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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 indigested 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, &

Abbreviations: AI, apoptotic index; AOM, azoxymethane; Bcl-2, B cell leukemia; CAT, catalase; GPX2, glutathione peroxidase 2; GST-π, glutathione *S*-transferase π; KGM, konjac glucomannan; PI, proliferative index; qPCR, quantitative real-time polymerase chain reaction; SCFA, short-chain fatty acid; SOD1, superoxide dismutase 1.

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Tanaka, 2009). 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 animal 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 CO_2 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. The cecal 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 immunohistological 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 (5×10^5/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*-laurylsarcosine, 0.1 mM Na_2EDTA, 2.5 M NaCl, 1% Triton X-100, 10% 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 Na_2EDTA), 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 (Ccnd1), 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 (5×10^5 cells/ml) in REzol™ 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 12,000*g* 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, 12,000*g* 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 Ccnd1 was Mm01344233_g1 (NM_011434.1), Mm00437992_m1

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