Soy Induces Phase II Enzymes But Does Not Inhibit Dimethylbenz[a]anthracene-Induced Carcinogenesis in Female Rats1,2,3

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ABSTRACT Isoflavones in soy may play a role in the prevention of cancer through their capacity to affect antioxidant or protective phase II enzyme activities. This study evaluated the effects of dietary isoflavone levels on the induction of antioxidant and phase II enzyme activities and inhibition of breast carcinogenesis. Female Sprague-Dawley rats (36 d) were fed one of four purified diets with casein, or with soy containing three levels of isoflavonoids (0.03, 0.4 or 0.81 mg/g diet; low, middle and high level of isoflavones, respectively). After 2 wk, enzyme activity was determined of rats (n = 6–7) from each diet group. Liver glutathione peroxidase and glutathione reductase activities, blood glutathione levels, kidney glutathione s-transferase and colon quinone reductase (QR) activities were greater in rats consuming the high isoflavone diet compared to rats consuming the casein diet. Kidney QR and liver, kidney, small intestine, and colon UDP-glucuronosyltransferase activities were greater in rats fed the high isoflavone diet compared to rats fed the casein and low-isoflavone diets. Liver and blood oxidized glutathione were lower in rats fed the high-isoflavone diet compared to those fed the low-isoflavone diet. A subset of rats (n = 86) was fed the purified diets for 2 wk and intubated with dimethylbenz[a]anthracene or peanut oil and palpated weekly for tumors. At 13 wk, there was an inverse relationship (R² = 0.911, P < 0.09) between tumor incidence and increasing isoflavone intake. These data support the mechanism of soy and soy isoflavones as antioxidant and phase II enzyme inducers, but not as tumor inhibitors. J. Nutr. 129: 1820–1826, 1999.

KEY WORDS: rats • soy • carcinogenesis • phase II enzymes

Evidence shows that the consumption of soybean preparations (soybean chips, soybean paste, soy sauce) inhibits tumor-rogenesis in experimental animals (Baggott et al. 1990, Messina and Barnes 1991, Nagahara et al. 1992). Various soybean preparations fed to rodents resulted in a reduced incidence and delayed appearance of rat mammary carcinogenesis induced by dimethylbenz[a]anthracene (DMBA)5 or methylnitrosourea (Baggott et al. 1990, Barnes et al. 1990) and inhibition of mouse forestomach neoplasia induced by benzo[a]pyrene (Nagahara et al. 1992). Recent work involving chemoprevention by soy focused on its major phytoestrogens, genistein and daidzein, and mechanisms explaining protective effects. Suggested mechanisms involve alteration of the ontogeny of the mammary gland (Lamartiniere et al. 1998), alteration in hormonal status and regulation of the menstrual cycle (Cassidy et al. 1994), protease inhibition (Maki et al. 1994), or antioxidant activity (Record et al. 1995).

The protective effects of soy may also be exerted in other steps involved mechanistically in cancer development, such as phase I and II metabolism of carcinogens. Induced levels of the phase II enzymes, glutathione s-transferase (GST, E.C. 2.5.1.18) and quinone reductase (QR, E.C. 1.6.99.2) were proposed as suitable biomarkers for identifying compounds likely to inhibit carcinogenesis (Prochaska and Fernandes 1993; Sparnins et al. 1982). There is evidence for a relationship between induction of phase II enzyme activity by dietary nonnutritive compounds and anticarcinogenic effects in the DMBA-induced animal tumor model (Elegbede et al. 1993). There is also initial evidence that soy or its isoflavones can induce antioxidant and phase II enzymes. In a recent study, soy flour and soy protein isolate increased phase II enzyme activity, especially QR and UDP-glucuronosyl transferase (UDPGT, E.C. 2.4.1.17) in various tissues of rats fed soy for 1 and 2 wk (Appelt and Reicks 1997). Mouse hepatic UDPGT activity was increased after animals were fed processed soybean flakes at 8 and 25% of the diet for 90 d (Mirsalis et al. 1993). In a preliminary study, increases in GST, QR and UDPGT activities were observed in liver from rats fed isolated soy protein and basal diet supplemented with the isoflavones, genistein and daidzein (Staack and Jeffery 1994). Cai and Wei (1996) showed small but significant increases in hepatic GST activity of mice fed genistein as well as the antioxidant enzymes.

