Chemopreventive Effects of Soy Protein and Purified Soy Isoflavones on DMBA-Induced Mammary Tumors in Female Sprague-Dawley Rats

Andreas I. Constantinou, Daniel Lantvit, Michael Hawthorne, Xiaoying Xu, Richard B. van Breemen, and John M. Pezzuto

Abstract: There are conflicting reports on the effect of soy and its components on mammary carcinogenesis in adult female rats, mainly because of different rodent models that are used in chemoprevention studies. The present study was undertaken to compare the tumor-preventative effects of soy protein isolate (SPI) and two of its isoflavones in a “standard” model that had been used for the identification of many chemopreventive agents. Six groups of female Sprague-Dawley rats were provided with modified cornstarch AIN-76A diets supplemented as follows: no additional agents (control), purified genistein (200 mg/kg diet), purified daidzein (200 mg/kg diet), genistein + daidzein (100 mg/kg diet each), SPI containing normal levels of isoflavones (SPI-n), or SPI depleted of isoflavones (SPI-d). Mammary carcinomas were induced by 7,12-dimethylbenz[a]anthracene (DMBA) introduced 1 wk after the animals began consuming the experimental diets. At the end of the study (120 days after DMBA treatment), no significant differences were found among the six groups with respect to tumor incidence or survival, nor was there a significant reduction in tumor multiplicity in the genistein or genistein + daidzein group. However, there was a 32% reduction in tumor multiplicity in the daidzein and SPI-n groups relative to the control group (P < 0.05). The most effective diet was SPI-d, which produced a 50% reduction in tumor multiplicity relative to the control (P < 0.01). The difference between the SPI-d group and the daidzein or SPI-n group was not significant. Median tumor latency was increased by 33 days in the control group to 68 days in the daidzein group and to 72 days in the SPI-d group, but these differences were not statistically significant. These results show that daidzein and SPI (with normal or low levels of isoflavones) are effective inhibitors of DMBA-induced mammary tumors in adult rats.

Introduction

Populations consuming predominantly plant-based diets tend to have lower incidence and mortality rates of several cancers, including breast cancer, than populations consuming mainly animal-based diets. An inverse relationship has been reported between the consumption of soybean products and breast cancer risk in premenopausal women (1). Striking differences were found when the incidence of breast cancer in women from Japan and regions of China who derive a large percentage of their daily caloric intake from soybeans was compared with breast cancer incidence in women from Western industrialized countries who consume little or no soy products. However, steep increases in breast cancer incidence and mortality rates were reported in urban areas of China, Japan, and Singapore, concomitant with increased consumption of animal fat (2). Breast cancer rates in Asian-American women who adopt Western diets mimic rates in women who normally consume such diets (3), suggesting that the difference in cancer rates is influenced by dietary, rather than genetic, factors.

However, chemoprevention studies in female rats fed soy diets (or diets containing purified soy components) have given conflicting results, providing little support for suggestions based on epidemiological data. These studies have involved whole soybeans, textured soy protein, soy protein isolates (SPI) containing natural or low levels of isoflavones, and purified soy components that were evaluated in chemically induced mammary carcinogenesis models with rats (4–9). The differences between the models include the age of the rat at the time of test agent administration, the route of administration, the type and dose of the carcinogen, the rat strain, the dose of soy or its components, and the tumor parameters being measured. In prepubertal rats, genistein injections suppressed 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammary tumors by accelerating terminal differentiation of the mammary gland (10). Adult female rats whose mammary gland can no longer undergo differentiation are not expected to respond in this manner when exposed to soy or its isoflavones (11,12).

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Table 1. Composition of Experimental and Control Diets.a,b

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (DMBA control)</th>
<th>Group 2 (genistein)</th>
<th>Group 3 (daidzein)</th>
<th>Group 4 (genistein + daidzein)</th>
<th>Group 5 (SPI-n)</th>
<th>Group 6 (SPI-d)</th>
<th>Group 7 (Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIN-76A (17.4% casein protein)</td>
<td>840</td>
<td>840</td>
<td>840</td>
<td>840</td>
<td>840</td>
<td>840</td>
<td>840</td>
</tr>
<tr>
<td>SPI-n (87.0% soy protein)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>160</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SPI-d (80.4% soy protein)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>160</td>
<td>0</td>
</tr>
<tr>
<td>Casein (85%) protein</td>
<td>160</td>
<td>160</td>
<td>160</td>
<td>160</td>
<td>0</td>
<td>0</td>
<td>160</td>
</tr>
<tr>
<td>Genistein</td>
<td>0</td>
<td>0.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Daidzein</td>
<td>0</td>
<td>0</td>
<td>0.2</td>
<td>0.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total protein</td>
<td>282</td>
<td>282</td>
<td>282</td>
<td>282</td>
<td>285</td>
<td>284</td>
<td>282</td>
</tr>
</tbody>
</table>

