated with decreased secondary bile acid levels by all fibers, reduced fecal β-glucuronidase activity by cellulose, and increased short-chain fatty acid levels by soluble dietary fibers.

KEYWORDS: Cellulose; pectin; konjac glucomannan; β-glucuronidase; bile acid; toxicity

INTRODUCTION

Cancers of the colon and rectum are the third most common type worldwide, and rates of this cancer increase with industrialization and urbanization (*1*). Epidemiological studies have supported the conclusion that increased intake of food containing dietary fiber is associated with decreased risk of colorectal cancer (*1*). The protective effects of dietary fibers against colorectal cancer may vary with their chemical and physical characteristics. Cellulose, an insoluble fiber, has consistently protected experimentally induced colon cancer (*2*). In contrast, the protective effects of soluble dietary fibers vary with their source, dose, and type of fat (*2*, *3*).

Although mechanisms whereby dietary fibers modulate the colorectal cancer remain to be illustrated. It is generally believed that insoluble dietary fibers dilute fecal toxin content, decrease transit time, and increase stool weights (*1*), which lead to decreased exposure of colonocytes to potential mutagens. On the other hand, the fermentation of soluble fibers in the colon may cause substantial increase in short-chain fatty acids, which have been shown to increase the apoptosis and to suppress the growth of colon cancer cells (*4*−*6*). Another uncertain role of insoluble and soluble dietary fibers in fecal mutagens relates to the bacterial enzymes that hydrolyze pro-carcinogen into carcinogens (*7*).

Fecal water, the aqueous phase of human feces, contains the components most likely to interact with epithelium cells in the colon and is considered a marker of colorectal cancer (*8*). Increased cytotoxicity of fecal water, namely, increased cell death, is thought to enhance the proliferation rate of colonic epithelial cells (*9*). Genotoxicity of fecal water causes DNA damage of colonocytes, which subsequently leads to mutation. Our previous studies have found that the addition of cellulose, konjac glucomannan (KGM), oligofructose, and inulin to fiber-free diet reduced the toxicity of fecal water obtained from BALB/cJ mice toward Caco-2 cells, a model for colonocytes (*10*, *11*). These results suggest that either cellulose or certain soluble fibers, that

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is, KGM and fructan oligosaccharides, reduce the toxicity of fecal water and, subsequently, may exert chemopreventive action against colorectal cancer. The toxicity of fecal water may relate to its components, that is, secondary bile acids, short-chain fatty acids, and others (*8*). Primary bile acids excreted from the liver to the intestines are transformed by the bacteria in the colon to secondary bile acids that are thought to affect cancer development by tumor-promoting activity, inducing DNA damage and pro-inflammatory response by activating nuclear factor κB (*12*). However, the effects of insoluble and soluble fibers on fecal water bile acids have not been compared.

The main goal of this study was therefore to compare the effects of cellulose and three soluble fibers, pectin, KGM, and inulin, on the toxicity of fecal water obtained from BALB/cJ mice toward Caco-2 cells. We also compared the effects of these dietary fibers on fecal bacterial enzymes that relate to toxin formation and fecal water components including bile acids and short-chain fatty acids.

MATERIALS AND METHODS

Animals and Diets. Six-week-old male BALB/cJ mice purchased from the National Laboratory Animal Breeding and Research Center (Taipei, Taiwan) were housed in solid-bottomed plastic cages with wood shavings for bedding in a room maintained on a 12 h light−dark cycle (8:00 a.m.−8:00 p.m.) at 24 ± 1 °C and 50% humidity. All animals were allowed free access to water and food during the study. Animal care followed the guidelines of the National Research Council (*13*) and was approved by the Institutional Animal Care and Use Committee at Chung Shan Medical University. After a 10 day adaptation period, mice were randomly divided into five groups (n = 12 per group) and fed a fiber-free AIN-93 diet or an AIN-93 modified diet containing konjac cellulose (Sigma Chemical Co., St. Louis, MO), pectin (from apple, 70−75% esterification, Sigma Chemical Co.), KGM (Fukar Co., Taiwan), or inulin (Sentosa Co., Taiwan). The composition of the diets was as follows (g/kg): casein, 200.0; corn starch, 529.5; corn oil, 70.0; dietary fiber, 50 (adjusted based on the purity of the fiber source); AIN-93G mineral mix, 35; AIN-93 vitamin mix, 1.0; L-cystine, 3.0; choline bitartrate, 2.5; butylated hydroxytoluene, 0.014. The amount of sucrose was adjusted accordingly. The purities of cellulose, pectin, KGM, and inulin were 99.9, 94, 80, and 85.5%, respectively. The powder diet was mixed with an equal weight of distilled water and made into pellets. Food intake was weighed every day, and body weight was measured twice weekly. Feces voided by each mouse during days 18−21 were collected in ice-bathed tubes, weighed, and homogenized to be individual fecal composites. A 10th of the composite was removed for fecal short-chain fatty acid analysis, whereas the remaining samples were lyophilized and stored at −20 °C. The lyophilized fecal composite was used for fecal bacterial enzyme assay and for preparation of the fecal water.

