Effects of konjac glucomannan, inulin and cellulose on acute colonic responses to genotoxic azoxymethane

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ABSTRACT

Mice were fed low-fibre, or that supplemented with soluble fibre (konjac glucomannan, KGM; inulin), or insoluble fibre (cellulose) to determine how these three fibres modulated the acute colonic responses to an azoxymethane (AOM) treatment. Results indicated that KGM and inulin exerted greater anti-genotoxic effects compared to cellulose and up-regulated the gene expressions of glutathione S-transferase and antioxidant enzymes. The apoptotic index in the distal colon was the greatest and the expression of Bcl-2 was the lowest in the KGM group 24 h after the AOM treatment. On the other hand, the proliferative index and expression of Cyclin D1 were lower in all fibre groups. Furthermore, KGM increased cecal short-chain fatty acid contents, and both KGM and inulin increased fecal probiotic concentrations. This study suggested that soluble fibres were more effective than cellulose on ameliorating AOM-induced genotoxicity by up-regulating antioxidant enzyme genes, and enhancing epithelium apoptosis by down-regulating Bcl-2.

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1. Introduction

Colon cancer is the leading cause of death worldwide, and dietary factors are known to be capable of regulating the colon carcinogenesis (Ferlay et al., 2010). Epidemiological studies have suggested the reverse association between intake of dietary fibre, the indigestible parts of plant materials, and the risk of colon cancer (Aune et al., 2011; Dahm et al., 2010). Underlying potential mechanisms, whereby dietary fibres may influence the development of colon carcinogenesis, include increased fecal bulk, reduced colonic transit time and diluted fecal toxin contents, which consequently reduce the exposure of colonic mucosa to the luminal carcinogens (American Institute for Cancer Research, 2007; Spiller, 2001). In addition, the interaction between dietary fibre and colonic microbiota and bile acids, and the production of short-chain fatty acids (SCFA) resulting from fermentation, are believed to protect against colon cancer development (Young, Hu, Le, & Nyskohus, 2005). Butyrate, in particular, is one of the SCFA that serves as the major energy source of colonocytes (Roediger, 1982) and has been shown to enhance apoptosis and inhibit proliferation in the colonic cells in vitro (Chai, Evdokiou, Young, & Zalewski, 2000; Zhang et al., 2010).

Konjac glucomannan (KGM), derived from the tubers of Amorphophallus konjac C. Koch, is composed of β-1,4-linked D-glucose and D-mannose units joined together with branches through β-1,6-glucosyl units (Doi, 1995). The viscous polymer can be processed into various vegetarian food products and commonly consumed in the Asian countries such as Japan and Taiwan. Inulin, a mixture of fructo-oligosaccharides derived from the tuber of chicory (Cichorium intybus), is a well-known prebiotic and widely used as a supplement in functional food. Both KGM and inulin have been shown to increase the production of SCFA and stimulate the growth of bifidobacteria and lactobacilli in animal and human studies (Chen, Cheng, Wu, Liu, & Liu, 2008; Chen, Lin, & Wang, 2010). In addition, these two soluble fibres have also been shown to up-regulate the antioxidant enzymes in the colon (Wu & Chen, 2011b). On the other hand, cellulose, a poorly-fermented insoluble fibre, increases fecal bulk and may therefore reduce the fecal toxic concentration, but does not increase the fecal butyrate level (Chen et al., 2010).

Azoxymethane (AOM) is commonly used to induce experimental animal model of colon carcinogenesis (Rosenberg, Giardina, &
The AOM is metabolized into methylazoxymethanol that causes DNA adducts (Weisburger, 1971). The potential cellular defense mechanisms, such as antioxidant machinery and apoptosis, and the compensatory response to apoptosis, such as cell proliferation, may occur after the DNA damage (Bellamy, Malcomson, Harrison, & Wyllie, 1995; Fan & Bergmann, 2008). Therefore, it is generally considered that increased DNA damage or/and insufficient apoptosis response against the DNA damage leads to an increased risk of carcinogenesis. We have previously demonstrated that supplementation of KGM, inulin or cellulose into a low-fibre diet reduced acute DNA damages in Caco-2 cell, a colonocyte cell line model, caused by fecal water treatment (Chen et al., 2008), as inulin exerted greater suppressive effect compared to KGM and cellulose. However, effects of these three fibres, on colonic DNA damage, antioxidant enzymes, apoptotic and proliferative responses induced by AOM, have not been shown in vivo.