a: Values are g/kg. SPI-n, soy protein isolate containing normal levels of isoflavones; SPI-d, soy protein isolate depleted of isoflavones.
b: Rats in all groups, except Group 7, were treated with 7,12-dimethylbenz[a]anthracene (DMBA) at 50 days of age.

The chemopreventive properties of soy are often attributed to the isoflavones genistein and daidzein, which are found uniquely in soy among the foods consumed by humans. These were originally recognized as phytoestrogens with estrogenic and antiestrogenic effects (13). Genistein, in particular, has been shown to inhibit enzymes that promote cell proliferation, such as protein tyrosine kinases (PTK) (14,15) and topoisomerase II (16), to inhibit angiogenesis (17) and oxidative stress (18,19), and to induce cell differentiation (20–22). With the exception of the estrogenic stimuli, the remaining properties of genistein can exert antiproliferative effects. Daidzein does not inhibit PTK or topoisomerase II (23). Despite the plethora of in vitro and in vivo data, it is unclear whether purified genistein and daidzein alone or as components of SPI can effectively inhibit DMBA-induced mammary tumors in female rats.

In a previous study, using the direct-acting carcinogen N-methyl-N-nitrosourea as an inducer of mammary tumors in adult Sprague-Dawley rats, we evaluated the effect of purified genistein and daidzein and found both isoflavones to be only marginally effective (8). Recently, Cohen et al. (5) evaluated intact and isoflavone-depleted soy proteins against N-methyl-N-nitrosourea-induced mammary carcinogenesis and found only trends toward inhibition by intact soy protein and isoflavone-depleted soy protein. We currently report the first study to evaluate the effect of soy and its main isoflavones in a single experiment in adult rats using a carcinogen (DMBA) that requires metabolic activation. We compared SPI containing normal levels of isoflavones (SPI-n) or depleted of isoflavones (SPI-d), as well as the purified isoflavones genistein and daidzein individually or in combination, in the DMBA rat mammary carcinogenesis model. We found that SPI (regardless of the isoflavone levels) and purified daidzein are effective inhibitors of DMBA-induced mammary tumors.

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Materials and Methods

Chemicals, Agents, and Soy Protein

All chemicals, unless specified otherwise, were purchased from Sigma Chemical (St. Louis, MO). Genistein and daidzein were purchased from Indofine Chemical (Somerville, NJ). Two types of SPI were contributed by Protein Technologies International (St. Louis, MO). One preparation contained levels of isoflavones that are normally found in soybeans, here designated SPI-n (product number FXP-H-0086A, lot number GC6-XRM-004), and the other contained low levels of isoflavones as a result of being washed with alcohol, here designated SPI-d (product number FXP-H-0088, lot number C7E-XRP-9001). According to the supplier, SPI-n was composed of 87% protein, 4.2% water, 4.8% fat, and 4.2% ash. SPI-d was composed of 86.4% protein, 4.8% water, 3.5% fat, and 4.4% ash. According to the supplier’s data, the total concentration (free and conjugated) of isoflavonoids in SPI-n was 2.89 mg/g isolate, the total concentration of genistein was 1.85 mg/g, total concentration of daidzein was 0.89 mg/g, and total concentration of glycitein 0.15 mg/g. The total concentration (free and conjugated) of isoflavonoids in SPI-d was 0.2 mg/g isolate, total concentration of genistein was 0.12 mg/g, total concentration of daidzein was 0.06 mg/g, and total concentration of glycitein was 0.02 mg/g. Total isoflavone levels in the various experimental diets were assessed in our laboratory after acid hydrolysis. The measured values in diets containing purified isoflavones were generally lower than those expected on the basis of the analysis provided by Protein Technologies International (Table 1).

