Soy Isoflavonoid Aglycons Genistein and Daidzein Do Not Increase the Cytochrome P-450 Content of the Liver Microsomes of Mice

Taro Kishida, Kunitaka Nashiki, Toru Izumi,† and Kiyoshi Ebihara*

Department of Biological Resources, Faculty of Agriculture, Ehime University, Matsuyama 790-8566, Japan

Mice (4-week-old, male ddy) were fed four isonitrogenic diets for 21 days: purified diet (C diet); fermented soybean (400 mg of soy isoflavonoids/kg; FSB); fermented soybean extract (400 mg of soy isoflavonoid aglycones/kg; FSBE); C with indole-3 carbinol (I3C) (2500 mg of I3C/kg; I3C). The I3C and FSB diets significantly increased the cytochrome P-450 content of hepatic microsomes in comparison with the C diet, while the FSBE diet did not. Other mice were fed seven diets for 21 days: C; C with 100 mg or 200 mg of genistein, 100 mg or 200 mg of daidzein, or 100 mg of genistein + 100 mg of daidzein/kg; I3C diet. Genistein and daidzein did not change the liver cytochrome P-450 content. There was no synergistic effect of the combined feeding of genistein and daidzein. The increase in the cytochrome P-450 content with the FSB diet depends on chemicals other than genistein and daidzein. Genistein and daidzein do not induce cytochrome P-450.

Keywords: *Fermented soybean; genistein; daidzein; P-450; mice*

INTRODUCTION

Cytochrome P-450 comprises a group of drug-metabolizing enzymes involved in the synthesis of cholesterol, steroids, and other important lipids such as prostacyclins and thromboxane A2 in the liver. It has been well established that dietary constituents modulate the activity and content of cytochrome P-450 in liver microsomes (Guengerich, 1984). Modulation of the activity and content of cytochrome P-450 in liver microsomes may be important in terms of human health since these enzymes activate and inactivate a wide range of xenobiotics.

Flavonoids are a group of polyphenolic compounds that modulate various biological activities and influence the cytochrome P-450 activity and content in liver microsomes (Li et al., 1994; Zhai et al., 1998). Variations of the heterocyclic ring C of flavonoids give rise to flavonols, flavones, catechins, flavanones, anthocyanidins, and isoflavonoids. More than 4000 flavonoids have been identified. Many flavonoids modify the activity of drug-metabolizing enzymes (Buening et al., 1981; Siess et al., 1989, 1992, 1995; Sousa and Marletta, 1985; Li et al., 1994). The effect of a flavonoid on the activity of drug-metabolizing enzymes is complex and depends on the structure of the flavonoid (Smith and Yang, 1994).

Sariaslani and Kunz (1986) reported that soybean flour induced cytochrome P-450 in *Streptomyces griseus*. Iitoi et al. (1992) showed that feeding various kinds of miso (soybean paste) tends to increase cytochrome P-450 activity in rats. Kishida et al. (2000) also found that soybean fermented by *Aspergillus awamori* increased the cytochrome P-450 content in the liver microsomes of mice. Soybeans contain isoflavonoids. Therefore, the increase in the cytochrome P-450 content of liver microsomes by dietary soybean flour, miso, and fermented soybean may be mediated by soy isoflavonoids. However, information on the effects of soy isoflavonoids on the cytochrome P-450 activity and content in liver microsomes is limited. Soybean contains various chemical compounds in addition to soy isoflavonoids. The increase in the cytochrome P-450 content of the liver microsomes by dietary soybean flour, miso, and fermented soybean may be mediated by chemical compounds other than soy isoflavonoids.

Therefore, we carried out experiments to evaluate the effect of the two main isoflavonoids in soybean, genistein and daidzein, on the cytochrome P-450 content of the liver microsomes of mice.