Fecal Water Preparation. Lyophilized fecal composites were rehydrated to 3-fold their original fecal weight and centrifuged at 36000*g* for 2 h (*10*). The supernatant fluid, that is, fecal water, was collected and used immediately for incubation with Caco-2 cells.

Cell Culture. Caco-2 cells were obtained from Bioresource Collection and Research Center (Hsinchu, Taiwan) and were cultured in Dulbecco's modified Eagle's medium (DMEM, containing 10% fetal bovine serum, 4 mM L-glutamine, 1.5 g/L $NaHCO_3$, 4.5 g/L glucose, 0.01 g/L human transferrin, and 1 mM sodium pyruvate (GIBCO/BRL, Gaitherburg, MD) at 37 °C in a humidified incubator under 5% CO_2 and 95% air. The cells were harvested at approximately 90% confluence (approximately 10^6 cells/10 cm dish). For use in the assay of fecal water toxicity, cells were detached with trypsin−EDTA, centrifuged for 5 min at 200*g*, and resuspended in Hanks balanced salt solution (HBSS; 1.3 mM $CaCl_2$, 5.4 mM KCl, 0.4 mM KH_2PO_4, 0.5 mM $MgCl_2 \cdot 6H_2O$, 0.4 mM $MgSO_4 \cdot 7H_2O$, 136.7 mM NaCl, 4.2 mM $NaHCO_3$, and 0.3 mM $NaH_2PO_4 \cdot H_2O$) at a concentration of 10^6 cells/mL.

Cytotoxicity. An aliquot of the cell suspension (900 μL) was incubated with 100 μL of fecal water or HBSS buffer (as control) at 37 °C in a gently shaking water bath for 1 or 3 h (*10*). An aliquot (400 μL) was taken to assess cell viability by trypan blue exclusion staining (*14*), and the rest of the mixture was centrifuged (600*g*, 10 min) to collect the cells used for the comet assay. Each value presented was calculated from three separate experiments, each of which included triplicate assay.

Table 1. Effects of Fiber-Free, Cellulose, Pectin, Konjac Glucomannan (KGM), and Inulin Diets on Daily Body Weight Gain, Feed Intake, and Feed Efficiency of BALB/cJ Mice during the Experiment[a]

diet	weight gain (g/day)	feed intake (g/day)	feed efficiency[b] (%)
fiber-free	0.3 ± 0.1	3.6 ± 0.1a	6.9 ± 2.2
cellulose	0.3 ± 0.2	3.6 ± 0.1a	7.8 ± 2.2
pectin	0.3 ± 0.1	3.7 ± 0.1a	7.2 ± 1.9
KGM	0.2 ± 0.1	3.8 ± 0.2b	6.3 ± 1.8
inulin	0.3 ± 0.1	3.7 ± 0.2a	7.4 ± 2.0

[a] Data are expressed as means ± SE (n = 12 animals per group). There was no significant difference between treatments according to one-way ANOVA followed by Tukey's test ($p < 0.05$). [b] Feed efficiency (%) = [weight gain (g/day)/feed intake (g/day)] × 100%.

Comet Assay of DNA Damage. The comet assay was used to determine DNA damage as described previously (*10*, *11*, *15*). The treated cells were suspended in low melting point agarose in phosphate-buffered saline (PBS) at 37 °C and placed onto a frosted glass microscope slide precoated with a layer of 1% normal melting point agarose. After application of a third layer of 1% normal melting point agarose, the slides were immersed in cold-lysing solution (10 mM Tris, 2.5 M NaCl, 100 mM Na_2EDTA, 1% sodium *N*-laurylsarcosine, 1% Triton X-100, and 10% dimethyl sulfoxide) for 1 h at 4 °C and then placed in an electrophoresis tank for 15 min in the alkaline solution. Electrophoresis (30 mA, 25 V) was performed for 20 min. Each value presented was calculated from three separate experiments, each of which included two slides with at least 120 comets. The image was analyzed by computer by using the Interactive Image Analysis Comet Assay III (Perceptive Instruments, Haverhill, U.K.), and DNA strand breaks were described as tail moment, where tail moment = %DNA in tail × tail length.