The main goal of this study was to examine effects of two soluble fibres (KGM, inulin) and one insoluble fibre (cellulose), over 24 h, after the AOM administration on colonic DNA damage, cell cycle homeostasis, and gene expression of related cellular mechanisms in mice. We also determined the SCFA in the cecum and fecal microbiota.

2. Methods and materials

2.1. Animals

Male C57BL/6j mice were obtained at 5 weeks of age from the National Laboratory Animal Breeding and Research Center (Taipei, Taiwan). Every three mice were housed in a solid-bottomed plastic cage, with stainless wire-bar lid and wood shavings for bedding, in a animal holding room maintained on a 12-h light-dark cycle at 24 ± 1 °C and 50% humidity. All animals were allowed free access to water and food in the study. Animal care followed the guidelines of the National Research Council (1985) and was approved by the Institutional Animal Care and Use Committee (IACUC) of Chung Shan Medical University (approved number 1077).

2.2. Experimental design

After 1 week of acclimatisation, mice (6-week-old) were randomly divided into four groups (n = 12 per group) and fed either modified AIN-76 (American Institute of Nutrition, 1977) high-fat (20% corn oil, w/w) low-fibre (1% cellulose) diet or that supplemented with another 5% (w/w) fibre derived from KGM (80%, Fukar Co., Taipei, Taiwan), inulin (85.5%, Sentosa Co., Taipei, Taiwan), or cellulose (99.9%, Sigma Chemical Co., St. Louis, MO) for 3 weeks. The composition of the low-fibre diet was as follows (g/kg): casein, 200; corn starch, 540; corn oil, 200; AIN-76A mineral mix, 35; AIN-76A vitamin mix, 10; methionine, 3; choline bitartrate, 2; cellulose, 10. The amount of corn starch was substituted by dietary fibre, with correction of the purities to formulate the fibre-supplemented diet. Daily food intake and body weight were recorded throughout the study. Mice were individually housed and fecal outputs were collected during days 17–21. Mice were anaesthetized with CO2 before or 24 h after a single intraperitoneal injection of AOM (10 mg/kg body weight, Sigma) on day 22. A midline incision was made to dissect the cecum from where the contents were removed and weighed. Theecal contents were immediately stored at −20 °C for further analysis of SCFA. The entire colon was then removed and flushed clean with ice-cold sterile saline. Segments (0.5 cm) of the distal colon were fixed in 10% (v/v) buffered formalin overnight and embedded in paraffin for further immunohistochemical examination. The remaining colons were immediately processed for colonocyte isolation.

2.3. Isolation of colonocytes

The colonocytes were isolated according to the method described by Pool-Zobel et al. (1993) with slight modification. Briefly, colonic tissues were washed in a phosphate buffered saline containing penicillin (10 units/ml, Gibco Life Technologies, Foster City, CA) and streptomycin (10 mg/ml, Sigma) at 37 °C with shaking, for three times, each for 10 min. Tissues were then treated with collagenase (type XI, 125 units/ml, Sigma) for 30 min at 37 °C and was then centrifuged at 800 g for 10 min to collect the colonocytes. Half of the isolated colonocytes were used to determine the DNA damage, while the other halves were processed for RNA isolation to determine the expression of target genes.