MATERIALS AND METHODS

Fermented Soybean (FSB). FSB was prepared from commercial defatted soybean. The defatted soybean was steamed at 100 °C for 90 min. Aliquots (100 g) of the steamed soybean meal were cooled and mixed with 0.1 g of rice flour. *Aspergillus awamori* were inoculated on each aliquot (Hishiroku Co., Kyoto, Japan) at a density of 8×10^7 spores/g of steamed soybean meal. These were incubated at 33−38 °C for 48 h. Following this incubation, water was added to bring the product to 50% content, and this was further incubated at 45 °C for 41 h. This product was designated FSB.

Determination of Isoflavonoid Content in FSB. The amount of daidzin, genistin, daidzein and genistein in the FSB was determined by high-performance liquid chromatography (HPLC) according to the method of Kitada et al. (1986). Briefly, 2 g of FSB was refluxed for 1 h in 20 mL of 800 mL of methanol/L of distilled water in a boiling water bath. The extract was centrifuged at 1500*g* at 5 °C for 20 min. The supernatant was obtained and evaporated to dryness using a rotary evaporator (model RE52B, Yamato, Tokyo, Japan). After evaporation, the dried extract was dissolved in 5 mL of 800 mL of methanol/L of distilled water. Aliquots were filtered through a membrane filter (cellulose acetate, pore size 0.45 mm; DISMIC-13cp; Toyo Roshi, Tokyo, Japan) before analysis by HPLC (model 655, Hitachi, Tokyo, Japan). Separation of the isoflavonoids was performed on a reverse-phase column

* Corresponding author. Telephone no.: 81-89-946-9847. Fax no.: 81-89-946-9847. E-mail address: ebihara@agr.ehime-u.ac.jp.

† Present address: Kikkoman Corp., Research and Development Division, Noda 278-0037, Japan.

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Table 1. Composition of Test Diets (g/kg of Diet) in Experiment 1

component	diet			
	C	FSB	FSBE	I3C
casein	200		200	200
fermented soybean[a]		269.9		
corn oil	50	50	50	50
mineral mixture[b]	35	35	35	35
vitamin mixture[b]	10	10	10	10
sucrose	200	200	200	200
corn starch	505	235.1	503.67	502.5
fermented soybean extract[c]			1.33	
indole-3-carbinol				2.5

[a] Soybean fermented by *Aspergillus awamori*, which contains 113 mg of genistin, 33 mg of daidzin, 704 mg of genistein and 632 mg of daidzein/kg of fermented soybean. [b] Based on AIN-76 (1997). [c] Fermented soybean extract, which contains 155 mg of genistein, 127 mg of daidzein and 18 mg of glycitein/g of fermented soybean extract.

(LiChrosord RP-18, 5 mm, 25 cm long × 4.6 mm i.d.; Merck Co., Inc., Whitehouse Station, NJ) using a mixture of 2.5% acetic acid/acetonitrile (5:2, v/v). The flow rate was 1 mL/min. Daidzin, genistin, daidzein, and genistein were monitored by the UV absorbance at 250 nm with the use of a spectrophotometer (model L-7400, Hitachi, Tokyo, Japan).

Fermented Soybean Extract (FSBE). FSBE which is rich in the two major isoflavonoid aglycones, genistein and daidzein, provided by Kikkoman Co. (SoyAct, Noda, Japan), was used as the source of soy isoflavonoid aglycones. FSEB was produced by the fermentation of soy, followed by ethanol/water extraction and purification. FSBE contains 155 mg of genistein, 127 mg of daidzein, and 18 mg of glycitein per g. The content of genistin, daidzin, and glycitin was each not more than 1 mg/g.

Animals and Diets. This study was approved by the Laboratory Animal Care Committee of Ehime University. The mice were maintained in accordance with the guidelines for the care and use of laboratory animals of Ehime University.

Male std:ddy mice aged 4 weeks (Japan SLC, Hamamatsu, Japan) with an initial weight of approximately 20 g were used in the experiments. The mice were housed individually in cages and were kept at 23 ± 1 °C with a 12-h light/dark cycle (light, 0700 h−1900 h). The mice were acclimated by feeding a commercial solid diet (MF, Oriental Yeast Co., Osaka, Japan) for 7 days. Then, the mice were divided into groups and allowed free access to the test diet and water. Each test diet contained the same level of protein.