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Abbreviations used: DMBA, dimethylbenz[a]anthracene; GSH Px, glutathione peroxidase; GSH, reduced glutathione; GSH Rd, glutathione reductase; GST, glutathione s-transferase; GSSG, oxidized glutathione; Hgb, hemoglobin; 4 HNE, 4-hydroxy-2-nonenal; QR, quinone reductase; UDPGT, UDP-glucuronosyltransferase.
glutathione peroxidase (GSH Px, E.C. 1.11.1.9) and glutathione reductase (GSH Rd, E.C. 1.6.4.2) in skin and small intestine. Further study of the dose-response relationship between soy, its isoflavones and carcinogen-metabolizing enzyme activity is warranted given the previous animal studies documenting induction and the evidence that induction of phase II enzymes may inhibit carcinogenesis. Since many studies involved feeding large amounts of soyfoods or high concentrations of isoflavones, it is important to examine the dose-response relationship when soy is fed at more moderate doses. It is not known whether feeding dietary isoflavones from soy at levels that are more consistent with the typical human intake or are relatively low compared to those used in other studies induces phase II enzymes or inhibits DMBA-induced tumorigenesis. The purpose of the current study was to determine whether there was a time and dose-dependent effect of dietary soy isoflavones in soy protein on antioxidant and phase II enzyme activity, and tumor development induced by DMBA in female Sprague-Dawley rats.

MATERIALS AND METHODS

Animals. Female Sprague-Dawley rats [n = 111 (SASCO, Omaha, NE), 36-d-old and weighing 75–100 g, were housed individually in stainless steel cages and divided into four experimental groups. The temperature-controlled room was maintained at 25°C with a light cycle of 12 h on and 12 h off. Rats were given free access to AIN-93G purified diets and distilled water. Body weight and food consumption were recorded weekly. Animal care and use complied with the Guide for the Care and Use of Laboratory Animals (NRC 1985).

Diets. The casein control diet composition (Dyets, Inc., Bethlehem, PA) was the same as the AIN-93G diet (Table 1; Reeves et al. 1993). Experimental diets contained soy protein isolate with three levels of isoflavones (0.03, 0.4 or 0.81 mg/g diet; low, middle or high level of isoflavones, respectively) to provide 20% total protein (donated by Protein Technologies International, St. Louis, MO). To maintain equal content of l-tartaric acid, l-cystine, vitamins and minerals, these levels were adjusted in soy-based AIN-93G purified rodent diets (Reeves 1997). Dietary isoflavone concentrations were analyzed using HPLC by P. Murphy at Iowa State University (Murphy et al. 1981, 1982).

Experimental design. Rats were randomly assigned to one of the four diet treatments and fed the experimental diets for 2 wk. At 2 wk, a subset of rats (n = 6–7 rats per group) was killed by CO2 asphyxiation and tissues were collected and stored at −80°C until further analysis for enzyme activity. Also at 2 wk, the remaining rats (age 50 d) were gavaged with 10 mg of DMBA suspended in 0.475 mL of peanut oil (n = 14–15 rats per group) or peanut oil only (n = 7 rats per group). These rats were fed the same experimental diets until they were killed at 127 d of age (13 wk). Enzyme activity in the first subset of rats was used as an estimate of induction of enzyme activity that could be expected at 2 wk in the subset of rats that received carcinogen treatment.

Chemicals. All chemicals were obtained from Sigma Chemical Company (St. Louis, MO) at the highest-purity level available.