Fecal Bacterial Enzyme Activity. β-Glucosidase, β-galactosidase, and β-glucuronidase activities were measured from the release of 2-nitrophenol from synthetic substrates as described by Marteau et al. (*16*). An aliquot (0.5 g) of dry fecal composite was homogenized in PBS (pH 7.4) in an ice bath for 1 min, followed by centrifugation at 10000*g* and 4 °C for 10 min. An aliquot (0.5 mL) of supernatant was mixed with 0.25 mL of substrate (52 mmol/L) at 37 °C for 2, 5, and 10 min, respectively, from which the initial reaction rate was determined. The reaction product was measured at 405 nm by comparison to 4-nitrophenol. The protein content in the fecal sample was determined by a protein assay reagent (Life Science Research, Hercules, CA). The enzyme activity was expressed as international units (IU) per milligram of protein.

Bile Acid in Fecal Water. Hyodeoxycholic acid (Sigma Chemical Co., St. Louis, MO) as internal standard was added to 0.5 mL of fecal water. After a 1 h mild alkaline hydrolysis with 1 mL of NaOH (5 N) at 90 °C, bile acids were extracted and derivatized according to the method described by Czubayko et al. (*17*). Samples were dissolved in cyclohexane before they were injected onto a gas chromatograph (GC-14B, Shimadzu Corp., Kyoto, Japan) fitted with a capillary silica column (HP-5, 0.25 mm × 60 m, Agilent Technology, Santa Clara, CA) and a flame ionization detector. The injector and detector temperatures were 300 and 320 °C, respectively. The oven temperature was 150 °C for 3 min, increasing to 270 °C at 30 °C/min and then maintained at 270 °C for 64 min, with the carrier, N_2, at 2 mL/min. Peak areas were analyzed with a C-R6A Chromatopac (Shimadzu Corp.).

Short-Chain Fatty Acids in Fecal Water. Short-chain fatty acid were extracted by ether and measured using a gas chromatograph (GC-14B, Shimadzu Corp.) fitted with a glass capillary column (0.25 mm × 30 m, Stabilwax-DA, Restek Corp., Bellefonte, PA) and a flame ionization detector as described previously (*18*).

Statistical Analysis. Values are expressed as means ± SE and were analyzed by using Student's t test for two-group comparisons or one-way ANOVA followed by Tukey's test for comparisons of group means. A p value of < 0.05 was considered to be statistically significant.

RESULTS

The daily feed intake, weight gain during the study, and feed efficiency (g of daily weight gain/g of daily feed intake) did not

Table 2. Effects of Fiber-Free, Cellulose, Pectin, Konjac Glucomannan (KGM), and Inulin Diets on Fecal Weights and Moisture in BALB/cJ Mice[a]

diet	wet weight (g/day)	dry weight (g/day)	moisture (%)
fiber-free	0.49 ± 0.15a	0.18 ± 0.02a	59.0 ± 4.8b
cellulose	0.73 ± 0.10c	0.38 ± 0.06b	48.2 ± 6.8a
pectin	0.74 ± 0.07c	0.27 ± 0.02a	62.6 ± 6.9b
KGM	0.62 ± 0.10b	0.24 ± 0.02a	59.5 ± 8.9b
inulin	0.63 ± 0.16b	0.23 ± 0.03a	61.8 ± 7.0b

[a] Data are expressed as means ± SE (n = 12 animals per group). Different letters within a column denote significant differences between treatments according to one-way ANOVA followed by Tukey's test ($p < 0.05$).

