A Rice Protein Isolate Alters 7,12-Dimethylbenz[a]Anthracene-Induced Mammary Tumor Development in Female Rats

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Summary The effects of a rice protein isolate (RPI) on 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammary tumor progression were investigated in female Sprague-Dawley rats. At 6 weeks of age, rats were fed a casein, RPI or soybean protein isolate (SPI) diet, respectively. After 1 week, DMBA was administered orally at the dose of 30 mg/kg body weight. The mean tumor number per tumor-bearing rat at autopsy was significantly lower only in rats fed RPI than in those fed casein. Palpable tumors at the mid point of the experiment were significantly lower in rats fed RPI and SPI than in those fed casein. Serum estradiol-17β concentrations were lower in rats fed the SPI (but not in those fed RPI) than in those fed casein. In a further experiment, no differences were found in hepatic microsomal DMBA-arylhydrocarbon hydroxylase activity after 7 days of feeding the respective diets. These results suggest that RPI exerts its inhibitory effect on DMBA-induced mammary tumorigenesis irrespective of changes in circulating estrogens or modulation of hepatic DMBA metabolism.

Key Words breast cancer, dietary protein, rice protein isolate, carcinogens (dimethylbenz[a]anthracene, DMBA), estradiol-17β, fecal steroid excretion, rats

It is believed that some of disparity in breast cancer rates between Western and Asian countries is due to hormonal status, especially that of estrogen (1). Goldin et al. (2) showed that fecal estrogen excretion of Oriental women was twice as high as that of Caucasians while the plasma estrogen concentrations in the latter were higher than in Orientals. Experimental studies into nutritional factors responsible for these differences have concentrated on examinations of fat and energy intake as these differ between the more affluent Westernized countries and those in the Orient
More recently, attention was also paid to the possible protective role of plant proteins in diet-related cancers. Experimental studies support the importance of protein source and Hawrylewicz et al. (6) have reported that the incidence of mammary tumors induced by N-nitrosomethylurea was lower in rats fed a diet containing soybean protein compared with those fed a diet containing casein. These works suggested that the lower methionine content of soybean protein was responsible for the difference. A protective effect of soybean protein was recorded also by Barnes et al. (7) who noted that the number of palpable mammary tumors induced by 7,12-dimethylbenz[a]anthracene (DMBA) was lower in rats fed with crude or purified soybean proteins compared with those fed casein. In this model, tumor development is dependent primarily on prolactin and estrogens (8). Barnes et al. (7) have attributed the slower tumor progression to phytoestrogens present in soybeans. However, a note of caution must be mentioned as Carroll (3) and Hsueh and Park (9) reported that the tumor incidence after DMBA treatment was the same in rats fed soybean protein or casein. To date, the effects of soybean protein on the DMBA-induced mammary tumor appear to be rather contradictory.

Soybeans are only one of the sources of plant protein in Asian diets and rice is at least as important but has received much less attention. Previously, we have prepared a rice protein isolate (RPI) diet and found that it reduced the serum cholesterol concentration in rats (10). Further studies showed also that feeding RPI increased fecal excretion of neutral and acidic sterols to the same degree as soybean protein isolate (SPI) (T. Morita and S. Kiriyama, unpublished observations). These observations prompted us to hypothesize that RPI could affect mammary cancer risk through lowering of circulating estradiol-17β concentrations through increasing its fecal excretion. In the present study, we have compared the effects of RPI and SPI on mammary tumor progression in female rats exposed to DMBA. We have also examined the effects of these proteins on hepatic DMBA arylhydrocarbon hydroxylase activity which effects the conversion of DMBA to DMBA-epoxide, the carcinogenic form.