2.4. Comet assay

The DNA damages of colonocytes were determined using the Comet assay as described previously (Wu & Chen, 2011b). The viability of isolated colonocytes was determined using the trypan blue assay (Phillis, 1973). With ≥90% cell viability, cells (5 × 10³/ml) were suspended in 1% (w/v) low-melting-point agarose which was layered onto a layer of 1% (w/v) normal-melting-point agarose on a frosted glass slide. After application of a third layer of 1% normal-melting-point agarose, the slides were immersed in a cold lysing solution (10 mM Tris, 1% sodium N-lauroylsarcosine, 0.1 mM Na₂EDTA, 2.5 M NaCl, 1% dimethylsulphoxide, pH 10) for 1 h at 4 °C. After being washed with a saline solution, the slides were allowed to unwind for 20 min in an alkaline solution (0.3 M NaOH, 1 mM Na₂EDTA), followed by electrophoresis at 25 V and 300 mA for 20 min. Duplicate slides were prepared from each mouse, and the DNA breakages from at least 100 cells per slide were determined. The image was analysed using the Interactive Image Analysis Comet Assay III (Perceptive Instrument, Haverhill, Suffolk, UK). DNA damage was denoted as tail moment (% of DNA in tail × tail length).

2.5. Relative gene expressions

The gene expressions of antioxidant enzymes, superoxide dismutase 1 (SOD1), catalase (CAT), glutathione peroxidase 2 (GPX2), detoxification enzyme, glutathione S-transferase π (GST), B cell leukemia (Bcl-2) oncogene that suppresses cell apoptosis (Willis, Day, Hinds, & Huang, 2003), and Cyclin D1 (Ccdn1), a cell cycle regulator that controls transition from the G1 to S phase (Fu, Wang, Li, Sakamaki, & Pestell, 2004), were determined by using quantitative real-time polymerase chain reaction (qPCR). The RNAs was isolated according to the method described previously Ferlay et al. (2010). Briefly, colonocytes were homogenised (5 × 10³ cells/ml) in RErzol™ C&T reagent (PROtech Technology, Taipei, Taiwan). After addition of 0.2 ml chloroform, the samples were vigorously mixed for 15 s, followed by centrifugation 12,000g for 15 min at 4 °C. The supernatant was mixed with an equal volume of isopropanol (J. T. Baker, Deventer, The Netherlands), and the RNA pellet was precipitated with centrifugation, 12,000g 10 min at 4 °C. After washing with 75% ethanol, the RNA was dissolved in RNA-free water for further complementary DNA (cDNA) synthesis. The quality of RNA were determined by the 260/280 nm absorbance. The cDNA was synthesized using random primers (Applied Biosystems Life Technologies) in a thermal cycler (TaKaRa Biomedical, Shuzo, Japan).

The qPCR was performed using TaqMan gene expression assays (Applied Biosystems) with the StepOne Real-Time PCR System (Model 7700, Applied Biosystems). The assay identification (accession number of NCBI gene reference shown in parenthesis) of primers for the target genes SOD1, CAT, GPX2, GST, Bcl-2, and Ccdn1 was: Mm01344233_m1 (NM_011434.1), Mm00437992_m1 (NM_000997.2), Mm00437992_m1 (NM_000997.2), and Mm00437992_m1 (NM_000997.2).
counts/g feces. Nagayasu, Kobayashi, & Sakurai, 2002). The nucleic acid stain 4 (Wang, Cao, & Cerniglia, 1996), and clostridia (Nagahama, 0.25 m, Stabilwax-DA, Restek Corp., Bellefonte, PA) with a flame ionisation detector and peak areas were collected with a C-R6A Chromatopac (Shimadzu Corp.). 2.9. Statistical analysis

Values were presented as means ± SEM and analysed using SPSS version 14.0 (SPSS Inc., Chicago, IL). The diet effects at a time point were determined using one-way ANOVA followed by Tukey’s test. The time effect of AOM within each dietary group was determined using the Student’s t-test. A P value < 0.05 was considered statistically significant.

3. Results

The growth and physical activity were normal in all groups throughout the experimental period. The calorie intake was 56.4 ± 3.4, 51.8 ± 2.7, 52.2 ± 1.1 and 52.6 ± 1.6 kJ/d in the low-fibre, KGM, inulin and cellulose group, respectively. The daily weight gain of low-fibre, KGM, inulin and cellulose group was 0.20 ± 0.01, 0.16 ± 0.01, 0.15 ± 0.01, and 0.17 ± 0.02 g/d, respectively. Both caloric intake and weight gain were similar across groups.