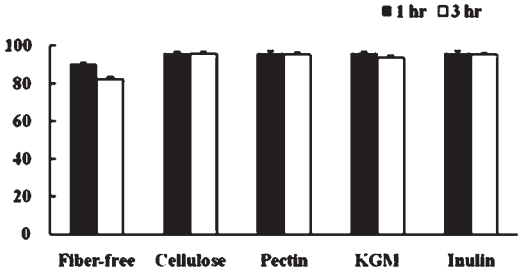

Figure 1. Effects of fecal water from different dietary fiber diets on the cell viabilities of Caco-2 cells. The cells were treated with HBSS solution (control) or fecal water at 37 °C for 1 and 3 h in a shaking water bath. Data are expressed as means ± SE (n = 12 animals per group) of three separate experiments. *, $p < 0.01$; #, $p < 0.001$, significantly different from the other groups.

differ among groups (**Table 1**). The wet fecal weight was lowest in mice fed the fiber-free diet. The wet fecal weight was significantly elevated by KGM and inulin by approximately 30% and elevated by approximately 50% by cellulose and pectin (**Table 2**). Cellulose diet led to significantly greater dry fecal weight, but lower fecal moisture (%), than did fiber-free and soluble fiber diets.

The Caco-2 cell survival rate (%) was suppressed only by the fiber-free diet to ~90% of the control level with 1 h of treatment and to ~82% with 3 h of treatment (**Figure 1**) and was not different across fiber groups. Because the cell survival rates were maintained above 90% at 1 h, DNA-damaging effects of fecal waters were determined at this time point. The DNA damage (expressed as tail moment) caused by the fecal water from the fiber-free group was approximately 4-fold that caused by the control medium (**Figure 2**). The cellulose and KGM diets almost diminished, whereas the pectin and inulin diets completely diminished, the DNA damage caused by the fecal water.

The KGM and inulin diets elevated the fecal β-glucosidase activities as compared with the fiber-free or pectin diet (**Table 3**). The β-galactosidase activity was reduced in mice fed the cellulose, KGM, and inulin diet and increased in mice fed the pectin diet. The β-glucuronidase activity was reduced only by the cellulose diet, whereas other soluble fiber diets did not modulate this enzyme activity.

The level of cholic acid, the major primary bile acid, was lowest in the fecal water from the fiber-free group and was elevated in the ascending order cellulose, inulin < KGM < pectin group (**Table 4**). The cholic acid level was drastically elevated to 10-fold level by pectin as compared to that in the fiber-free group. The level of chenodeoxycholic acid, the other primary bile acid measured, increased in the ascending order cellulose < pectin group. In terms of secondary bile acids, cellulose, KGM, and inulin diets, but not pectin diet, effectively reduced the deoxycholic acid level, whereas all dietary fiber diets significantly reduced the lithocholic acid level, as compared with the fiber-free diet. The total

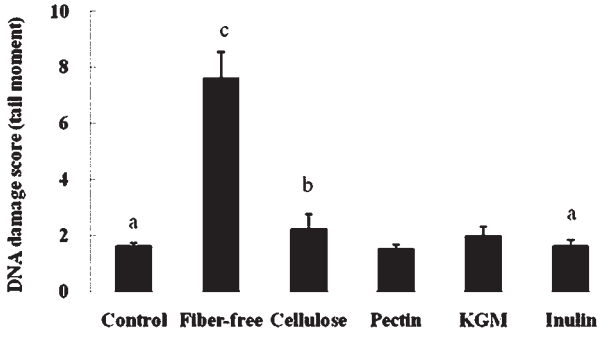

Figure 2. DNA damage induced by fecal water in Caco-2 cells. The cells were preincubated in HBSS solution (control) or with fecal water at 37 °C for 1 h in a shaking water bath. Data are expressed as means ± SE (n = 12 animals per group) of three separate experiments, each of which included 2 slides with at least 120 comets. Different letters denote significant differences between treatments according to one-way ANOVA followed by Tukey's test ($p < 0.05$).

Table 3. Effects of Different Dietary Fiber Diets on Fecal Enzyme Activities in BALB/cJ Mice[a]

	IU/mg of protein		
diet	β-glucosidase	β-galactosidase	β-glucuronidase
fiber-free	0.62 ± 0.09a	1.48 ± 0.18ab	3.96 ± 0.52b
cellulose	0.73 ± 0.17a	0.97 ± 0.29a	2.53 ± 0.44a
pectin	0.65 ± 0.10a	2.50 ± 0.29b	3.90 ± 0.40b
KGM	2.13 ± 0.20b	1.05 ± 0.29a	4.10 ± 0.45b
inulin	1.58 ± 0.29b	1.20 ± 0.13a	4.04 ± 0.51b

[a] Data are expressed as means ± SE (n = 12 animals per group). Different letters within a column denote significant differences between treatments according to one-way ANOVA followed by Tukey's test ($p < 0.05$).

secondary bile acid level was significantly reduced by cellulose, pectin, KGM, and inulin diets for 43.7, 20.6, 34.0, and 48.5%, respectively, as compared with the fiber-free diet. Even though the pectin diet caused the greatest total bile acid level in the fecal water, it led to the lowest proportion of secondary to total bile acid among the fiber groups.