**EXPERIMENTAL**

*Materials.* Rice flour (obtained as 70–35% milling fraction) of *Oryza sativa* L. Japonica (cv. Miyamanishiki) was purchased from Otokoyama breweries (Asahikawa, Japan). Rice protein isolate was isolated from this flour after treatment with a heat stable α-amylase (Termamyl 120L, Novo Laboratories, Sapporo, Japan) and was analysed as described previously (10). The composition of the isolate used in these studies is shown in Table 1. DMBA, estradiol-17β and 4-androstene-3,17-dione were purchased from Nacalai Tesque (Kyoto, Japan). [G-3H]DMBA (specific radioactivity, 69.5 Ci/mmol), [2,4,6,7-3H(n)]-estradiol-17β (specific radioactivity, 103.5 Ci/mmol) and [1β-3H]-4-androstene-3,17-dione (specific radioactivity, 24.3 Ci/mmol) were purchased from Amersham Life Science (Buckinghamshire, England). Rabbit antiserum to estradiol-17β-6-carboxymethyl-
Table 1. Chemical composition of rice protein isolate produced by Termamyl 120L digestion (RPI). 1

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>RPI (g/100 g)</th>
<th>Ingredient</th>
<th>RPI (g/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>79.1</td>
<td>Ash</td>
<td>1.2</td>
</tr>
<tr>
<td>Lipids</td>
<td>1.0</td>
<td>Total dietary fiber</td>
<td>6.8</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>2.8</td>
<td>Moisture</td>
<td>9.3</td>
</tr>
</tbody>
</table>

1Values are means from 16 batches. 2 Determined by the Kjeldahl method (24) using a conversion factor, 6.25. 3 Lipids were extracted by the method of Folch et al. (25) and measured gravimetrically after removing the solvent. 4 Calculated by the difference, subtracting protein, ash, water, lipids and total dietary fiber from RPI. 5 Determined by the direct ignition method (525°C, overnight). 6 Analyzed by the method of Prosky et al. (26). Water-soluble dietary fiber was not included in this fraction. 7 Determined from the loss in weight after drying at 105°C for 24 h.

Animal care. The studies were approved by the Hokkaido University Animal Use Committee, and all rats were maintained in accordance with the guidelines for the care and use of laboratory animals, Hokkaido University. Female rats (37 days of age) of the Sprague-Dawley strain (purchased from Shizuoka Laboratory Animal Center, Hamamatsu, Japan) were used in all experiments. They were housed in individual cages with screen-bottoms of stainless steel in a room with controlled temperature (23±1°C) and lighting (lights on: 0800–2000 h). The animals were adapted to the experimental treatment by the feeding of the standard casein-sucrose diet for 5 days and then divided into groups and fed the experimental diets ad libitum. They were allowed free access to water. Body weight and food intake were recorded daily in the morning before replenishing the diet. The composition of the standard and experimental diets is shown in Table 2.

Experiment 1: Fifty-four rats (42 days of age) weighing 135 g (pooled SE = 2) were divided into 3 groups of 18 rats and allowed free access for 126 days to one of the three diets (casein, RPI or SPI, Table 2). The dietary protein concentration was adjusted to 30% by weight because we have that maximal growth of weanling rats occurs at 30–40% RPI (10). The animals were fed the diets for 1 week and then were given by mouth a single dose of DMBA (30 mg/kg of body weight) in 0.5 ml of corn oil. To minimize the effects of the experimental diets on absorption of the carcinogen, all rats were fed a standard diet from one day before to one day after administration of DMBA, then they were returned to the respective test diets. After the experimental treatment, rats were examined weekly for palpable mammary tumors once a week. Feces were collected for 3 days (days 67–70 of the experimental period) to determine the fecal excretion of total bile acids, neutral sterols and estradiol-17β. The experiment was terminated on 14 days after tumor incidence in each experimental groups reached a plateau (i.e., 119 days after...
Table 2. Composition of the standard and experimental diets.

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Standard diet (g/kg)</th>
<th>Experimental diets (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Casein</td>
<td>RPI</td>
</tr>
<tr>
<td>Casein</td>
<td>250</td>
<td>300</td>
</tr>
<tr>
<td>RPI</td>
<td>—</td>
<td>300</td>
</tr>
<tr>
<td>SPI</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Sucrose</td>
<td>647</td>
<td>547</td>
</tr>
<tr>
<td>Corn oil</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Lard</td>
<td>—</td>
<td>50</td>
</tr>
<tr>
<td>Salt mixture</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Vitamin E granules</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

