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Partial hydrolysis enhances the inhibitory effects of konjac glucomannan from *Amorphophallus konjac* C. Koch on DNA damage induced by fecal water in Caco-2 cells

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ABSTRACT

This study determined effects of diets containing 5% (w/w) unhydrolysed konjac glucomannan (KGM), and its acid-hydrolysate, fractions 1 and 2 (F1, F2), with degrees of polymerisation 8 and 4, respectively, on the cytotoxicity and DNA damage of fecal water-treated Caco-2 cells. Oligofructose was used as a positive control. In addition, the possible mechanisms for these effects were investigated. Results indicated that KGM, F1, F2 and oligofructose diets similarly increased the survival rate of fecal water-treated Caco-2 cells compared with the FF diet. F2 exerted the greatest protective effects, amongst KGM-based fibres, on fecal water-induced DNA damage. The prebiotic effects of F1 and F2, were also better than that of KGM. However, the fecal water from the group fed KGM, rather than partially-hydrolysed KGMs, exerted the greatest ferrous ion-chelating ability. In conclusion, partially-hydrolysed KGMs exerted greater protective effects than did the unhydrolysed KGM on fecal water-induced DNA damage, mainly by their prebiotic effects.

1. Introduction

Konjac glucomannan (KGM) is a well-known highly viscous dietary fibre extracted from the tuber of *Amorphophallus konjac* C. Koch of the botanical family Araceae (Tye, 1991). It is mainly composed of a β-(1 → 4)-linked D-glucosyl and D-mannosyl polymer with a mean molecular weight of $9.0 \pm 1.0 \times 10^5$ g mol^{-1} (Ratcliffe, Williams, Viebke, & Meadows, 2005). Recent studies have shown that addition of KGM to a low fibre diet could improve bowel habit in children (Loening-Baucke, Miele, & Staiano, 2004) and adults (Chen, Cheng, Liu, Liu, & Wu, 2006). In addition, KGM is also a prebiotic fibre in animals (Chen, Fan, Chen, & Chan, 2005) and humans (Chen et al., 2006). We have previously shown that BALB/c mice fed a 5% KGM diet, as compared to a fibre-free diet, could reduce the toxicity of water-soluble fecal material in Caco-2 cells (Yeh, Lin, & Chen, 2007).

Although KGM exerts many beneficial physiological functions, its high viscosity limits its application in food. Downsizing, by hydrolysing the glucomannan polymer or by pulverising of natural particles, increases the solubility of KGM and may expand its application. The change of particle size (Li, Xia, Wang, & Xie, 2005; Onishi et al., 2007) or degree of polymerisation (DP) (Chen et al., 2005) of KGM may modulate its physiological function. For example, pulverisation of native KGM (grain size 657.3 mm) to a small-sized KGM (grain size 23.7 mm) increases the swelling rate, which may promote the anti-obesity effect (Li et al., 2005). Similarly, small-sized KGM rather than native KGM suppresses the development of allergic rhinitis-like symptoms and IgE response in mice (Onishi et al., 2007). Furthermore, we have previously demonstrated that a raw KGM hydrolysate (average DP of 12) exerts a greater prebiotic effect than does the native KGM in BALB/c mice (Chen et al., 2005). Therefore, partial hydrolysis of KGM may enhance its beneficial physiological effects; however, the optimal DP of this fibre, for various functions, remains unclear.

Anti-carcinogenic effects of prebiotic dietary fibres are associated with probiotics, such as bifidobacteria and lactobacilli (Pool-Zobel et al., 1996); those have been found to decrease fecal water-induced DNA damage in the colonic mucosa (Burns & Rowland, 2004). In agreement with that, we recently proposed that the prebiotic

Abbreviations: FF, fibre-free; KGM, konjac glucomannan; DP, degree of polymerisation; F1 and F2, fractions 1 and 2; FPG, formamidopyrimidine DNA glycosylase; *C. perfringens*, *Clostridium perfringens*.

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characteristic is one of the possible mechanisms whereby KGM decreases the toxicity of faeces (Yeh et al., 2007). In addition, our previous study also suggests that the anti-toxic effect of KGM on colonic cells is partly due to its ferrous ion-chelating ability (Yeh et al., 2007). However, the optimal DP of KGM hydrolysate, for decreasing the toxicity of fecal water toward intestinal cells and for the mechanisms mediating the anti-toxic effect, has not been investigated.

The main goal of the present study was therefore to compare effects of native KGM and its partial hydrolysate, fractions 1 and 2 (F1 and F2) (average DPs 8 and 4, respectively), on the toxicity of fecal water obtained from BALB/c mice toward Caco-2 cells, derived from a human colon adenocarcinoma (Sambuy et al., 2005). We also compared the effect of DP on fecal microflora in BALB/c mice and on ferrous ion-chelating ability of fecal water. Oligofructose, a well-known prebiotic, was used as a positive control in this study.