Preparation of the Diets

The basal diet was semipurified AIN-76A, which is devoid of soy. On the basis of information from the supplier, the basal diet has the following composition (g/kg): 200 protein (87% casein), 3 DL-methionine, 500 sucrose, 150 cornstarch, 50 corn oil, 50 fiber, 35 mineral mix, 10 vitamin mix, 2.0 choline bitartrate, and 0.01 ethoxyquin. Additional protein casein (16 g/kg diet) was added in the diets that did not contain SPI to maintain a constant protein content of 28% in all diets (Table 1). Diets were prepared in our facilities by making initially 1 kg of premix and then adding specific amounts of casein, SPI-n, SPI-d, genistein, daidzein, or basal diet to make 10-kg lots using a LiquidSolid Blender (Patterson Kelly, East Stroudsburg, PA). Diets were stored at 20°C until use. Fresh diets were made every 2 wk. Routine sampling and analysis of diets from different batches and
different areas of the same batch have demonstrated the uniformity of diet preparation. Rats were fed the experimental diets 1 wk before DMBA administration and remained on these diets for the duration of the study (120 days).

Analysis of Isoflavones in the Diets

The quantities of genistein and daidzein in the animal diets were determined by high-performance liquid chromatography (HPLC) using a method similar to that described by Franke et al. (24). Briefly, samples were freeze-dried, homogenized, and extracted by acid hydrolysis, yielding total aglycone content. Clear aliquots were diluted in ethanol and injected into the HPLC apparatus. Separation of 10-μl aliquots was over a NovaPak C18 reverse-phase column (5 μm) coupled to direct-connect guard column. Elution was performed at a flow rate of 0.8 ml/min in mobile phase of 60% methanol-40% 0.1 M ammonium acetate, pH 4.6. Detection was at 260 nm.

Analysis of Isoflavones in Rat Serum

Rat serum (0.5 ml) was mixed with 0.25 ml of 0.5 M triethylammonium acetate (pH 7.0), 80 μl of β-glucuronidase (200 U/ml), 80 μl of arylsulfatase (5 U/ml), and internal standard 7-hydroxyflavone (30 nM). The mixture was incubated in a sealed container for 18 h at 37°C, and then 0.25 ml of 10% aqueous trichloroacetic acid was added. The sample was extracted three times with 2-ml portions of ethyl acetate, and the organic phases were combined, centrifuged, and evaporated to dryness under vacuum. The residue was stored at −20°C until quantification using liquid chromatography-mass spectrometry. The residue was redissolved in 5 μl of dimethyl sulfoxide and 20 μl of methanol.

HPLC separation was carried out using a Hewlett-Packard (Wilmington, DE) C18 5-μm column (2.1 × 100 mm) at a flow rate of 0.2 ml/min. The solvent system consisted of a linear gradient from water (containing 0.1% formic acid) to acetonitrile as follows: 0–20% acetonitrile in 5 min, 20–50% from 5 to 20 min, and then 100% acetonitrile after 20 min. The entire HPLC eluate was analyzed on-line using positive ion electrospray mass spectrometry (model 1946A LC/MSD, Hewlett-Packard, Palo Alto, CA). Genistein, daidzein, and 7-hydroxyflavone were monitored using selective ion monitoring at mass-to-charge ratios of 271, 255, and 239, respectively. The electrospray parameters included a nitrogen nebulizer gas temperature of 280°C, fragmentor voltage of 120 V, nitrogen drying gas at 6.0 l/min, and capillary voltage of 4,000 V. The limit of detection for genistein and daidzein was 0.05 pmol.

Experimental Mammary Tumor Induction

Virgin female Sprague-Dawley (SD/VAF) rats (n = 130), 35 days old, were purchased from Harlan Sprague Dawley (Indianapolis, IN). All rats were fed soy-free AIN-76A diet (Harlan/Teklad, Madison, WI) until 43 days of age, at which time they were placed on experimental diets. Rats were then randomized to equalize the initial weight to one of six experimental groups of 20 animals each as follows: control (Group 1), genistein (200 mg/kg diet, Group 2), daidzein (200 mg/kg diet, Group 3), genistein (100 mg/kg diet) + daidzein (100 mg/kg diet, Group 4), 16% SPI-n (Group 5), and 16% SPI-d (Group 6). At 50 days of age, all rats in Groups 1–6 received a single intragastric dose of DMBA (15 mg) in sesame oil. A seventh group of 10 animals did not receive DMBA and continued being fed the basal diet (AIN-76A). During the experimental period (120 days), the animals were weighed weekly. Palpation of mammary tumors began 4 wk after animals received DMBA and continued until termination of the study. The date of appearance and location of every palpable tumor were recorded. Animals were observed daily to assess their general health. At 120 days after DMBA treatment, rats were sacrificed by CO2 asphyxiation, and mammary tumors were removed from the animals and weighed. Each tumor was divided into two portions: one was snap-frozen in liquid nitrogen and stored at −70°C for biochemical analysis, and the other was fixated in 10% buffered formalin for histopathological examination (25). In addition, intact livers were removed and snap-frozen.