1 Purchased from New Zealand Dairy Board, Wellington, New Zealand. Casein contained 127.3 mg nitrogen/g. 2 Rice protein isolate produced by Termamyl 120L (heat stable α-amylase) digestion. Contained 126.6 mg nitrogen/g. 3 Soybean protein isolate, purchased from Fuji Oil (Osaka, Japan). Soybean protein isolate contained 132 mg nitrogen/g. 4 Granulated beet sugar, supplied from Nippon Beet Sugar Manufacturing, Obihiro, Japan. 5 Mixed with 0.2 ml of Chocola A (Eisai, Tokyo, Japan) and 0.05 ml of Chocola D (Eisai) to provide 7.66 μmol of retinyl palmitate and 0.051 μmol of ergocalciferol per kg diet, respectively. The former contains 38.3 mM retinyl palmitate and the latter, 1.01 mM ergocalciferol. 6 The salt mixture was identical with mineral mixture 2 formulated by Ebihara et al. (27). 7 The vitamin mixture was prepared according to the AIN-76 vitamin mixture (AIN 1977) except for menadione (0.29 mmol/kg diet) (AIN 1980). 1-Ascorbic acid was added to stabilize thiamine during storage of the diet (28.39 mmol/kg diet) (28). This vitamin mixture was made without all-rac-α-tocopheryl acetate. 8 Four milliliters of a chloride solution (3.58 mM) in 50% (v/v) ethanol were added per kg diet. 9 “Juvela granule” (Eisai) containing 423 mmol all-rac-α-tocopheryl acetate/kg.

DMBA administration). On the last day of feeding, food was withdrawn at 10:00 h and blood was collected from the abdominal aorta under anesthesia with Nembutal (pentobarbital sodium, Abbott Laboratories, North Chicago, IL, USA). Blood was collected at 1200–1500 h, after which ovaries were removed rapidly, weighed and homogenized with a Teflon homogenizer (800 rpm, 5 strokes) in 20 volumes of 0.1 M potassium phosphate buffer (pH 7.4). The homogenate was centrifuged at 400 × g at 4°C for 15 min and the supernatant was removed, frozen in liquid N2 and stored at −80°C for the assay of aromatase activity. Estradiol-17β was assayed on serum prepared from aortic blood.

Experiment 2: Fifteen rats weighing 125 g (pooled SE = 1) were divided into 3 groups of 5 animals and were allowed free access to one of the three experimental diets (Table 2) for 1 week. At the end of that period, rats were killed by decapitation and livers were immediately perfused through the portal vein with ice-cold 0.154 M NaCl solution. Then, livers were removed and rinsed in ice-cold

0.25 M sucrose-0.05 M Tris/HCl buffer (pH 7.4). Then 2 g of liver was homogenized in 10 ml of the same buffer using a Teflon homogenizer (800 rpm, 5 strokes). The homogenate was centrifuged at 10,000 × g for 20 min to remove debris, the supernatant removed and then centrifuged again at 100,000 × g for 60 min. The pellet was resuspended in 0.05 M Tris/HCl buffer (pH 7.4) and assayed for microsomal protein, cytochrome P-450 and DMBA-arylhydrocarbon hydroxylase.