2. Materials and methods

2.1. Dietary fibres

The konjac powder (purity 80.0%, Fukar Co., Taipei, Taiwan) was boiled in 0.2 N HCl (25 g/l) for 40 min in a round-bottom flask with a reflux condenser and then condensed in vacuum to 1/10 of its original volume. After this, raw KGM hydrolysate was composed to make a 75% ethanol solution (v/v); it was centrifuged at 10,000*g* for 30 min at 4 °C. The pellet was denoted as F1. F2 was precipitated from the same condensed solution with raw KGM hydrolysate by adjusting its ethanol concentration to 90% (v/v). F1 and F2 were successively washed with ethanol to remove the residual acid, lyophilised, and stored in desiccators until used. The sugar composition of each fraction was reported in our previous study (Wang, Lai, Chen, & Chen, 2008), and the mean DPs of F1 and F2, calculated as the ratios of total sugar content (Dubois, Gilles, Hamilton, Robers, & Smith, 1956) and reducing sugar content (Aued, Carvalho, Tavares, Zanelatto, & Bacetti, 1990), were 7.8 ± 0.2, and 3.9 ± 0.1, respectively. The purities of F1 and F2 were 94.0% and 97.7%, respectively. Oligofructose syrup contained 67.0% of oligofructose (Taiwan Sugar Co., Taipei, Taiwan).

2.2. Animals and experimental design

Seven-week-old male BALB/c mice (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 (0800–2000) 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 (National Institutes of Health, 1985) and was approved by the Institutional Animal Care and Use Committee at the Chung Shan Medical University.

After a 10 days adaptation period, the mice were randomly divided into five groups (n = 8/group) and fed a fibre-free AIN-93 diet (FF) or an AIN-93 modified diet containing KGM, F1, F2, or oligofructose (Taiwan Sugar Co., Taipei, Taiwan). The composition of the diets was as follows (g/kg): casein, 200.0; corn starch, 529.5; sucrose, 100.0; corn oil, 70.0; AIN-93G mineral mix, 35; AIN-93 vitamin mix, 1.0; L-cystine, 3.0; choline bitartrate, 2.5; butylated hydroxytoluene, 0.014; and dietary fibre (corrected for its purity), 50. The powdered 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. Faeces voided were collected in ice-bathed tubes, weighed, lyophilised, and stored at −20 °C during days 18–21. The faeces voided from 0900 to 0930 on day 21 were placed directly into the anaerobic solution for analysis of fecal microflora.

2.3. Fecal water preparation

In an adaptation of the method described previously (Rieger, Parlesak, Pool-Zobel, Rechkemmer, & Bode, 1999), lyophilised fecal composites were rehydrated to 3-fold their original fecal weight. The samples were centrifuged at 36,000*g* for 2 h. The supernatant fluid, i.e., fecal water, was collected and used immediately for incubation with Caco-2 cells.

2.4. Cell culture

Caco-2 cells were obtained from the 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 of $NaHCO_3$, 4.5 g/l of glucose, 0.01 g/l of human transferrin, and 1 mM sodium pyruvate (Gibco, Carlsbad, CA, USA) at 37 °C in a humidified incubator under 5% CO_2 and 95% air according to the method described previously (Yeh et al., 2007). 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 Hank's 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 $MgSO_4{\cdot}7H_2O$, 137 mM NaCl, 4.2 mM $NaHCO_3$, and 0.3 mM $NaH_2PO_4{\cdot}H_2O$) at a concentration of 2×10^6 cells/ml. 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 3 h (Rieger et al., 1999). An aliquot (400 μl) was taken to assess cell viability by trypan blue exclusion staining (Freshney, 2005), and the rest of the mixture was centrifuged (600*g*, 10 min) to collect the cells used for the comet assay.

2.5. Comet assay

The comet assay (Pool-Zobel, Bub, Muller, Wollowski, & Rechkemmer, 1997) was used to determine DNA damage. According to the method described previously (Yeh et al., 2007), 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% dimethylsulphoxide) for 1 h at 4 °C. Afterwards, the slides were divided into two subgroups. One of these was treated with formamidopyrimidine glycosylase (FPG, 50 μl, 1 mg/ml) at 37 °C for 30 min for quantification of the oxidised nucleic acids in Caco-2 cells. FPG is a specific enzyme for nicking the DNA at sites of oxidised purine (Collins, Ma, & Duthie, 1995). All of the slides were placed in an electrophoresis tank, and the DNA was allowed to unwind for 15 min in the alkaline solution, followed by electrophoresis. Each value presented was calculated from three batches of experiments, each of which included two slides with at least 50 comets (Collins et al., 1995). The image was analysed (by computer) by using the Interactive Image Analysis Comet Assay III (Perceptive Instrument, Haverhill, Suffolk, UK), and DNA strand breaks were described as tail moment, where

$$\text{Tail moment} = \%\text{DNA in tail} \times \text{tail length}.$$

2.6. Enumeration of intestinal microflora

According to the method described previously (Yeh et al., 2007), Brucella blood agar (Pankuch & Appelbaum, 1986), Lactobacilli MRS agar, bifidobacteria iodoacetate medium-25 (Munoa & Pares, 1988), and modified differential clostridial agar supplemented