Experiment 1: This experiment involved 4 groups of 6 mice each. The mice were fed the FSB diet, FSBE diet, indole-3 carbinol (I3C) diet, or control diet (C diet) for 21 days. The composition of each test diet is shown in Table 1. Each test diet contained the same level of protein. The FSB diet contained 400 mg of soy isoflavonoids/kg of diet. The FSBE diet contained 400 mg of soy isoflavonoid aglycones/kg of diet. On day 21, the mice were sacrificed by decapitation at 1000 h within a 30-min period. The liver was removed and homogenized in the ice cold buffer described below. The amount of cytochrome P-450 in the homogenate of each liver was determined.

Experiment 2: This experiment involved 7 groups of 6 mice each. The composition of the control diet (C diet) was the same as that in experiment 1. Mice were fed one of the following seven test diets for 21 days: C diet; C diet supplemented with 100 mg of genistein/kg of diet; C diet with 100 mg of daidzein/kg of diet; C diet with 200 mg of genistein/kg of diet; C diet with 200 mg of daidzein/kg of diet; C diet with 100 mg of genistein + 100 mg of daidzein/kg of diet; C diet with 2500 mg of I3C/kg of diet. In the various experimental diets, the gelatinized corn starch in the C diet was reduced by an amount equal to the amounts of genistein and daidzein added to the diet. Genistein and daidzein were synthesized according to the method of Wähälä et al. (1995) and were each >99% pure by HPLC. The mice were given free access to the experimental diet and water. The level of food intake was recorded daily for each mouse in the morning before replacing the diet by determining the reduction in the weight of the food jar. The body weight of each mouse was recorded at the beginning and at the end of the experimental period. On day 21, the mice were sacrificed by decapitation at 1000 h within a 30-min period. The liver was removed and weighed. The liver was homogenized in the appropriate ice-cold buffer described below. The amount of cytochrome P-450 in the homogenate of each liver was determined.

Table 2. Effect of Soybean Fermented by *Aspergillus awamori*, Fermented Soybean Extract, and Indol-3-carbinol on Body Weight Gain, Food Intake, Liver Weight, and Cytochrome P450 in Ddy Mice (Experiment 1)[a]

diet	liver wt (mg/100 g of BW)	cytochrome P-450 nmol/(mg of) protein/min
C	4.58 ± 0.16[a]	0.73 ± 0.06[a]
FSB	5.08 ± 0.23[ab]	1.31 ± 0.07[b]
FSBE	4.43 ± 0.12[a]	0.93 ± 0.09[a]
I3C	5.44 ± 0.38[b]	2.18 ± 0.10[c]

[a] Mean initial body weight, 33.1 g (range, 32.7−33.3 g). Each value represents the mean ± SEM, $n = 6$. In each column, values with different superscript letters are significantly different at $p < 0.05$, as determined by Duncan's new multiple range test.

Measurement of Hepatic Cytochrome P-450. The fresh liver of each mouse was homogenized in 6 vol. of 0.1 mol Tris/HCl buffer (pH 7.4) containing KCl (7.4 g/L) and EDTA (0.372 g/L) in a Potter-Elvehjem homogenizer (Asahi Techno Glass Co., Tokyo, Japan). The homogenate was centrifuged for 15 min (10 000*g*) at 4 °C. The postmitochondrial supernatant was centrifuged for 90 min (105 000*g*) at 4 °C. The microsomal pellets were suspended in 0.1 mol Tris/HCl buffer (pH 7.4) containing glycerin (200 mL/L) and EDTA (0.372 g/L). In this suspension, the total cytochrome P-450 content was determined by the dithionite difference method of Omura and Sato (1964). The amount of microsomal protein was determined according to the method of Lowry and Rosebrough (1951), with bovine serum albumin as the standard.

Statistical Analysis. Data were evaluated by ANOVA followed by the Duncan's new multiple range test using the statistical package software Super ANOVA (Abacus Concepts Inc., Berkeley, CA). A difference was considered to be statistically significant at a level of $p < 0.05$.