Sample collection. Rats were asphyxiated with CO2, and blood was collected via cardiac puncture. Microsomal isolation was done according to Crankshaw et al. (1979). Livers were immediately perfused with homogenization buffer (50 mmol/L Tris, pH 7.5, 150 mmol/L KCl, 0–4°C), excised and weighed. The livers were homogenized using a Polytron tissue homogenizer. Phenylmethylsulfonyl fluoride (1 mmol/L) was added prior to homogenization. Standards, differential centrifugation was used to isolate microsomes and cytosol. Microsomal pellets were resuspended and washed with buffer (100 mmol/L tetradsodium pyrophosphate and 10 mmol/L of EDTA, pH 8.8 0–4°C) at 100,000 × g for 70 min. Final pellets were resuspended in Tris buffer (50 mmol/L, pH 7.5, 0–4°C) containing 50% glycerol and 0.1% BHT. A small portion of the liver was frozen in liquid nitrogen for later determination of GSH. Small intestine, kidney and colons were removed, flushed with homogenization buffer, and frozen in liquid nitrogen for microsomal and cytosol separation. All samples were stored at −80°C until further analysis.

Sample analysis. Assays were completed using various spectrophotometric methods (Gilford Response UV-VIS Spectrophotometer, Oberlin, OH). Cytosolic GST activity was assayed using 1-chloro-2,4-dinitrobenzene as the substrate (Coomes et al. 1983; Habig et al. 1974). Cytosolic NAD(P)H:(quinone acceptor) QR activity was assayed according to the method of Pochapska (1994). Microsomal UDPGT activity was determined using an NAD+ linked assay (Mulder and Van Doorn 1975). GST Px activity was measured according to the method of Lawrence and Burk (1976). GST Rx activity was assayed according to the method of Worthington and Rosemeyer (1974). Total and oxidized glutathione (GSSG) concentrations were determined as previously described (Anderson 1989). Cytosolic and microsomal protein were measured using the Bio-Rad protein reagent according to directions included with the kit (Bio-Rad Laboratories, Richmond, CA).

Evaluation of mammary tumor development. Developing mammary tumors were palpated and measured with a vernier calipers weekly beginning 4 wk after DMBA administration. Appearance, location and size of mammary tumors were noted. Mammary tumor development was assessed according to the following parameters: the rate of tumor appearance (the mean number of palpable tumors in each treatment group/wk); the final number of mammary carcinomas at the termination of the experiment (13 wk after DMBA administration); the percentage of tumor incidence (the number of rats with mammary tumors/total number of rats given carcinogen treatment); and the mean mammary tumor latency period (as determined by palpation, the time between DMBA administration and the initial appearance of each palpable tumor). At the termination of the experiment, all palpable and nonpalpable tumors were excised and preserved in 10% formalin and processed for histopathological analysis. Tumor pathology was characterized by H. Kurtz, veterinary pathologist, at the University of Minnesota. Only confirmed tumors are reported in the results.

Statistical methods. Data were analyzed by analysis of variance, and then differences in means were determined according to Tukey-Kramer multiple comparisons test with In Stat. 2.0 (GraphPad Software, San Diego, CA). Data were transformed via logarithmic transformation when necessary to meet assumptions for parametric analysis. Linear correlation and Chi-squared trend were calculated for

### Table 1

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>AIN-93G</th>
<th>AIN-93G + Soy protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornstarch (&gt;85% protein)</td>
<td>396.406</td>
<td>397.486</td>
</tr>
<tr>
<td>Casein (&gt;85% protein)</td>
<td>200.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Soy protein isolate (&gt;90% protein)</td>
<td>0.000</td>
<td>200.000</td>
</tr>
<tr>
<td>DEXTROSE: Dextrinized cornstarch (90-94% tetrasaccharides)</td>
<td>132.000</td>
<td>132.000</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100.000</td>
<td>100.000</td>
</tr>
<tr>
<td>Soybean oil (no additives)</td>
<td>70.000</td>
<td>70.000</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50.000</td>
<td>50.000</td>
</tr>
<tr>
<td>Mineral mix (AIN93G-MX)</td>
<td>35.000</td>
<td>35.000</td>
</tr>
<tr>
<td>Vitamin mix (AIN93G-VX)</td>
<td>10.000</td>
<td>10.000</td>
</tr>
<tr>
<td>L-Cysteine</td>
<td>2.540</td>
<td>1.500</td>
</tr>
<tr>
<td>l-Methionine</td>
<td>2.540</td>
<td>2.400</td>
</tr>
<tr>
<td>Choline bitartrate (41.1% choline)</td>
<td>2.500</td>
<td>2.500</td>
</tr>
<tr>
<td>Tert-butylhydroquinone</td>
<td>0.014</td>
<td>0.014</td>
</tr>
</tbody>
</table>