Statistical Analysis

Only histologically confirmed mammary tumors were used in the data analysis. A significant inhibition of tumor induction as achieved by administration of an inhibitor was defined as a statistically significant decrease in tumor incidence, multiplicity, or latency period. The statistical significance of differences between mean tumor multiplicities was assessed using one-way analysis of variance Armitage's test for trend in proportions. Tumor incidence curves were generated by the life table method and compared by log-rank analysis (26).

Results

The levels and stability of genistein and daidzein in the diets were monitored by HPLC analysis. About 57% of the isoflavones in SPI are present in their aglycone form, and the remaining are present as β-glucoside conjugates. To measure the total isoflavone content, all diets were hydrolyzed in acid to extract the aglycone forms before HPLC analysis (24). Recovery of the two soy isoflavones from the diets ranged from 86 to 98%. The reported and measured total (free + conjugated) concentrations of genistein and daidzein are shown in Table 2. In the SPI-n diet, we detected 233 mg/kg genistein and 104 mg/kg daidzein, substantially lower than the reported values of 296 and 142 mg/kg, respectively. The measured values were closer to the reported values in the diets that contained the pure aglycones (i.e., 200 vs. 196 mg/kg for genistein), suggesting that the measured values may be due to incomplete hydrolysis of the
conjugated forms or overestimation of the concentrations by the supplier of the SPI diets. When the diets were tested by HPLC analysis after 3 wk of storage at room temperature, the free isoflavone levels were within 95% of their original values, suggesting that the isoflavones are stable in the diet under these storage conditions.

The isoflavone concentrations in the rat serum after 4 wk of feeding were analyzed using liquid chromatography-mass spectrometry, and the data are shown in Table 3. The doses of SPI-d and SPI-n that were used in the chemoprevention study were based on a preliminary 6-wk dose selection study. The maximum tolerated dose was determined to be 20% for SPI-n and SPI-d (data not shown). Supplementation with 16% soy, representing 80% of the maximum tolerated dose, was selected, and this dose produced no adverse effects as indicated by weight gain. In the dose selection study, the maximum tested dose of genistein or daidzein was 200 mg/kg diet. Because these doses did not produce toxicity, they were selected for the long-term study. Previous studies used genistein doses of 150 mg/kg diet in rats without any toxic effects (27).

The effects of the SPI diets on tumor incidence, mean tumor multiplicity, mean body weight, and survival are shown in Fig. 1. Supplementation of the basal diet with 16% SPI-n reduced final tumor incidence from 100% in the control group to 95% (Fig. 1A, Table 4). Diets supplemented with SPI-d reduced incidence to 89.5%. These differences were not statistically significant. However, the incidence rate (which takes into consideration the rate of tumor appearance) was significantly reduced in the SPI-d group (P = 0.042) compared with the control group. Mean tumor multiplicity was reduced by both experimental diets (Fig. 1B). The SPI-d diet reduced tumor multiplicity from 8.0 to 4.0, a reduction that is significant (P = 0.0053). The effect of the SPI-n diet on tumor multiplicity was also significant (P = 0.041; Table 4). Tumor latency was also increased by both soy diets, with the SPI-d diet being more effective, causing a 19.6-day delay in the median appearance of the first tumor, compared with 13.8 days for the group taking the SPI-n diet. There were no significant differences in body weight (Fig. 1C) or survival (Fig. 1D).

The effects of diets containing pure genistein, daidzein, or their combination on tumor incidence, mean tumor multiplicity, mean body weight, and survival are shown in Fig. 2. Neither dietary genistein nor daidzein significantly reduced tumor incidence, but the latter significantly reduced the rate of incidence (P = 0.030) as determined by log-rank analysis (Fig. 2A). Tumor multiplicity was significantly reduced (P = 0.021) only in the daidzein group (Fig. 2B). Tumor latency was increased by ~14 days by both diets (Table 4). No significant differences among the groups were evident in body weight gain (Fig. 2C) or in tumor weight (Table 4). Survival was slightly reduced in the genistein group, but it was not different from control in the daidzein group (Fig. 2D).