Analytical procedures. Estradiol-17β was assayed by radioimmunoassay using the method of Butcher et al. (11) with a slight modification. Two milliliters of serum were pipetted into a 15-ml polyethylene tube which contained 1,000 cpm of [2,4,6,7-3H]estradiol-17β as recovery standard. Estradiol-17β was extracted by vigorous shaking with 4 × 2 ml of diethyl ether for 2 min each. The ether layer was removed, pooled and evaporated to dryness under nitrogen gas and the dried extract was dissolved in 0.1 ml of benzene:methanol (85:15, v/v) and placed on a Sephadex LH-20 (Pharmacia, Uppsala, Sweden) column (inner diameter: 7 mm). The column was washed with 5 ml of freshly prepared benzene:methanol mixture before samples (0.1 ml) were applied. Fresh solvent was applied to the column and the first 3 ml of the first eluate were discarded. Two milliliters of the second eluate (containing estradiol-17β) were collected in 15-ml polyethylene tubes. The solvent was removed with nitrogen gas and 0.15 ml of 0.02 M sodium phosphate buffer (pH 7.4) containing 0.5% bovine serum albumin was added and the residue was dispersed by sonication for 5 min. The resulting suspension was used for radioimmunoassay. Aliquots of the dispersate (0.1 ml) and graded amounts of estradiol-17β standard solution (6.25, 12.5, 25, 50, and 100 pg dispersed in 0.02 M sodium phosphate buffer as described above) were added to each tube. Then, 0.1 ml of 1:3,333 antisera (adding titer) and 0.1 ml of [3H]estradiol-17β (15,000 cpm) were added to each tube, mixed well and incubated at 4°C for 90 min. After incubation, the tubes were placed on ice and the free and bound steroids were separated by the addition of 0.5 ml of 0.345% ice-cold dextran-charcoal suspension (Norit A:dextran T-70 = 10:1 in distilled water). The tubes were shaken briefly and allowed to stand for 30 min at 4°C. After centrifugation at 5,000 × g for 5 min, 0.6 ml of supernatant containing the antibody-bound steroids was added to vials containing 5 ml of Atomlight (New England Nuclear, Boston, USA). Radioactivity was measured with a Beckmann LS 9000 scintillation spectrophotometer (Tokyo, Japan). Estradiol-17β is expressed as pmol/ml serum after correction of procedural losses. When no unlabeled steroid was present, the antibody bound 30–40% of the added radioactive estradiol-17β. In our assay condition, the sensitivity of assay was 6.25 pg of estradiol-17β. Samples of feces (50 mg dried feces) were extracted 4 times with 2 ml of chloroform/methanol (1:1, v/v) solution at 4°C for 48 h with continuous shaking for the determination of estradiol-17β. Extracts were pooled, and evaporated to dryness under nitrogen gas. The residue was suspended in 4 ml of 30% ethanol by sonication for 10 min and unconjugated estradiol-17β was extracted 4 times with 2 ml of diethyl ether. After removing the solvent under

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N₂, the residue was dissolved in 0.1 ml of benzene:methanol solution (85:15, v/v). Estradiol-17β was separated and measured as described above.

Ovarian aromatase activity was measured according to the method of Thompson and Sitteri (12) as ³H₂O released from the 1β-position of androstenedione. Aromatase activity is expressed as pmol of ³H₂O released·mg protein⁻¹·min⁻¹. Arylhydrocarbon hydroxylase activity was assayed using [G⁻³H]DMBA as a substrate by the method of Nebert and Gelboin (13). Activity of DMBA-arylhydrocarbon hydroxylase is expressed as pmol of DMBA hydroxylated·mg microsomal protein⁻¹·min⁻¹.

Chemical analyses of RPI were performed by the methods described previously (10). Fecal neutral sterols and total bile acids were determined by the methods of Morita and Kiriyama (10) and Nishimura et al. (14), respectively. Cytochrome P-450 content was determined by the dithionite difference method of Omura and Sato (15). Protein content was measured by the procedure of Lowry et al. (16) with bovine serum albumin as a standard. Serum cholesterol concentration was determined by using a commercial assay kit (Cholesterol C-Test Wako, Wako Pure Chemical Industries, Osaka, Japan).

Statistical methods. The statistical differences in tumor incidence between groups were determined by chi-square analysis with Yates' correction (17). Other statistical differences were determined by ANOVA and Duncan's multiple range test (18). Differences were considered significant at the 5% level. The significance of relationships between data was established by linear regression analysis (19).

RESULTS

Experiment 1

Changes in body weight of rats fed the three diets are shown in Fig. 1. Final body weights for rats fed the casein, SPI and RPI diets were unaffected by treatment with mean values of 294, 286 and 277 (pooled SE=9) g, respectively. Food consumption was similar in all groups with mean cumulative intakes of 1,558, 1,544 and 1,626 (pooled SE=37) g for the casein, SPI and RPI groups, respectively.