1 Dyets, Inc. Bethlehem, PA
number of tumors developed in each diet group/wk and percentage of tumor incidence, respectively. A probability level of $P < 0.05$ was used to determine statistical significance. Values are means ± SEM.

**RESULTS**

Daily food consumption and body weight were not significantly different among rats in experimental diet treatment groups. Mean intake of food at 2 and 13 wk was 16.0 ± 0.3 and 18.5 ± 0.6 g/day, respectively (n = 111 and 86 rats, respectively). Mean body weight for rats killed at 2 wk was 154.5 ± 2.5 g (n = 25). Mean body weight for rats killed at 13 wk was 236.5 ± 2.0 g (n = 86). Relative liver weight in rats at 2 and 13 wk (4.43 ± 0.08 and 3.43 ± 0.07 g/g body wt, respectively) also did not vary among experimental groups.

Intake ($\mu$g/g body wt · d) of selected isoflavones (daidzin, genistin, M-genistin, A-genistin, daidzein and genistein) was significantly higher in rats fed for 2 wk compared to rats fed for 13 wk across the high-level isoflavone groups (Table 2). Isoflavone intake varied with dose at both 2 and 13 wk.

Glycitin activity (Table 3) was significantly greater in the liver of rats fed the high isoflavone diet at 2 wk compared to rats fed the middle-level isoflavone diet (P < 0.05). The specific activity of GST in the kidney of rats fed the high and high-level isoflavone diets was 183% of the value for rats fed the casein diet for 2 wk. Greater activity was observed in the small intestine of rats fed the high isoflavone diet compared to rats fed the casein diet for 2 wk. Greater activity was observed in the kidney of rats fed the middle and high-isoflavone diets was 183% of the value for rats fed the casein diet for 2 wk. Greater activity was observed in the small intestine of rats fed the high isoflavone diet compared to rats fed the casein diet for 2 wk. Greater activity was observed in the small intestine of rats fed the high isoflavone diet compared to rats fed the casein diet for 2 wk. Greater activity was observed in the small intestine of rats fed the high isoflavone diet compared to rats fed the casein diet for 2 wk. Greater activity was observed in the small intestine of rats fed the high isoflavone diet compared to rats fed the casein diet for 2 wk. Greater activity was observed in the small intestine of rats fed the high isoflavone diet compared to rats fed the casein diet for 2 wk. Greater activity was observed in the small intestine of rats fed the high isoflavone diet compared to rats fed the casein diet for 2 wk. Greater activity was observed in the small intestine of rats fed the high isoflavone diet compared to rats fed the casein diet for 2 wk. Greater activity was observed in the small intestine of rats fed the high isoflavone diet compared to rats fed the casein diet for 2 wk. Greater activity was observed in the small intestine of rats fed the high isoflavone diet compared to rats fed the casein diet for 2 wk. Greater activity was observed in the small intestine of rats fed the high isoflavone diet compared to rats fed the casein diet for 2 wk. Greater activity was observed in the small intestine of rats fed the high isoflavone diet compared to rats fed the casein diet for 2 wk. Greater activity was observed in the small intestine of rats fed the high isoflavone diet compared to rats fed the casein diet for 2 wk. Greater activity was observed in the small intestine of rats fed the high isoflavone diet compared to rats fed the casein diet for 2 wk. Greater activity was observed in the small intestine of rats fed the high isoflavone diet compared to rats fed the casein diet for 2 wk. Greater activity was observed in the small intestine of rats fed the high isoflavone diet compared to rats fed the casein diet for 2 wk. Greater activity was observed in the small intestine of rats fed the high isoflavone diet compared to rats fed the casein diet for 2 wk. Greater activity was observed in the small intestine of rats fed the high isoflavone diet compared to rats fed the casein diet for 2 wk. Greater activity was observed in the small intestine of rats fed the high isoflavone diet compared to rats fed the casein diet for 2 wk. Greater activity was observed in the small intestine of rats fed the high isoflavone diet compared to rats fed the casein diet for 2 wk. Greater activity was observed in the small intestine of rats fed the high isoflavone diet compared to rats fed the casein diet for 2 wk. Greater activity was observed in the small intestine of rats fed the high isoflavone diet compared to rats fed the casein diet for 2 wk. Greater activity was observed in the small intestine of rats fed the high isoflavone diet compared to rats fed the casein diet for 2 wk. Greater activity was observed in the small intestine of rats fed the high isoflavone diet compared to rats fed the casein diet for 2 wk. Greater activity was observed in the small intestine of rats fed the high isoflavone diet compared to rats fed the casein diet for 2 wk. Greater activity was observed in the small intestine of rats fed the high isoflavone diet compared to rats fed the casein diet for 2 wk. Greater activity was observed in the small intestine of rats fed the high isoflavone diet compared to rats fed the casein diet for 2 wk. Greater activity was observed in the small intestine of rats fed the high isoflavone diet compared to rats fed the casein P < 0.05).