RESULTS

Isoflavonoid Content in the FSB. The FSB that was prepared contained 113 mg of genistin, 33 mg of daidzin, 704 mg of genistein, and 632 mg of daidzein per kg. The percentage of aglycone out of the total isoflavonoids by weight in the FSB was 89.0%.

The liver weight and the cytochrome P-450 content of the mice that were fed the C, FSB, FSBE, or I3C diet for 7 days are shown in Table 2. The liver weight of the mice fed the I3C diet was significantly higher than that of mice fed the C diet or FSBE diet. The cytochrome P-450 content of the hepatic microsomes of mice fed the I3C or FSB diet was significantly higher than that of mice fed the C or FSBE diet. The cytochrome P-450 content of the mice fed the I3C diet was significantly higher than that of mice fed the FSB diet.

In the second experiment, mice were fed the C diet supplemented with various amounts of genistein and/or daidzein or I3C for 21 days. The body weight gain, food intake, liver weight, and cytochrome P-450 content in the hepatic microsomes of mice fed the various diets are summarized in Table 3. Genistein or daidzein in the diet did not cause a reduction in food intake nor a reduction in body mass gain in comparison with the respective level in mice fed the C diet. Genistein or

Table 3. Effect of Genistein, Daizein, and Indol-3-carbinol on Body Weight Gain, Food Intake, Liver Weight, and Cytochrome P-450 in Ddy Mice (Experiment 2)[a]

diet	body wt gain (g/21 d)	food intake (g/21 d)	liver wt. (mg/100 g of BW)	cytochrome P-450 nmol/(mg of protein/min)
C[b]	12.4 ± 1.0^{ab}	105 ± 2^{a}	4.92 ± 0.20^{a}	0.71 ± 0.04^{a}
G-100[c]	16.3 ± 1.0^{c}	119 ± 3^{b}	5.41 ± 0.32^{a}	0.70 ± 0.11^{a}
D-100[d]	13.3 ± 0.7^{ab}	106 ± 3^{a}	5.72 ± 0.13^{a}	0.84 ± 0.03^{a}
G-200[e]	15.4 ± 0.5^{bc}	114 ± 2^{ab}	5.31 ± 0.12^{a}	0.75 ± 0.07^{a}
D-200[f]	13.6 ± 0.5^{abc}	109 ± 4^{a}	5.62 ± 0.18^{a}	0.87 ± 0.08^{a}
GD-200[g]	14.0 ± 1.6^{abc}	114 ± 4^{ab}	5.76 ± 0.34^{a}	0.79 ± 0.05^{a}
I3C[h]	11.2 ± 0.8^{a}	107 ± 3^{a}	6.92 ± 0.56^{b}	1.56 ± 0.07^{b}

[a] Mean initial body weight, 27.1 g (range, 24.5−29.7 g). Each value represents the mean ± SEM, $n = 6$. In each column, values with different superscript letters are significantly different at $p < 0.05$, as determined by Duncan's new multiple range test. [b] C, C diet. [c] G-100, C diet supplemented with 100 mg of genistein/kg of diet. [d] D-100, C diet supplemented with 100 mg of daidzein/kg of diet. [e] G-200, C diet supplemented with 200 mg of genistein/kg of diet. [f] D-200, C diet supplemented with 200 mg of daidzein/kg of diet. [g] GD-200, C diet supplemented with 100 mg of genistein and 100 mg of daidzein/kg of diet. [h] I3C, C diet supplemented with 2500 mg of indole-3-carbinol/kg of diet.

daidzein in the diet did not significantly affect the liver weight nor cytochrome P-450 content of the hepatic microsomes in comparison with the respective level in mice fed the C diet. There was no synergistic effect of the combination of genistein and daidzein in the diet on the cytochrome P-450 content. I3C in the diet did not cause a significant reduction in food intake nor a significant reduction in body mass gain in comparison with the respective level in the mice fed the C diet. However, I3C in the diet significantly increased both the liver weight and cytochrome P-450 content of the hepatic microsomes in comparison with the respective level in mice fed the C diet.