The cumulative incidence of palpable mammary tumor in rats is shown in Fig. 2. Tumor became palpable day 29, 35 and 42 after DMBA administration in rats fed the casein, SPI and RPI diets, respectively. The mean latency period for the appearance of palpable tumors did not differ among groups and averaged 63, 76 and 73 (pooled SE=5) days for the casein, SPI and RPI diets, respectively. Cumulative tumor incidences in rats fed RPI were significantly lower on day 84 (p<0.007), 91 (p<0.045) and 98 (p<0.074) after DMBA administration than in rats fed casein. Nevertheless, the cumulative tumor incidence continued to increase in rats fed the RPI diet and the difference between this group and rats fed casein disappeared 106 days after DMBA administration. Cumulative tumor incidence in rats fed the SPI diet tended to be lower than in those fed casein but the difference was significant.

Fig. 1. Change in body weight in rats fed the casein (●), rice protein isolate (▲) and soybean protein isolate ( ○) diets for 126 days. Each data represents the mean (n=18) of body weight. Average initial body weight, 135 g (range: 119–149 g). After 1 week-feeding of the respective diets, rats (50 days of age) were orally administered a single dose of 7,12-dimethylbenz[a]anthracene (DMBA) (30 mg/kg body weight). From 1 day before to 1 day after DMBA administration, all rats were fed the standard diet to eliminate the effects of the experimental diets on absorption of carcinogen.

only on day 70 (p < 0.046). The autopsy data are summarized in Table 3. The total number of mammary tumors in the casein diet-fed group were markedly greater than that in the RPI diet-fed group. Therefore, the total weight of tumors in the casein diet-fed group was almost two times greater than that in the RPI diet-fed group. Total tumor numbers and total weight of tumors in the SPI diet-fed group were similar to those of the casein diet-fed group. The mean tumor number per tumor-bearing rat when fed the RPI diet was significantly lower than when fed the casein diet. While tumor weight per tumor-bearing rat was highest in rats fed the casein diet and lowest in rats fed the RPI diet, the high variance meant that none of the differences were significant.

Serum concentrations of estradiol-17β and total cholesterol are shown in Table 4. Estradiol-17β was significantly lower in rats fed SPI than in those fed the casein diet. While concentrations in rats fed RPI decreased by 36% compared with those fed the casein diet, the high variance meant that the differences were not significant. A similar pattern was seen in plasma cholesterol with the order of concentrations being casein > RPI > SPI. In this instance, the differences among the three groups were all significant statistically.

The dry weight of feces from rats fed the RPI diet was significantly higher than from rats fed the casein and SPI diets (Table 5). Fecal bile acid (acidic sterol) excretion by rats fed the RPI and SPI diets was significantly higher than by rats fed
Fig. 2. Cumulative palpable tumor incidence in rats fed the casein (●), rice protein isolate (▲) and soybean protein isolate (○) diets for 126 days. Each data represents the percentage of cumulative palpable tumor incidence. Average initial body wt, 135 g (range: 119–149 g). After 1 week-feeding of the respective diets, rats (50 days of age) were orally administered a single dose of 7,12-dimethylbenz[α]anthracene (DMBA) (30 mg/kg body weight). From 1 day before to 1 day after DMBA administration, all rats were fed the standard diet to eliminate the effects of the experimental diets on absorption of carcinogen.

Table 3. Tumor numbers and weight in rats fed the respective diets for 129 days (Experiment 1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total No. per group</th>
<th>Mean No. per tumor-bearing rats</th>
<th>Total weight per group (g)</th>
<th>Mean weight per tumor-bearing rats (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>104</td>
<td>6.9a</td>
<td>139</td>
<td>8.2</td>
</tr>
<tr>
<td>RPI²</td>
<td>58</td>
<td>3.6b</td>
<td>68.1</td>
<td>4.6</td>
</tr>
<tr>
<td>SPI³</td>
<td>94</td>
<td>5.5ab</td>
<td>97.4</td>
<td>5.7</td>
</tr>
<tr>
<td>Pooled SE</td>
<td>0.9</td>
<td></td>
<td></td>
<td>2.1</td>
</tr>
</tbody>
</table>