Plasma GSH Px and GSH Rd (Table 4) activities did not differ among diet groups. However, liver GSH Px and GSH Rd activities were greater in rats fed the high isoflavone diet for 2 wk compared to rats fed the casein diet (P < 0.05). After 13 wk of consuming the high isoflavone diet, rats had lower hepatic GSH Px and GSH Rd activities compared to rats consuming the high isoflavone diet for 2 wk (P < 0.001). Liver total glutathione concentrations (Table 4) were not different among diet groups; however, the consumption of the high isoflavone diet resulted in higher amounts of percentage of GSSG compared to rats fed the low isoflavone diet (P < 0.05). Blood glutathione (GSH) concentrations (Table 4) were greater in rats fed the high isoflavone diet for 2 and 13 wk compared to rats fed the casein diet (P < 0.05). The percentage of GSSG in whole blood was lower in rats fed the high isoflavone diet for 2 and 13 wk compared to rats fed the casein diet (P < 0.05).
Tumor latency period was relatively constant among diet groups (Table 5). Rats fed the middle- and high-level isoflavone diets had 1.6 ± 0.3 and 1.7 ± 0.5 tumors/tumor-bearing rat, respectively, while rats fed the casein and low isoflavone diets had 2.7 ± 0.8 and 2.1 ± 0.6 tumors/tumor-bearing rat, respectively. These differences were not statistically significant. Tumor burden and tumor latency period (Table 5) were variable and not related to diet treatment. Rats fed the high isoflavone diet showed a trend toward lower tumor incidence by wk 13 compared to rats fed the casein diet (P < 0.09). There was also a trend toward decreasing numbers of tumors (Fig. 1, P < 0.10) and percentage of tumor incidence (P < 0.01).
**TABLE 5**

<table>
<thead>
<tr>
<th>Diet</th>
<th>n</th>
<th>Tumor incidence</th>
<th>Tumor latency period</th>
<th>Tumors/tumor bearing rat</th>
<th>Tumor burden tumor wt (g)/tumor-bearing rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>14</td>
<td>71.4</td>
<td>10.8 ± 0.4</td>
<td>2.7 ± 0.8</td>
<td>1.7 ± 1.5</td>
</tr>
<tr>
<td>Low isoflavone</td>
<td>14</td>
<td>57.1</td>
<td>9.6 ± 0.4</td>
<td>2.1 ± 0.6</td>
<td>3.4 ± 2.5</td>
</tr>
<tr>
<td>Middle isoflavone</td>
<td>15</td>
<td>53.3</td>
<td>9.8 ± 0.6</td>
<td>1.6 ± 0.3</td>
<td>2.9 ± 1.1</td>
</tr>
<tr>
<td>High isoflavone</td>
<td>15</td>
<td>40.0</td>
<td>10.1 ± 0.7</td>
<td>1.7 ± 0.5</td>
<td>1.6 ± 1.2</td>
</tr>
</tbody>
</table>