The DNA damage (tail moment) of colonocytes at 0 h was the greatest in the low-fibre group (Fig. 1), which was significantly decreased with dietary supplementation of KGM (P = 0.015) and inulin (P = 0.003), respectively. The single AOM injection significantly increased the DNA damage at 24 h as compared with the respective counterpart at 0 h (P < 0.05, respectively). The tail moment at 24 h was still the greatest in the low-fibre group, 4.5 ± 0.2, which was significantly reduced by all fibres. KGM, inulin and cellulose decreased the DNA damage by 27% (P < 0.001), 40% (P < 0.001) and 16% (P = 0.006), respectively, as compared with that in the low-fibre group.

Addition of dietary fibre into the low-fibre diet did not affect the expression of SOD1 in the isolated colonocytes at 0 h (Fig. 2A). The AOM treatment increased the SOD1 expression in all groups except the cellulose group. The SOD1 gene expression was enhanced to the greatest with KGM, from 1.54 ± 0.17 at 0 h to 2.88 ± 0.16 at 24 h (P < 0.001 vs. low-fibre at 24 h). However, cellulose group

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2.6. Immunohistochemical staining

The apoptosis and proliferation of colonic epithelium were determined by using the immunohistochemical staining. Paraffin sections (5 m) were dewaxed, rehydrated through descending alcohol concentration and treated with 20 g/ml proteinase K (Sigma) for 20 min. Endogenous peroxidase was removed by treatment with 3% hydrogen peroxide (H2O2) for 20 min. The epithelial cells undergoing apoptosis were determined by using the TUNEL method according to the manufacturer’s instruction (ApopTag S7101, Millipore, Temecula, CA) and counterstained with methyl green. The 3’ hydroxyl ends of broken DNA strand were enzymatically labelled with digoxigenin nucleotides and were then treated with anti-digoxigenin antibody bound to peroxidase. A negative control was prepared for each animal to monitor the non-specific reaction. The apoptotic index (AI), the ratio (%) of TUNEL-positive to total epithelium cells was determined from at least 40 crypts randomly selected from each animal.

The Ki-67 protein, a marker shown during cell proliferation, in the colonic epithelial cells was determined with a polyclonal antibody (Millipore). After inhibition of endogenous peroxidase activity by 3% H2O2, the Ki-67 antibody was then applied at 1:300 dilutions for 1 h at room temperature. The stains were shown by using a biotinylated secondary antibody and detection system (IHC Select Immunoperoxidase Secondary Detection System, Millipore) according to the manufacturer’s instruction. The proliferative index (PI), i.e. the ratio of Ki-67 positive to total cells, was determined in the whole crypt column and upper-third crypt, respectively.

2.7. Cecal SCFA

Cecal SCFA was extracted with methyl ether, according to the method described previously (Wu & Chen, 2011a), using 4-methyl-N-valeric acid (Sigma) as an internal standard. The re-dissolved sample was analysed by gas chromatography (GC-14B; Shimadzu Corp., Kyoto, Japan) using a glass capillary column (0.25 mm × 30 m, Stabilwax-DA, Restek Corp., Bellefonte, PA) with a flame ionisation detector and peak areas were collected with a C-R6A Chromatopac (Shimadzu Corp.).