¹Data are expressed as mean (n=18) and values not sharing a common superscript letter are significantly different (p<0.05), when analyzed by Duncan's multiple range test. ²Rice protein isolate, produced by Termamyl 120L digestion. ³Soybean protein isolate.

the casein diet. However, neutral sterol excretion was significantly higher only in rats fed the SPI diet so that total biliary steroid excretion was in the order SPI > RPI > casein. Regression analysis showed that there was a significant negative correlation between serum cholesterol concentration and fecal excretion of total steroids (r = −0.323, p < 0.05). Fecal excretion of estradiol-17β (free form) was

Table 4. Serum concentrations of estradiol-17β and cholesterol in rats fed the respective diets for 129 days (Experiment 1).  

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cholesterol (mM)</th>
<th>Estradiol-17β (pm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>2.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>200&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>RPI&lt;sup&gt;3&lt;/sup&gt;</td>
<td>1.97&lt;sup&gt;b&lt;/sup&gt;</td>
<td>128&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>SPI&lt;sup&gt;4&lt;/sup&gt;</td>
<td>1.57&lt;sup&gt;c&lt;/sup&gt;</td>
<td>78&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pooled SE</td>
<td>0.11</td>
<td>53</td>
</tr>
</tbody>
</table>

<sup>1</sup>Data are expressed as mean (n=18) and values not sharing a common superscript letter are significantly different (p<0.05), when analyzed by Duncan's multiple range test. <sup>2</sup>Serum concentration of estradiol-17β was determined by radio immunoassay method described in EXPERIMENTAL. <sup>3</sup>Rice protein isolate, produced by Termamyl 120L digestion. <sup>4</sup>Soybean protein isolate.

Table 5. Fecal weight, fecal excretion of bile acids, neutral sterols and estradiol-17β in rats fed the respective diets for 129 days (Experiment 1).  

<table>
<thead>
<tr>
<th>Groups</th>
<th>Fecal dry weight (g/day)</th>
<th>Neat neutral steroids (µmol/day)</th>
<th>Acidic steroids (µmol/day)</th>
<th>Total steroids (µmol/day)</th>
<th>Estradiol-17β (µmol/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>1.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>RPI&lt;sup&gt;3&lt;/sup&gt;</td>
<td>2.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.33&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SPI&lt;sup&gt;4&lt;/sup&gt;</td>
<td>1.48&lt;sup&gt;c&lt;/sup&gt;</td>
<td>21.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.34&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pooled SE</td>
<td>0.08</td>
<td>1.3</td>
<td>1.3</td>
<td>2.3</td>
<td>0.03</td>
</tr>
</tbody>
</table>

<sup>1</sup>Data are expressed as mean (n=18) and values not sharing a common superscript letter are significantly different (p<0.05), when analyzed by Duncan's multiple range test. <sup>3</sup>Rice protein isolate, produced by Termamyl 120L digestion. <sup>4</sup>Soybean protein isolate.

similar in rats fed the RPI and SPI diets and 300% more than that in rats fed the casein diet. A weak negative correlation was also observed between serum estradiol-17β concentration and fecal excretion of estradiol-17β (r = -0.239, p < 0.1), but it was not significant.

Ovary weight was the same in all groups (110, 109 and 105 mg for casein, RPI and SPI diet-fed groups, respectively) and there was no effect of diet on ovarian aromatase activity (0.053, 0.071 and 0.059 (pooled SE=0.012) pmol·min<sup>-1</sup>·mg protein<sup>-1</sup> for casein, RPI and SPI diet-fed groups, respectively).

**Experiment 2**

Food intake and body weight gain were unaffected by dietary treatment (data not shown). Liver weight was significantly higher in rats fed casein diet (8.0 g) than in those fed RPI (7.0 g) or SPI (7.0 g) diet although microsomal protein did not differ among groups. Neither cytochrome P-450 content nor DMBA-aryl-
hydrocarbon hydroxylase activity of liver microsomes was affected by dietary treatment (data not shown).