1 Values for tumor latent period, number of tumors/tumor-bearing rat, and tumor burden are means ± SEM. Tumor incidence was measured at the termination of the experiment (13 wk). Values are for palpable and nonpalpable tumors discovered upon termination of the experiment. None of the values are significantly different. There were no tumors in rats not treated with DMBA (peanut oil only).

= 0.911, P < 0.09) in rats treated with carcinogen with increasing isoflavone level. There were no tumors present in the rats not treated with DMBA (peanut oil only) (data not shown).

**DISCUSSION**

Ideal chemopreventers which are involved through the alteration of phase I or II detoxification enzymes should be present in common foods and effective at moderate doses (Morse and Stoner 1993). The present study indicates that dietary soy isoflavones at larger than moderate doses are needed to induce antioxidant and phase II detoxification enzymes in various tissues in female Sprague-Dawley rats. While a consistent dose-response relationship was not observed, in general, the highest dose of isoflavones was more effective than the middle and low doses in tissues where significant increases in activity were observed for various enzymes. The highest dose used based on intake of genistein was comparable to a dose two to three times higher than the estimated typical consumption for the Asian population consuming their traditional diet (about 20–80 mg/d of genistein or about 0.3 to 1.1 mg/kg body weight · d) for a 70 kg reference person (Adlercreutz et al. 1993; Barnes et al. 1995). For those not consuming soy as a staple in the diet, a 70 kg reference human consuming 114 g of tofu/d would consume about 0.34 mg genistein/(kg BW · d) (Wang and Murphy 1994). This is somewhat comparable to the level present in the low- to middle-level isoflavone diets [0.16 to 0.88 mg/(kg body weight of the rat · d)]. Since induction was observed mostly with the higher levels of isoflavones, the results from the present study indicate that in rodents, a higher dose than is typical of human consumption is needed to exert the clinical/biological effect of inducing phase II enzymes.

With the technology available, it was not possible to generate an isoflavone-free soy-protein-based diet; therefore, observed effects can not be unequivocally attributed to the isoflavone content of the diet. Other components in soy, which could have been responsible for the observed effects, were not measured. However, the level of these other components is likely to be constant in all three soy-based diets.

The ability to induce phase II enzymes without inducing or decreasing phase I enzyme activity also appears to be an ideal quality of a chemopreventive agent (Morse and Stoner 1993). In the present study, phase I metabolism was not examined, but others have shown that a variety of flavonoids can alter xenobiotic metabolism by both inhibition and induction of certain detoxification enzymes (Siess et al. 1992; Yannai et al. 1998). Helsby et al. (1997) showed that genistein and equol (40 mg/kg i.p. 4 d) inhibited rodent and human cytochrome P450 isozymes including CYP1A2-, CYP2E1-, and CYP3A1-dependent activities by noncompetitive mechanisms. Several flavonoids, quercetin, morin, and kaempferol, were reported to be potent inhibitors of cytochrome c reductase (P-450), which suggests that polyhydroxylated flavonoids, and maybe isoflavonoids, may reduce cancer risk by inhibiting the reduction of cytochrome P-450 (Buening et al. 1981). In the present study, GST, UDPGT and QR were induced in various tissues at different lengths of time, ranging from 2 to 13 wk of soy feeding. The results from the present study, when combined with the previously reviewed studies, indicate that soy isoflavones are able to alter both phase I and II metabolism of xenobiotics and thus may not meet the criteria for an ideal chemopreventer (Morse and Stoner 1993).