DISCUSSION

In the two experiments of our study, mice fed the I3C diet were used as the positive control. As shown in Tables 2 and 3, the liver weight and cytochrome P-450 content of the hepatic microsomes of mice fed the I3C diet were significantly higher than the respective level of mice fed the C diet, which is in agreement with the results of Bradlow et al. (1991).

Genistein and daidzein in the diet each did not cause a reduction in food intake nor a reduction in body weight gain in comparison with the respective value in mice fed the C diet (Table 3). However, Magee (1963) reported that genistein significantly reduced the body weight gain in rats fed a dietary level of 50 g of genistein/kg. Also, Ishida et al. (1998) and Toda et al. (1999) showed that daidzein suppressed food intake and body weight gain in ovariectomized rats when orally administered at a dose of 50 mg/(kg/day), whereas genistein did not. The difference between our results and the results of these previous studies may be due to the difference in the quantity of genistein intake.

The liver weight of mice fed the FSB diet was slightly higher than that of mice fed the C diet, although the difference was not significant (Table 2). Genistein and daidzein in the diet each did not affect the liver weight (Table 3).

As shown in Table 2, the cytochrome P-450 content of the hepatic microsomes of mice fed the FSB diet was significantly higher than that of mice fed the C diet. This result is in agreement with the result of our previous study (Kishida et al., 2000). Sariaslani and Kunz (1986) reported that soy flour increased the total cytochrome P-450 level in *Streptomyces griseus*. Iitoi et al. (1992) showed that various kinds of miso (soybean paste) tend to increase cytochrome P-450 activity in rats. Soybean contains isoflavonoids. When fed diets with different levels of isoflavonoid aglycones by varying the proportion of FSB and ethanol-extracted FSB, the cytochrome P-450 content of the hepatic microsomes increased in a dose-dependent manner with the content of isoflavonoids in diet (Kishida et al., 2000). Therefore, we speculated that the effects of soy isoflavonoids included increasing the cytochrome P-450 content of hepatic microsomes. However, the cytochrome P-450 content of hepatic microsomes did not increase in the mice fed the FSBE diet compared with that in mice fed the C diet (Table 2), although the FSBE and FSB diets contained equivalent amounts of soy isoflavonoids. Furthermore, genistein and daidzein each did not affect the cytochrome P-450 content of hepatic microsomes (Table 3). Also, the combined use of genistein and daidzein did not affect the cytochrome P-450 content (Table 3). Helsby et al. (1997) reported that the isoflavonoids equol and genistein do not induce xenobiotic-metabolizing enzymes in mice. Soybean contains various chemical compounds other than soy isoflavonoids. Therefore, it is likely that chemical compounds other than soy isoflavonoids contribute to the increases in the liver weight and cytochrome P-450 content of hepatic microsomes induced by FSB feeding.

Epidemiological studies have shown that frequent consumption of soybean is associated with a reduction in the risk for developing breast cancer. This protective effect has been attributed, in part, to genistein (Barnes, 1995). It has been suggested that this protection is mediated through its effect on the P-450 system (Guengerich, 1988; Obermeier et al., 1995).

In the present study, dietary genistein did not increase the cytochrome P-450 content of hepatic microsomes. The cytochrome P-450 enzymes are classified into families and subfamilies on the basis of sequence similarity. Approximately 1000 cytochrome P-450 sequences have been identified. Genistein may affect the P-450 enzyme profile.

Genistein and daidzein did not increase the cytochrome P-450 content but may induce specific cytochrome P-450 forms. Therefore, it is necessary to investigate the effects of genistein and daidzein on not only the cytochrome P-450 content of hepatic microsomes but also the cytochrome P-450 profile.

ABBREVIATIONS USED

FSB diet, fermented soybean diet; FSBE diet, fermented soybean extract diet; I3C, indole-3 carbinol; C diet, control diet; HPLC, high-performance liquid chromatography.

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