Impact of Dietary Polydextrose on the Daidzein Metabolism in Adult Mice

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Equol, a bacterial product from daidzein, has been shown to provide beneficial effects. The impact of polydextrose, a compound known to affect the intestinal flora, was studied for its impact on mouse intestinal flora and isoflavonoids in the cecum and plasma. We hypothesized that polydextrose would change the metabolism of isoflavonoids and intestinal flora in mice. Male mice were administered a 1% polydextrose solution (PD) in their drinking water which was provided ad libitum, and were compared with a control group (CO, water only). Both groups were fed the AIN-93M diet for 24 days. Plasma equol and cecal equol concentrations and in vitro equol production from daidzein with fecal flora of mice were measured. The plasma equol concentration was significantly higher in the PD group than in the CO group at 22 hr after the administration of daidzin. The concentration of equol in the cecum was significantly greater in the PD group than in the CO group at 22 hr after administration of daidzin. In the in vitro incubation of daidzein with the fecal flora of mice, equol concentrations were greater in the PD group. These results suggest that dietary polydextrose has the potential to affect equol production by altering the metabolic activity of the intestinal flora and/or the gut environment.

Key words: daidzein; equol; polydextrose; isoflavonoids; intestinal flora

INTRODUCTION

Beneficial effects of dietary fiber have been reported. Polydextrose, regarded as a kind of dietary fiber, is hardly digested in the small intestine and is fermented in the lower gut by intestinal flora (7). It has been reported that polydextrose tends to decrease the expression of mucosal COX-2 (cyclo-oxygenase-2), possibly reducing the risk of colon cancer-promoting conditions developing in the distal intestine (6). Polydextrose also seems to have prebiotic effects. There were substantial changes in the distribution of fecal anaerobes after polydextrose intake: Bacteroides species (B. fragilis, B. vulgatus, and B. intermedius) decreased, whereas Lactobacillus and Bifidobacterium species increased (8). Recently much attention has focused on the effects of equol. Equol is a bacterial metabolite of the isoflavone, daidzein (12). Human gastrointestinal bacteria seem to play an important role in isoflavone metabolism (12, 2) including the soy isoflavone daidzein (4). Equol is considerably more estrogenic than daidzein (13). Prostate cancer is known to be responsive to estrogen therapy, and it appears that equol or an unknown factor regulating the metabolism of daidzein is involved in the biology of prostate cancer (1). There are few reports on food components that may affect the equol production and bioavailability in vivo. As polydextrose seems to affect the intestinal flora, it is possible that polydextrose affects the metabolism of isoflavonoids via modification of the intestinal flora in the gut.

In this report, we tested the hypothesis that dietary polydextrose affects daidzein metabolism in adult mice. We investigated the effects of dietary polydextrose on plasma and cecal equol in mice orally administered daidzin.

MATERIALS AND METHODS

Materials

Daidzein, daidzin and equol were purchased from LC laboratories (Woburn, MA, United States). β-Glucuronidase type H-5 was obtained from Sigma (St. Louis, MO, United States). Polydextrose was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Treatment of animals

Male Crj: CD-1 (ICR) mice (5 weeks old) were purchased from Charles River Japan, Inc. (Kanagawa, Japan). All mice were specific pathogen-free (SPF), and the animals were housed under conventional conditions in our laboratory. The mice were randomly divided into two groups of nine animals each. The animals were housed in suspended stainless-steel cages with wire mesh
bottoms, in a room kept at 24 ± 0.5°C and a relative humidity of 65%, with 12-h periods of light and dark. They were fed an MF diet (Oriental Yeast Co., Ltd. Tokyo, Japan) for 3 weeks, then the diet was replaced with an AIN-93M diet (11) for 20 days. The AIN-93M diet is usually used as the standard diet for mice. After 20 days, all animals were administered a 1% polydextrose solution (PD) or water (CO) (control group) in their drinking water which was provided *ad libitum*, and both groups continued to receive the AIN-93M diet for 24 days. All mice were given the diet *ad libitum*. Body weight and food consumption were measured during the experiment. Figure 1 shows the animal experiment schedule. Twenty-one days after starting the administration of polydextrose or water, mouse feces were collected and immediately processed for *in vitro* incubation of daidzein with the fecal flora of mice. Twenty-four days after the experimental diet feeding period, a daidzin solution (40 mg • ml⁻¹) was freshly prepared as a 2-ml suspension in water and a 0.2-ml dose was administered to all the mice *via* a stomach tube. Six hours and thirty minutes later, four mice from each group were anesthetized with diethyl ether and blood samples were taken from the abdominal aorta and placed in heparinized tubes. Twenty two hours later five mice from each group were anesthetized with diethyl ether and blood samples were taken from the abdominal aorta and placed in heparinized tubes. The
plasma was separated from whole blood by centrifugation and stored at –80°C until HPLC analysis for isoflavonoids. The cecal contents were collected. Cecal contents were stored at –80°C until HPLC analysis for isoflavonoids. All animals were euthanized with diethyl ether.

All procedures involving mice in this study were approved by the Animal Care Committee of the National Food Research Institute of Japan, in accordance with the “Guidelines for Animal Care and Experimentation” of the National Food Research Institute of Japan.