2.8. Fecal microbiota

Fecal bacteria population were determined by using fluorescence in situ hybridization method (FISH), as described previously (Chen, Cheng, Liu, Liu, & Wu, 2006). The genotypic probes were specifically designed to target 16S rRNA of bifidobacteria (Jansen, Wildeboer-Veloo, Tonk, Franks, & Wellin, 1999), lactobacilli (Wang, Cao, & Cerniglia, 1996), and clostridia (Nagahama, Nagayasu, Kobayashi, & Sakurai, 2002). The nucleic acid stain 4’,6-diamidino-2-phenylindole was used for total bacterial counts (Chen et al., 2006). Probe fluorescence was detected with a Zeiss Axiostop2 microscope (Carl Zeiss, Jena, Germany) fitted for epifluorescence microscope with a 100 W mercury bulb (HBO 103), a 20× Plan-neofluar objective, a filter set 01, 09 and 20, and a cooled charge-coupled device video camera (MacroFire, Model S99831, Optronics, Goleta, CA). The microbial concentration is expressed as log10 counts/g feces.
slightly decreased SOD1 gene expression from 1.24 ± 0.23 at 0 h to 0.79 ± 0.03 at 24 h (P = 0.081). The relative gene expressions of CAT were greater in the KGM and inulin groups than that in the low-fibre and cellulose groups at 0 h (Fig. 2B). The AOM treatment decreased the CAT expressions in the KGM (P = 0.002) and cellulose (P = 0.004) groups, but not in the inulin group, and the inulin group had the greatest CAT expression among groups at 24 h. The relative gene expression of GPX2 was increased with either soluble or insoluble fibre supplementation at 0 h (Fig. 2C). The AOM treatment induced the relative GPX2 expression only in the low-fibre group, to a level similar to that shown in the fibre-supplemented groups at 24 h. The relative gene expression of GST was also enhanced with either type of fibre supplementation at 0 h (P < 0.05) (Fig. 2D). However, the relative GST expressions at 24 h were greater only in the KGM (P = 0.002) and inulin (P = 0.001) groups, but not in the cellulose group, as compared with that in the low-fibre counterpart.

The original (0 h) AI in the distal colon was similar among groups (Fig. 3). The AOM treatment significantly increased the AI in the low-fibre, KGM and inulin groups by one-fold (P < 0.001), ~120% (P < 0.001) and ~80% (P < 0.001), respectively, and slightly increased that in the cellulose group (P = 0.06). In addition, the AI at 24 h was greater only in the KGM (P = 0.002) and inulin (P = 0.001) groups, but not in the cellulose group, as compared with that in the low-fibre counterpart. The representative images of AOM-induced apoptosis are shown in the Supplementary data (A).

The PI of the whole crypt at 0 h was greater in mice fed either fibre-supplemented diet than that in the low-fibre group (Table 1). The AOM treatment significantly increased the PI in the whole-crypt only in the low-fibre group (P < 0.001), not in any fibre-supplemented groups. The PIs of the whole crypt at 24 h were significant lower in the KGM, inulin and cellulose groups for 30% (P < 0.001), 19% (P = 0.004) and 31% (P < 0.001), respectively, than that in the low-fibre counterpart. We further examined the PI of the upper-third crypt. The PI in the low-fibre group was the greatest among groups at either time point. Addition of KGM, inulin and cellulose into the low-fibre diet significantly decreased that by 26% (P = 0.001), 35% (P = 0.007) and 37% (P = 0.006), respectively, at 0 h, and 42% (P < 0.001), 34% (P = 0.004) and 40% (P < 0.001), respectively, 24 h after the AOM treatment. The representative images of Ki-67 positive stains are shown in the Supplementary data (B).

The relative expression of Bcl-2, an anti-apoptotic gene, at 0 h was similar among groups (Fig. 4A). However, the Bcl-2 gene expression was significant lower in the fibre-supplemented group
compared to the low-fibre counterpart 24 h after the AOM treatment. KGM exerted the greatest suppressive effect on Bcl-2 gene expression, followed by the inulin and then cellulose groups. Furthermore, the relative expression of Cyclin D1, a proliferation-related gene, at 0 h was increased with all types of dietary fibre examined in this study, and was the greatest in the cellulose group (Fig. 4B). However, the relative gene expression of Cyclin D1 at 24 h, in the low-fibre group, was significantly up-regulated as compared to that at 0 h (P < 0.001), and was greater (P < 0.05) than that in either fibre-supplemented group.

The cecal acetate and propionate contents were not affected by any dietary fibre examined in this study at 0 h, but the butyrate contents were greater in the KGM (P = 0.047) and inulin (P < 0.001) groups as compared to that in the low-fibre counterpart, respectively (Table 2). Most individual cecal SCFA content was not affected with the AOM treatment, except that the butyrate content was significantly increased with the AOM treatment in the KGM group by more than one-fold. In addition, the KGM group had the greatest acetate, propionate, butyrate and the total SCFA contents among groups at 24 h.