### Discussion

It is unclear whether the cancer-preventive effects of soy-containing diets rely on their high content of genistein/daidzein, some other ingredient, or a combination of ingredients. This uncertainty led us to test the effectiveness of iso-

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**Table 2. Total (Free + Conjugated) Isoflavone Levels as Analyzed in Experimental Diets and Compared With Expected Values**

<table>
<thead>
<tr>
<th>Group No. and Diet&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Total genistein</th>
<th>Total daidzein</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) DMBA control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2) Genistein</td>
<td>200</td>
<td>0</td>
</tr>
<tr>
<td>3) Daidzein</td>
<td>0</td>
<td>200</td>
</tr>
<tr>
<td>4) Genistein + daidzein</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>5) SPI-n</td>
<td>296</td>
<td>142</td>
</tr>
<tr>
<td>6) SPI-d</td>
<td>19.2</td>
<td>9.6</td>
</tr>
<tr>
<td>7) Control</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HPLC Analysis</th>
<th>Total genistein</th>
<th>Total daidzein</th>
</tr>
</thead>
<tbody>
<tr>
<td>196</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>172</td>
<td></td>
</tr>
<tr>
<td>233</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td>5.1</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Values are mg/kg diet. ND, not determined; HPLC, high-performance liquid chromatography.

<sup>b</sup> Diets were prepared as described in Materials and Methods and shown in Table 1.

<sup>c</sup> Based on quantities added on values provided by supplier (Protein Technologies International).

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**Table 3. Total (Free + Conjugated) Isoflavone Concentrations in Rat Serum as Determined by LC-MS Analysis**

<table>
<thead>
<tr>
<th>Group No. and Diet&lt;sup&gt;b&lt;/sup&gt;</th>
<th>LC-MS Analysis&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total genistein</td>
</tr>
<tr>
<td>1) DMBA control</td>
<td>2.4</td>
</tr>
<tr>
<td>2) Genistein</td>
<td>901.8</td>
</tr>
<tr>
<td>3) Daidzein</td>
<td>1.3</td>
</tr>
<tr>
<td>4) Genistein + daidzein</td>
<td>ND</td>
</tr>
<tr>
<td>5) SPI-n</td>
<td>924.6</td>
</tr>
<tr>
<td>6) SPI-d</td>
<td>22.5</td>
</tr>
<tr>
<td>7) Control</td>
<td>2.7</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values are pmol/ml serum. LC-MS, liquid chromatography-mass spectrometry.

<sup>b</sup> Diets were prepared as described in Materials and Methods and shown in Table 1.

<sup>c</sup> Analysis was performed after 4 wk of feeding of experimental diets.
Figure 1. Effect of soy protein isolate with normal level of isoflavones (SPI-n) and soy protein isolate depleted of isoflavones (SPI-d) on percent incidence of observable mammary tumors (A), mean number of tumors per tumor-bearing rat (B), mean body weight (C), and survival (D). Female Sprague-Dawley rats (n = 20) were started on basal AIN-76A diet (open circles) or 16% SPI-n (filled squares), or 16% SPI-d (filled inverted triangles) 1 wk before administration of 7,12-dimethylbenz[a]anthracene (DMBA). Rats were maintained on the diet throughout the experiment.

Figure 2. Effect of purified soy isoflavones on percent incidence of observable mammary tumors (A), mean number of tumors per tumor-bearing rat (B), mean body weight (C), and survival rate (D). Female Sprague-Dawley rats (n = 20) were started on basal AIN-76A diet (open circles), 200 mg genistein/kg diet (filled inverted triangles), 200 mg daidzein/kg diet (filled squares), or 100 mg genistein/kg diet + 100 mg daidzein/kg diet (filled diamonds) 1 wk before administration of (DMBA). Rats were maintained on the diet throughout the experiment.
Table 4. Effect of Soy Protein Isolates and Purified Isoflavones on Tumor Incidence, Multiplicity, Latency, and Weight\a