Pectin, KGM, and inulin, but not cellulose, diets significantly elevated the levels of acetate, propionate, n-butyrate, and total short-chain fatty acids in fecal water, as compared with the fiber-free diet (**Table 5**). Pectin, KGM, and inulin diets at least doubled the acetate level as compared with the fiber-free diet. The inulin diet tripled the propionate level, whereas KGM and pectin diets similarly doubled the level shown in the fiber-free diet. The inulin diet caused a 3-fold increase in the fecal butyrate level, whereas KGM and pectin doubled its level, as compared with the fiber-free diet.

DISCUSSION

We showed that either the soluble or insoluble fiber investigated in the current study equally prevented the toxicity of fecal water from mice, using Caco-2 cells as an assessment tool. Pectin and inulin diminished, and KGM and cellulose significantly attenuated, the fecal water-induced DNA damage of Caco-2 cells. Cellulose is the only fiber that suppressed the fecal β-glucuronidase activity. The level of secondary bile acids was improved by all fibers determined in the present study, in which cellulose, KGM, and inulin were more effective than pectin. However, pectin and inulin caused the lowest proportion of secondary bile acid to total bile acid. The n-butyrate level was elevated only by soluble fiber in the descending order inulin > pectin, KGM.

Table 4. Effects of Different Dietary Fiber Diets on Bile Acid Profiles in Fecal Water Obtained from BALB/cJ Mice[a]

	μmol/L						
diet	cholic	chenodeoxycholic	deoxycholic (*a*)	lithocholic (*b*)	*a* + *b*	total (*c*)	*a* + *b*/*c* (%)
fiber-free	5.55 ± 0.20a	2.22 ± 0.04a	33.10 ± 3.80b	32.63 ± 3.00c	65.73 ± 7.10c	73.74 ± 8.30ab	97.9 ± 2.1c
cellulose	18.97 ± 2.44b	4.86 ± 0.05b	23.14 ± 0.44a	13.81 ± 0.42a	36.95 ± 1.00a	61.27 ± 7.82a	90.5 ± 2.1b
pectin	58.31 ± 4.10e	8.30 ± 0.40c	37.70 ± 1.12b	14.47 ± 0.65a	52.17 ± 1.98b	121.28 ± 4.30c	80.4 ± 2.0a
KGM	38.45 ± 2.80d	3.13 ± 0.32a	23.35 ± 0.74a	20.00 ± 1.27b	43.35 ± 2.00a	87.15 ± 5.20b	88.4 ± 1.2b
inulin	24.29 ± 3.75b	1.97 ± 0.30a	17.47 ± 1.21a	16.38 ± 2.04a	33.85 ± 3.40a	61.16 ± 5.2a	81.2 ± 1.5a

[a] Data are expressed as means ± SE (*n* = 12 animals per group). Different letters within a column denote significant differences between treatments according to one-way ANOVA followed by Tukey's test ($p < 0.05$).

Table 5. Effects of Fiber-Free, Cellulose, Pectin, Konjac, and Inulin Diets on Fecal Short-Chain Fatty Acid Concentrations in BALB/cJ Mice[a]

	μmol/g of wet feces				
diet	acetate	propionate	isobutyrate	*n*-butyrate	total
fiber-free	20.5 ± 2.3a	3.9 ± 1.3a	0.6 ± 0.2	5.2 ± 0.8a	31.4 ± 4.2a
cellulose	28.4 ± 2.7a	3.2 ± 1.1a	0.6 ± 0.1	5.7 ± 1.0a	37.8 ± 4.2a
pectin	57.0 ± 5.4b	7.3 ± 1.9b	0.6 ± 0.2	11.7 ± 1.7b	76.6 ± 5.3b
KGM	54.4 ± 5.3b	7.1 ± 1.5b	0.9 ± 0.05	12.7 ± 2.4b	72.0 ± 5.6b
inulin	52.0 ± 4.3b	11.9 ± 2.6c	1.1 ± 0.5	22.3 ± 1.8c	87.3 ± 5.8b

[a] Data are expressed as means ± SE (*n* = 12 animals per group). Different letters within a column denote significant differences between treatments according to one-way ANOVA followed by Tukey's test ($p < 0.05$).