Addition of cellulose into the low-fibre diet increased total bacteria (log10 CFU/g) from 10.36 ± 0.03 to 10.98 ± 0.05 (P = 0.001) and 10.99 ± 0.04 (P < 0.001), respectively. Furthermore, addition of KGM and inulin into the low-fibre diet increased total bacteria (log10 CFU/g feces) from 10.50 ± 0.05 to 11.74 ± 0.03 (P = 0.015) and 11.78 ± 0.05 (P = 0.006), respectively. However, none of the dietary fibre examined in the present study significantly changed the cecal clostridia concentration.

### Table 1

<table>
<thead>
<tr>
<th>Proliferative index (%)</th>
<th>Low-fibre</th>
<th>KGM</th>
<th>Inulin</th>
<th>Cellulose</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>0 h</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole crypt</td>
<td>30.3 ± 2.0*</td>
<td>40.0 ± 2.3*</td>
<td>37.7 ± 0.5*</td>
<td>38.9 ± 2.3*</td>
</tr>
<tr>
<td>Upper-third crypt</td>
<td>55.2 ± 1.6*</td>
<td>40.6 ± 1.9*</td>
<td>36.0 ± 3.0*</td>
<td>34.6 ± 2.5*</td>
</tr>
<tr>
<td><strong>24 h</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole crypt</td>
<td>44.8 ± 1.6**</td>
<td>31.5 ± 1.1**</td>
<td>36.3 ± 1.4**</td>
<td>30.8 ± 2.5**</td>
</tr>
<tr>
<td>Upper-third crypt</td>
<td>59.0 ± 2.8**</td>
<td>34.5 ± 1.4**</td>
<td>39.0 ± 2.4**</td>
<td>35.3 ± 2.1**</td>
</tr>
</tbody>
</table>

1 Data are expressed as means ± SEM (n = 6 per group). Different superscript letters denote significant differences across dietary groups as analysed by one-way ANOVA followed by Tukey’s test. **P** < 0.001, *P* < 0.001, P < 0.05.

### Table 2

<table>
<thead>
<tr>
<th>µmole/cecum</th>
<th>Low-fibre</th>
<th>KGM</th>
<th>Inulin</th>
<th>Cellulose</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>0 h</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetate</td>
<td>10.3 ± 0.7</td>
<td>12.4 ± 3.8</td>
<td>13.0 ± 2.9</td>
<td>7.7 ± 1.1</td>
</tr>
<tr>
<td>Propionate</td>
<td>1.2 ± 0.3</td>
<td>1.4 ± 0.5</td>
<td>1.6 ± 0.4</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>Butyrate</td>
<td>0.6 ± 0.1*</td>
<td>0.9 ± 0.1b</td>
<td>1.3 ± 0.1*</td>
<td>0.8 ± 0.1b</td>
</tr>
<tr>
<td>Total SCFA</td>
<td>12.1 ± 0.7</td>
<td>14.7 ± 4.2</td>
<td>15.9 ± 3.3</td>
<td>9.3 ± 1.3</td>
</tr>
<tr>
<td><strong>24 h</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetate</td>
<td>9.0 ± 0.9a</td>
<td>13.4 ± 1.2b</td>
<td>12.4 ± 1.0b</td>
<td>8.9 ± 0.7a</td>
</tr>
<tr>
<td>Propionate</td>
<td>0.7 ± 0.2a</td>
<td>1.7 ± 0.4b</td>
<td>1.5 ± 0.2ab</td>
<td>0.8 ± 0.1ab</td>
</tr>
<tr>
<td>Butyrate</td>
<td>0.6 ± 0.1a</td>
<td>1.9 ± 0.3a</td>
<td>1.1 ± 0.2ab</td>
<td>0.9 ± 0.1a</td>
</tr>
<tr>
<td>Total SCFA</td>
<td>10.2 ± 1.1a</td>
<td>17.0 ± 1.8a</td>
<td>15.0 ± 1.3a</td>
<td>10.5 ± 0.7a</td>
</tr>
</tbody>
</table>

1 Data are expressed as means ± SEM (n = 6 per group). Different superscript letters denote significant differences across dietary groups as analysed by one-way ANOVA followed by Tukey’s test. **P** < 0.001, *P* < 0.001, P < 0.05.