<table>
<thead>
<tr>
<th>Group No. and Diet</th>
<th>Final Tumor Incidence, %</th>
<th>Mean Tumor Multiplicity</th>
<th>Median Latency, days</th>
<th>Mean Tumor Weight, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) DMBA control</td>
<td>100.0</td>
<td>8.0 ± 1.0</td>
<td>53.0 ± 2.4</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>2) Genistein</td>
<td>90.0</td>
<td>6.1 ± 1.0</td>
<td>67.0 ± 7.2</td>
<td>1.4 ± 0.4</td>
</tr>
<tr>
<td>3) Daidzein</td>
<td>100.0</td>
<td>5.4 ± 0.7*</td>
<td>67.7 ± 5.6</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>4) Genistein + daidzein</td>
<td>100.0</td>
<td>7.8 ± 0.7</td>
<td>56.9 ± 4.0</td>
<td>1.7 ± 0.5</td>
</tr>
<tr>
<td>5) SPI-n</td>
<td>95.0</td>
<td>5.5 ± 0.9*</td>
<td>66.8 ± 7.0</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>6) SPI-d</td>
<td>89.5</td>
<td>4.0 ± 0.7\†</td>
<td>72.6 ± 7.6</td>
<td>1.3 ± 0.4</td>
</tr>
</tbody>
</table>

\a: Values are means ± SE.

\b: Statistical comparisons were made for Groups 2-6 vs. control (Group 1) as follows: Mean tumor incidence by Fisher's exact test, mean tumor multiplicity (determined as no. of tumors/no. of tumor-bearing animals) by Armitage's test for trends in proportions, and median tumor latency and mean tumor weight by unpaired t-test. Statistical significance is as follows: *, P < 0.05 vs. Group 1; †, P < 0.01 vs. Group 1.

Lated genistein and daidzein and compare their effects with the effect of all other soy components in a "standard" chemoprevention protocol. We used the same animal model and exactly the same protocol that our group used previously to evaluate other chemopreventive agents (reviewed in Ref. 28). In the present study, the most effective diet among those tested was SPI-d, which, when given at 16% of total diet, reduced tumor multiplicity by ~50%. The efficacy of the SPI-d diet is comparable to that of previously identified efficacious chemopreventive agents, such as difluoromethylornithine and N-(4-hydroxyphenyl)retinamide (29,30). DMBA is effective only after it is metabolized to the ultimate carcinogen; thus the model is appropriate for detecting chemopreventive agents the mode of action of which involves inhibition of phase I bioactivating enzymes or induction of phase II detoxification enzymes.

The observed higher chemopreventive efficacy of daidzein than of genistein was not anticipated. Daidzein is generally less effective than genistein in inducing biochemical and biological responses. For example, unlike genistein, daidzein does not inhibit PTK or topoisomerase II (14,23), and it is a weaker estrogen antagonist than genistein (12). Zhang and Wang (31) found that daidzein substantially enhanced nonspecific immunity in mice. After 7 days of consuming daidzein-containing diets (20-40 mg/kg), humoral and cell-mediated immunity were substantially enhanced in Swiss mice. Enhanced immune response may account for the chemopreventive action of daidzein in Sprague-Dawley rats, although alternative explanations are possible. It is possible that a known or yet unidentified daidzein metabolite is a potent inhibitor of carcinogenesis. For example, daidzein-7,4'-di-O-sulfate inhibited the main pathway of estrogenic steroid production with an inhibition constant of 1 μM, providing a biochemical basis for the chemopreventive role of dietary daidzein (32).

Genistein, as a topoisomerase II poison, can damage DNA (21,33,34), providing a potential mechanism for the production of genotoxic effects. Although DNA double-strand breakage (introduced by topoisomerase II poisons) is generally lethal, a subfraction of damaged cells can survive with permanent genetic alterations. If these alterations involve key regulatory genes such as transcription factors, oncogenes, or tumor suppressor genes, carcinogenesis may be initiated. The carcinogenic effect of etoposide chemotherapy in the case of acute myelocytic leukemia is an example of this delayed adverse effect of topoisomerase II poison-induced genetic toxicity (35). The determination that certain tissues, including the mammary gland, may accumulate higher levels of genistein than those found in serum further supports the possibility that this isoflavone may produce low levels of DNA damage via a topoisomerase II-mediated mechanism (36).