The current study is the first indicating that pectin, similar to inulin, exerted strong protective effects against the cyto- and genotoxicity of fecal water toward Caco-2 cells. In agreement with these observations, previous studies have indicated that pectin increases colonocyte apoptosis and decreases the occurrence of aberrant crypt foci in rat models of experimentally induced colon cancer (*3*, *19*−*21*). The preventive role of pectin on fecal water toxicity may be mediated by several mechanisms. First, pectin diet caused a great increase in short-chain fatty acids, which may protect human colonocytes from DNA damage (*22*, *23*). Second, our results showed that pectin reduced the secondary bile acid level and its proportion to total bile acid in the fecal water. The secondary bile acids have been shown to damage the tight junction of colon cells (*24*, *25*) and cause DNA damage and oxidative stress (*26*). Therefore, decreased secondary bile acid in the fecal water could reduce the toxicity of fecal water toward colonocytes. The roles of pectin on β-glucuronidase activity in rat models of experimental carcinogenesis have been inconsistent (*27*, *28*). In this study, pectin failed to reduce the β-glucuronidase activity, indicating that fecal bacteria enzymes did not contribute to the beneficial effect of pectin. However, pectin increased fecal weight, which could dilute toxin levels in the fecal water.

Inulin, similar to pectin, exerted strong protective effects against fecal water toxicity, in agreement with previous observation (*10*, *11*). This study is the first study to investigate how inulin modulated the fecal water component. The role of inulin on fecal bile acid excretion is rarely studied. Although a previous study indicated that inulin increased the cecal pool of bile acids (*29*), we showed a decreased bile acid level and ratio of secondary to total bile acid in the fecal water. An acidic fecal environment resulting from short-chain fatty acids may facilitate the precipitation of hydrophilic deconjugated bile acids and inhibit their enzymatic formation from primary bile acids (*30*). In addition, inulin increased fecal weight, which could dilute toxin levels in the fecal water. On the other hand, inulin increased fecal β-glucosidase activity, in agreement with a previous study (*29*), but did not reduce β-glucuronidase activity.

The protective efficiency of KGM on fecal toxicity was similar to that of cellulose. However, the mechanisms whereby KGM and cellulose modulate fecal water toxicity were not identical. KGM caused less bulky effect, but greater fecal moisture content, than did cellulose. In addition, KGM, unlike cellulose, may protect cells from fecal water toxicity by increasing the short-chain fatty acid level. In agreement with other soluble fibers, KGM did not reduce β-glucuronidase activity in this study. In contrast to the promotive effect of KGM on total bile acids in feces (*31*), KGM decreased the secondary bile acid levels in the aqueous phase of feces in the current study, which could be due to the reduction of pH by increased short-chain fatty acids level, as described previously.

Insoluble fiber cellulose exerted significant protective effects on cyto- and genotoxicity of fecal water, in agreement with a previous study (*10*). The previous study suggested that the bulky effect of cellulose was likely to be a major mechanism (*10*). This study further demonstrated that cellulose reduced the bacterial β-glucuronidase, which in turn reduced transformation of conjugated glucuronides to toxic aglucuronides. In addition, cellulose also decreased the fecal water secondary bile acid level, which also diminished the toxicity of fecal water toward Caco-2 cells. In contrast, cellulose did not promote any individual short-chain fatty acid level. Therefore, the protective effect of cellulose on the cyto- and genotoxicity of fecal water is likely to be mediated by its inhibitory effect on β-glucuronidase activity and reduction in the secondary bile acid level probably through the bulky effect.

In summary, the present study suggests that cellulose, pectin, KGM, and inulin exert chemoprotective effects in the colonocytes, which may prevent the occurrence of colorectal tumors. However, soluble fibers exert these beneficial effects mainly through fermentation and reduction in the secondary bile acid level, whereas cellulose effects are mainly through β-glucuronidase activity, bulky effect, and reduction in secondary bile acid level.

ABBREVIATIONS USED

KGM, konjac glucomannan; DMEM, Dulbecco's modified Eagle's medium; HBSS, Hanks balanced salt solution; PBS, phosphate-buffered saline.

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