Analysis of plasma and cecal isoflavonoids

The analysis of plasma isoflavonoids was performed as follows. A total of 200 µl of plasma was added to 200 µl of β-glucuronidase type H-5 solution (35 mg/ml) in 0.2 M sodium acetate buffer (pH 5.0). Next, the mixture was incubated at 37°C in a shaking water bath for 2 h, followed by treatment with 3.6 ml of methanol/acetic acid (100/5, v/v), vortexing for 30 s, sonication for 30 s, vortexing again for 30 s, and centrifugation at 5,000 × g for 10 min. The supernatants were transferred to an eggplant-type flask and evaporated completely using a rotary evaporator. The sample was then dissolved in the mobile phase of the HPLC system in a volume equivalent to the original plasma sample and filtered through a 0.2-µm filter. During HPLC analysis, 20 µl of each preparation was injected into a 250 × 4.6 mm Capcell Pak C18-5 µ column (Shiseido, Tokyo, Japan). To detect isoflavonoids, a JASCO MD-1515 photodiode array detector (JASCO, Co., Ltd., Tokyo, Japan) was used to monitor the spectral data from 200 to 400 nm for each detector (JASCO, Co., Ltd., Tokyo, Japan) was used to monitor the spectral data for isoflavonoids. All animals were euthanized with diethyl ether.

We used the same HPLC analytical method as described above. The anaerobic broth used in this experiment was prepared as follows. Brain heart infusion (37 g), agar (1 g), L-cysteine HCl (0.5 g), and Na2CO3 (4 g) were dissolved in 1 liter distilled water. Aliquots of broth (9 ml) were distributed into test tubes, gassed with O2-free CO2 gas, sealed with a butyl rubber stopper, and sterilized by autoclaving.

RESULTS

General observations

No significant differences were observed between the PD group and the CO group in final body weight (g) or food consumption (g/day).

Plasma concentration of isoflavonoids

No significant difference was observed in the plasma equol concentrations between the PD and CO groups at 6.5 h after the administration of daidzin (Fig. 2). The plasma equol concentration was significantly greater in the PD group than in the CO group at 22 h after the administration of daidzin (P<0.05). No significant difference was observed in the plasma daidzein concentration between the two groups at 6.5 h or 22 h after administration of daidzin.

Cecal concentration of isoflavonoids

No significant differences were observed in the concentrations of equol in the cecum between the two groups at 6.5 h after administration (Fig. 3). The concentration of equol in the cecum was significantly greater in the PD group than in the CO group at 22 h after...
administration \((P<0.05)\). No significant difference was observed in the concentrations of daidzein in the cecum between the two groups at 6.5 h or 22 h after administration.

**In vitro incubation of daidzein with the fecal flora of mice**

There was a significant difference \((P<0.05)\) in equol concentrations between the two groups, with the PD group having the higher concentration (Fig. 4). The daidzein concentration was significantly lower in the PD group than in the CO group \((P<0.05)\).

**DISCUSSION**

Daidzein is metabolized to equol by the intestinal bacterial flora \((5)\). Both compounds have an estrogenic effect and may contribute to protection from cardiovascular disease. Conversion of daidzein to equol in the gut is important because of equol’s strong estrogenicity compared to daidzein.

In this report, we tested the hypothesis that dietary polydextrose affects daidzein metabolism in adult mice. The results indicate that plasma and cecal concentrations of equol were significantly lower in the CO group than in the PD group. A previous study demonstrated that intestinal flora strongly affects isoflavone metabolism \textit{in vivo} \((17)\). In a human study, it was reported that subjects with higher polyunsaturated fatty acids and a higher alcohol intake were more likely to be strong equol producers \((3)\). In animal studies, it has been suggested that the administration of \textit{Lactobacillus gasseri} is likely to influence the effect of isoflavonoids on mice \((14)\). It has also been reported that a combination of dietary fructooligosaccharides and isoflavone conjugates increases femoral bone mineral density and equol production in ovariectomized mice \((10)\). The composition of the diet may affect the production of equol from daidzein in the gut by modifying the metabolic activity and/or composition of the human and animal intestinal microflora.

Polydextrose has been shown to affect the intestinal
microflora, including increases in Lactobacillus and Bifidobacterium species observed in human fecal flora after ingestion of polydextrose (8). However, many of the equol-producing bacteria harboured in the gut do not belong to the Lactobacillus and Bifidobacterium species (9, 16). In the present study, plasma equol concentrations were significantly greater in the PD group. In the in vitro incubation of daidzin with the fecal flora of mice, equol concentrations were also significantly greater in the PD group than in the control group. Cecal equol was significantly greater in the PD group at 22 h after administration of daidzin. These results suggest that equol productivity might be higher in the PD group. It had been reported that dietary polydextrose affects the large intestine in rats (18). Short-chain fatty acid production, notably that of butyrate, isobutyrate and acetate, increased with polydextrose ingestion (8).

Administration of polydextrose might change the equol production via the equol-producing bacteria in the intestinal flora of mice. This stimulating effect might be related to the enhancing effects of volatile fatty acid production by polydextrose.

Some dietary fiber affect the absorption of isoflavonoids. In an experiment with healthy human subjects, dietary seaweed increased equol production (15). Seaweed contains water-soluble dietary fiber. Since polydextrose is a water-soluble fiber, it might enhance the absorption of the equol produced in the gut. Thus, polydextrose administration might alter the gut environment. Our present study suggests that dietary polydextrose has the potential to affect the metabolism of equol by altering the metabolic activity of the intestinal flora and/or gut environment. The limitation of our study was that we could not identify the bacteria that affected the equol production after daidzin administration. Further studies are underway to examine what kinds of intestinal bacteria affect the equol production in mice fed dietary polydextrose.

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