DISCUSSION

The role of dietary and environmental factors in cancer risk is accepted. Dietary protein is one of these factors and in the past attention has been focused on absolute levels of intake. More recently, the type of protein has attracted more interest with the perception that plant proteins appear to be beneficial in certain sex-linked cancers. There have been a number of studies on the effects of soy and other proteins in human populations and animal models but we believe that this is the first examination of isolated rice protein in an accepted animal model of breast cancer. The present study showed that, although the final tumor incidence was the same among the three groups, tumor development was retarded by the RPI and SPI diets compared with the casein control in the middle of the experimental period. We found also that the mean tumor number per tumor-bearing rat only when fed the RPI diet was significantly lower than when fed the casein diet. Barnes et al. (7) reported that the number of palpable tumors in rats treated with DMBA and fed both purified and crude soybean proteins at dietary levels of 10 and 20% was 40% lower than in those fed a diet containing 20% casein. These data conflict with those found in the present study where mean tumor number in tumor-bearing rats fed SPI diet was 5.5 as compared with 6.9 in those fed the casein diet. Thus, our results are consistent with those obtained by Carroll (3) and Hsueh and Park (9) where the inhibitory effects of SPI on mammary tumor development were very weak relative to casein. The experimental conditions of the present study considerably differed from that of Barnes et al. (7). They administered DMBA at the dose of 15 mg per rat. This dose is approximately 3 times higher than those of Carroll (3) and the present study. This difference in dose of DMBA may reflect the differential results for the effects of SPI. DMBA-induced mammary tumors are primarily prolactin- and estrogen-dependent. Both RPI and SPI have inhibitory effects on enterohepatic steroid circulation (T. Morita and S. Kiriyama; unpublished data), so we presumed that their effects on tumor development could be mediated through alterations in estrogen excretion. In fact, the present results showed that both RPI and SPI diets significantly increased fecal excretions of estradiol-17β to the same degree as compared with the casein diet. However, a significant decrease in serum estradiol-17β concentration was observed in rats only when fed the SPI diet. There were no significant differences in serum estradiol-17β concentrations between the RPI- and casein-fed groups. Thus, the present data indicate that the inhibitory potencies of RPI and SPI on tumor progression are not necessary parallel with their lowering effects on estradiol-17β. However, Dao (20) suggested that hormone concentrations in the first week after carcinogen administration was critical in terms of hormonal requirements for the development of mammary tumors. Since we did not measure serum estradiol-17β or prolactin immediately after DMBA administration,

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we cannot completely exclude their possible involvement at this critical period in tumor development.

The feeding of RPI or SPI also reduced serum cholesterol concentrations relative to casein. Klurfeld and Kritchevsky (21) demonstrated that diet-induced hypercholesterolemia enhanced DMBA-induced mammary tumorigenesis, and reduction of serum cholesterol by changing diets from a cholesterol-supplemented diet to a cholesterol-free diet reduced tumor formation to the level seen in the control group. In this context, both RPI and SPI reduced serum cholesterol concentrations and tumor formation as well. Although the mechanism of the relationship between serum cholesterol concentration and mammary tumor development is still unclear, cholesterol may affect the endocrine and immune systems, thereby influencing mammary tumor development (22).

Clinton et al. (23) reported a lower mammary tumor incidence in rats fed a high protein diet and exposed to DMBA. They suggested that high protein diets induced an increase of hepatic arylhydrocarbon hydroxylase activity which would decrease the concentration of DMBA (and its metabolites) reaching the mammary gland. In the present study, we measured hepatic microsomal DMBA-arylhydrocarbon hydroxylase after 7 days of feeding the test diets (Experiment 2). This corresponds to the day before DMBA administration in Experiments 1 and 2. Hepatic microsomal DMBA-arylhydrocarbon hydroxylase activity was the same in all groups even when allowance was made for differences in liver weight. It appears unlikely that differential induction of arylhydrocarbon hydroxylase activity was responsible for the dietary differences in mammary tumor induction.

The present data showed that dietary RPI inhibited the development of mammary tumors induced by a model carcinogen (DMBA). However, we could not elucidate the inhibitory effect of RPI only in terms of alterations of circulating estrogen and sterols. Further studies on the mechanisms of inhibition of tumor development by rice protein and the relevance of the findings to human cancer risk are required.

REFERENCES