### 4. Discussion

To our knowledge, this was the first in vivo study which compared the effects of soluble and insoluble fibres on the DNA integrity of colonocytes and examined the underlying mechanisms in mice fed a Western-like diet. Colonocytes were constantly challenged with the toxicity of colonic contents, which could lead to carcinogenesis. The present results indicated that dietary fibres, especially soluble fibre, effectively ameliorated the genotoxicity of the high-fat low-fibre diet at 0 h. These results were in agreement with our previous in vitro results showing that dietary fibres reduced the DNA damage of Caco-2 cells induced by fecal water of mice fed high-fat diet (Chen et al., 2010; Yeh, Lin, & Chen, 2007). Besides, our previous study has indicated that dietary fibres, such as KGM and inulin, up-regulated the GPX2 expression in the distal colon (Wu and Chen, 2011b). In agreement with that, the present result indicated that both soluble and insoluble fibres up-regulated...
These protective effects of fibres were likely to be mediated by up-regulation of antioxidant and detoxified enzymes, GPX2 and GST, in the colonocytes.

Earlier studies have indicated that soluble fibres promoted colonic epithelium proliferation (Comalada et al., 2006; Hino et al., 2010). However, the role of fibre on colonic apoptosis has not been examined. The imbalance between cell proliferation and apoptosis could lead to risk in carcinogenesis. Therefore, we also compared effects of soluble fibres and cellulose on the apoptosis and proliferation in the distal colon in the basal state (without the AOM challenge). We found that in the matrix of a high-fat diet, all dietary fibres examined in the present study similarly increased the PI of the whole crypt at 0 h. Furthermore, in order to differentiate the normal cell proliferation in the basal crypt and the “risky” proliferation in the upper crypt (Morini et al., 2005), we further specifically measured the PI in the upper-third crypt. We then found that all dietary fibre examined presently significantly decreased, instead of increasing, PI of the upper third crypt. Therefore, we suggested that addition of dietary fibre, regardless of solubility, into a high-fat low-fibre diet may maintain the normal proliferation and differentiation of colonic epithelium cells. On the other hand, the present study also found that all dietary fibres tended to increase the apoptosis of colonic epithelium cells. Therefore, results regarding the cell apoptosis and proliferation in the distal colon suggested that all dietary fibres examined in this study promoted epithelium turnover without increasing the uncontrolled cell proliferation. The increased cecal butyrate contents, especially in the soluble fibre groups, could supply energy for normal turnover of normal colon epithelium (Roediger, 1982).

The present study further examined effects of dietary fibres on the colonic responses during the initiation stage of carcinogenesis caused by AOM. Results confirmed the genotoxic effect of AOM and indicated that soluble fibres were more effective than cellulose on reducing AOM-induced DNA damages with concordant up-regulation of the colonic antioxidant enzymes, including SOD1, CAT and GST. The antioxidant enzymes in the colonocytes were likely to ameliorate the genotoxicity derived from AOM. Therefore, this study suggested that KGM and inulin effectively ameliorated the AOM-induced DNA damage partially by promoting the antioxidant machinery in the colonocytes.

We further determined the epithelium apoptosis after the AOM treatment since this cell death response appears to be an innate biological mechanism for protection against tumorigenesis. We found that AOM induced the AI at 24 h in all dietary groups, which was in agreement with a previous observation (Hu, Martin, Le, & Young, 2002). Among fibres examined in this study, KGM had the greatest effects on both promoting AI as well as reducing the transcription of Bcl-2, which suggests that KGM could exert the greatest effect on up-regulating apoptotic mechanisms against the AOM challenge. The current study further found that all dietary fibres significantly reduced the AOM-induced cell proliferation in the upper-third and whole crypt, suggesting protective effect of either soluble or insoluble fibre on carcinogen-induced hyper-proliferation of the distal colon. Therefore, KGM effectively induced colonic epithelium apoptosis and all fibres examined presently reduced proliferation after the AOM challenge, which suggest their protective effects on the initiation of carcinogenesis.