In the current study, a clear trend (P < 0.09) toward a decrease in tumor incidence was observed as the dietary isoflavone concentration increased compared to those animals fed a casein diet. However, this decrease did not achieve statistical significance by wk 13 (P < 0.09). It is possible that had the study been continued for several weeks longer, the difference may have become statistically significant. In addition to having more tumors, rats consuming the casein diet also had larger numbers of adenocarcinomas and invasive tumors (i.e., come-
Several studies provide evidence for a proposed mechanism whereby an increase in phase II detoxification enzymes by soy could increase metabolism of DMBA, resulting in anticarcinogenic effects in this tumor model (Elegbede et al. 1993; Slaga 1983). Simultaneous detoxification of the ultimate reactive metabolite of DMBA, i.e., 3,4-dihydrodiol-1,2-epoxide occurs by phase II enzymes. Elegbede et al. (1993) showed that the anticarcinogenic activity of dietary monomeric monoterpenes such as, limonene and sobrerol, is mediated through the induction of hepatic GST and UDPGPT during initiation in DMBA-induced carcino genesis. Sparnins and Wattenberg (1981) suggested that GST enhancement of 75% above control values is correlated with a reduced carcinogenic response to benzo(a)pyrene-induced carcino genesis in the mouse fore stomach. Other studies suggest that an induction of GST and other biotransformation enzymes may be responsible for the anticarcinogenic properties of the phenolic antioxidants BHT and BHA (Benson et al. 1980, DeLong et al. 1985). Baggott et al. (1990) fed rats a diet consisting of 20% miso (Japanese soybean paste) which resulted in a reduced incidence and delayed appearance of DMBA-induced mammary tumors in female Sprague-Dawley rats compared to control rats (Baggott et al. 1990). While in the current study did not directly test for a cause and effect relationship between phase II induction and decreased DMBA-induced tumorigenesis, the literature shows that this is a viable explanatory mechanism.

The antioxidant effects of soy isoflavones may be another mechanism for decreased DMBA-induced tumors in rats. Hirose et al. (1994) reported a significant decrease in DMBA-induced tumors and increased survival in rats fed dietary antioxidants, especially green tea catechins. Singletary (1990) showed that rats fed 0.6% dietary BHT had increased liver GST and QR activities, which resulted in decreased binding of DMBA to mammary and liver DNA. Enzyme activities were not increased in mammary tissue which suggests that decreased DMBA-induced carcinogenesis may involve increased liver metabolism of DMBA. In the current study, an elevation in antioxidant enzyme activities, such as GSH Px and GSH Rd, seen in rats fed the high isoflavone diet for 2 wk (at time of carcinogenic exposure), may have reduced the amount of electronegative metabolites of DMBA produced in the liver, thus decreasing interaction with DNA and proteins in the mammary gland.

GSH can act as a nonenzymatic antioxidant and reduce cytotoxic aldehydes and other oxidative products directly. This decrease in oxidative products thus leads to protection of DNA and other macromolecules which otherwise might increase tumor formation (Fiala et al. 1985; Ishikawa and Sies 1989). Spitz et al. (1991) showed that an enhancement of GSH and GST levels in vitro resulted in increased detoxification of 4-hydroxy-2-nonenal (4HNE), a toxic aldehyde formed as a by-product of lipid peroxidation. These data suggested that the GSH-dependent pathway may be responsible for the cellular resistance and increased metabolism and detoxification of 4 HNE observed in vitro. In the current study, it is possible that the elevation of blood GSH in rats fed the high isoflavone diet at 2 wk and rats fed the low-, middle- and high-level isoflavone diets for 13 wk compared to the casein diet groups reduced the level of toxic oxidative products produced in the body and by tumors leading to protective effects.

In summary, these studies indicate that dietary soy consumption resulted in induction of phase II and antioxidant enzyme activities but did not result in a significant dose-dependent decrease in DMBA-induced mammary carcinogenesis. At 13 wk, tumor incidence was not significantly reduced (P < 0.10) by soy or isoflavone consumption. It is clear that several mechanisms may be involved in the inhibition of carcinogenesis by constituents in dietary soy products. Additional study is justified to determine which step in the carcinogenic process is affected or whether constituents in soy act at several steps involved in the process (i.e., initiation or promotion).

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LITERATURE CITED


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