Somewhat paradoxical is the effect of the diet that contained purified genistein and daidzein (Group 4 in Table 4). The two isoflavones were combined at half the concentrations used for the individual isoflavones to determine whether there was an additive effect. With this combination, no significant effect was observed. Although this observation is difficult to explain, one possibility is that genistein works through a mechanism different from daidzein, and neither isoflavone alone reaches the necessary concentration to provide a chemopreventive effect. When the data from the genistein + daidzein diet are taken together with the data from the diets with SPI-n and SPI-d, they strongly suggest that unknown factors that copurify with soy protein may promote the chemopreventive effects of isoflavones and also diminish their genotoxic effects.

Results presented here might be of relevance for prevention of breast cancer in women. The present study showed, for the first time, that a soy diet with normal levels of isoflavones can suppress DMBA-induced mammary carcinogenesis in adult female rats, but this effect can also be mediated by diets depleted of isoflavones. Thus soy ingredients other than isoflavones, perhaps those with strong antioxidant activity, may contribute to the chemopreventive efficacy of soy products. Additional studies are necessary to determine optimal daily doses, efficacy, and long-term safety of the soy isoflavones.

Acknowledgments and Notes

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References


Uptake and Metabolism of Hydroxymatairesinol in Relation to Its Anticarcinogenicity in DMBA-Induced Rat Mammary Carcinoma Model

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Abstract: The chemopreventive effects of hydroxymatairesinol (HMR), a lignan extracted from Norway spruce (Picea abies), on the development of mammary carcinoma induced by 7,12-dimethylbenz[a]anthracene (DMBA) was studied in rats. HMR administered via diet in an average daily dose of 4.7 mg/kg body wt starting before DMBA induction reduced tumor volume and tumor growth, but no significant reduction in tumor multiplicity (number of tumors/rat) was observed. The predominant histological type in the control group was type B (well-differentiated adenocarcinoma, 78%). The proportion of type B tumors decreased to 35% in the HMR group, while the type A (poorly differentiated) and type C (atrophic) tumor proportions increased. Anticarcinogenic effects of dietary HMR (4.7 mg/kg) were also evident when the administration started after DMBA induction and was seen as growth inhibition of established tumors. Dietary HMR supplementation significantly increased serum and urinary enterolactone and HMR concentrations but had no significant effect on the uterine weight, suggesting that HMR or its major metabolite enterolactone did not have an antiestrogenic effect. Further studies are warranted to further clarify and verify HMR action and the associated mechanisms in mammary tumorigenesis.

Introduction

Plant lignans, secoisolariciresinol and matairesinol, found in many edible plants, are transformed by intestinal microbes to mammalian lignans, enterodiol (END) and enterolactone (ENL), respectively (1–3). The inverse correlation between urinary and serum ENL content and the risk of breast cancer found in epidemiological studies (4–6) has raised interest in the possible chemopreventive action of ENL and its precursors. Secoisolariciresinol diglucoside (SDG), extracted from flaxseed, the richest known dietary source for lignans, is metabolized to END as well as to ENL and was shown to have chemopreventive properties in a 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammary tumor model (7). Dietary supplementation with SDG for 20 wk, starting 1 wk after DMBA induction, reduced tumor multiplicity (number of tumors/rat) and tumor incidence (number of animals with tumors). Furthermore, SDG inhibited the growth of established tumors (mean total tumor volume/animal) and the number of tumors appearing during late-stage carcinogenesis (8).

Hydroxymatairesinol (HMR) is a plant lignan structurally closely related to matairesinol. HMR can be extracted on a large scale from Norway spruce (Picea abies), thus giving us the opportunity to study its metabolism and biological activities in vivo (9). In rats, a dose-dependent increase in urine ENL concentrations was measured after oral exposure to HMR, showing that HMR acts as a precursor for ENL production in vivo (9). In a DMBA-induced rat mammary carcinoma model, oral administration of HMR (15 mg/kg body wt) starting 9 wk after induction with DMBA inhibited the growth of established tumors (mean total tumor volume/animal) and increased the proportion of stabilized and regressing tumors (9). In the present study, chemopreventive effects of dietary HMR supplementation starting before DMBA induction were compared with effects on late-stage progression, i.e., to already established tumors seen in the previous study. A semipurified diet was used as a basal diet to avoid the fiber-associated plant lignans present in open-formula diets (10). The chemopreventive effects of HMR were correlated with concentrations of ENL and HMR in serum and urine.

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