Butyrate could be involved in the anti-genotoxic effects of soluble fibres observed in this study before and after the AOM treatment. A previous study showed that butyrate protected against $\text{H}_2\text{O}_2$-induced genetic damage in primary colon cells (Abrahamse, Pool-Zobel, & Reckhemmer, 1999). This effect of butyrate may contribute to the significant lower cellular DNA damage in the KGM and inulin groups before the AOM treatment, and slightly lower damage in the cellulose group. Furthermore, soluble fibre-supplemented groups had an increased cecal butyrate content and decreased DNA damage of colonocytes after the AOM treatment, which supported the potential role of butyrate in the DNA repair process (Kerr et al., 2013). In addition, in vitro cell line studies have shown that butyrate activated the intrinsic pathway of apoptosis and sensitised cancer cells to apoptosis mediated by the extrinsic pathway (Pajak, Gajkowska, & Orzechowski, 2009; Wang, Luo, & Xia, 2009). Previous studies also suggested that the butyrate-induced apoptosis was primarily associated with regulation of gene expressions of pro- and anti-apoptotic proteins such as Bcl-2 protein family, by inhibiting the activity of histone deacetylase (Fung, Cosgrove, Lockett, Head, & Topping, 2012). The role of KGM in epithelial apoptotic responses was in agreement with the increased cecal butyrate content and decreased Bcl-2 gene expression. Therefore, the butyrate derived from fermentation of soluble fibre that occurred after the AOM treatment, could primarily modulate the cellular pathways to apoptosis instead of proliferation.

Another mechanism that could mediate the anti-genotoxic effect of KGM and inulin is the colonic microbiota. The present study, in agreement with previous studies, demonstrated the probiotic effects of KGM and inulin (Wu & Chen, 2011a; Yeh et al., 2007). Probiotic supplement is shown to reduce genotoxic potential of fecal water in patients with atopic dermatitis (Roessler, Forsten, Glei, Ouwehand, & Jahr, 2012). Therefore, the increased fecal bifidobacteria concentration in the soluble fibre-supplemented groups may lead to a lower fecal toxic load and ameliorate colonic DNA damages. In addition, recent studies have shown that bifidobacteria and lactobacilli have anticancer properties (Clark, Robien, & Slavin, 2012; Verma & Shukla, 2013). Although mechanisms have not been fully understood, studies suggest that probiotics or their metabolite may ameliorate the transformation of AOM to toxic methylazoxymethanol by reducing colonic β-glucuronidase activity (Matsumoto, Takata, & Komeiji, 1979; Wu and Chen, 2011a), inhibiting proliferation and inducing apoptosis of colonocytes (Kumar et al., 2013).

Addition of cellulose into the low-fibre diet also ameliorated the AOM-induced DNA damage. However, the efficacy of cellulose was not as great as soluble fibres. When compared with the low-fibre groups at 24 h, cellulose did not significantly enhance gene expressions of any antioxidant enzyme examined, increase cecal SCFA and fecal bifidobacteria or lactobacilli concentrations. However, cellulose significantly reduced the gene expressions of Bcl-2 and Cyclin D1, and PI. Therefore, we suggested that the cellulose could still contribute to the cellular signals to modulate the cellular response to AOM. However, these effects may not be mediated by SCFA and underlying mechanism remains to be investigated.

In summary, the current study indicated that both soluble fibres and cellulose maintained normal cell turnover of crypts at the distal colon in mice fed a high-fat low-fibre diet. As mice were attacked by a carcinogen (AOM), dietary fibres ameliorated colonic DNA damage, with efficacy in the order of inulin > KGM > cellulose. In addition, dietary fibres increased cellular apoptosis response to AOM, with efficacy in the order of KGM > inulin > cellulose. The greater effects of soluble fibres may be mediated by butyrate and probiotics.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2014.01.065.

References


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