Consumption of a Buckwheat Protein Extract Retards 7,12-Dimethylbenz[a]anthracene-Induced Mammary Carcinogenesis in Rats

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Female rats were examined for the effects of feeding buckwheat protein extract (BWPE) on the development of mammary tumor caused by administration of 7,12-dimethylbenz[a]anthracene. The percentage of rats with palpable mammary tumors and serum estradiol were lower in the BWPE-fed animals than the casein-fed ones, implying that BWPE intake retarded the mammary carcinogenesis by lowering serum estradiol.

Key words: buckwheat protein; mammary carcinogenesis; estradiol; rat

Buckwheat protein is known to consist of well-balanced amino acids with high biological value, although its digestibility is somewhat low. We have recently found that buckwheat protein extract (BWPE) had a strong hypcholesterolemic action in rats fed a cholesterol-enriched diet. The hypocholesterolemic activity of BWPE was reduced by the treatment by partial digestion of protease, implying that the lower digestibility of BWPE is at least in part responsible for the hypocholesterolemic activity. Feeding of BWPE also elevated stool weight and ameliorated atropine-induced constipation and the toxicity of amaranth (Food Red. No. 2) in rats. BWPE had anti-obese activity in rats. The development of chemically-induced colon tumor in rats was suppressed by BWPE intake (Ishikawa et al., unpublished data). Taken together, these physiological functions of BWPE appear to be similar to those of dietary fiber. Dietary fiber is known to suppress the development of mammary tumors. This effect has been considered to be mediated by reduced circulating estrogen. Thus, if BWPE has dietary fiber-like activity, it would be expected that consumption of BWPE suppresses the development of mammary tumors in female rats. Here we report the retardation of 7,12-dimethylbenz[a]anthracene (DMBA)-induced rat mammary tumor by BWPE, being associated with lower serum estradiol.

BWPE was prepared by the method described in our previous report. The composition of BWPE was as follows: water, 8.0%; protein, 52.5%; lipid, 11.0%; dietary fiber, 7.0% (measured by the method of Prosky et al.); ash, 5.2%; and starch, 6.3% (measured by a kit for the UV method for measuring starch, Boehringer Mannheim, Mannheim, Germany). The amino acid composition of BWPE was described in our recent report. The composition of fatty acids of lipid in BWPE was as follows (% of lipid): 16:0, 14.0; 18:0, 39.7; 18:2, 33.6, 18:3, 2.0, 20:0, 17; 20:1, 36; 22:0, 1.9; 24:0, 1.2. The composition of sterols of lipid in BWPE was as follows (% of lipid): β-sitosterol, 0.02; isofucosterol, 0.003; campesterol, 0.002. Casein was purchased from Oriental Yeast Co. (Tokyo, Japan). DMBA was purchased from Nacalai Tesque Inc. (Kyoto, Japan).

Female Sprague-Dawley rats (4 wk of age) weighing approximately 70 g (Charles River Japan Inc., Hino, Japan) were used. Room temperature was kept at 24°C with a 12-light: dark cycle (lights on 0800–2000 h). The animals were maintained according to the Guide for the Care and Use of Laboratory Animals established by Hiroshima University. All rats had free access to deionized water and diets. For 1 wk, rats were fed a diet according to the formula recommended by the American Institute of Nutrition. DMBA was administered orally at the dose of 30 mg/kg body weight 2 d before the rats were given the experimental diet. When the rats received DMBA, the vaginal opening was not observed. The rats (20 animals per diet group) were fed on the experimental diet containing either casein or BWPE (Table 1) for 61 d. Food consumption and body weight

Table 1. Composition of Experimental Diets (%)

| Ingredient      | Casein | BWPE
|-----------------|--------|------
| Casein†         | 23.0   | 38.1 |
| BWPE            |        |      |
| DL-Methionine   | 0.3    | 0.3  |
| Corn oil        | 10.0   | 5.8  |
| Salt mixture†   | 3.5    | 3.5  |
| Vitamin mixture | 1.0    | 1.0  |
| Cellulose       | 5.0    | 5.0  |
| Corn starch     | 37.0   | 26.1 |
| Sucrose         | 20.0   | 20.0 |
| Choline bitartrate | 0.2  | 0.2  |

† Casein, (N × 6.25); 87.0%.
‡ BWPE (buckwheat protein extract), (N × 6.25); 52.5%.
§ AIN-76 diet (AIN 1977).
were recorded every morning. At the end of the experimental period, the feed was removed from each cage (8:00), and blood samples were collected (13:00–15:00) from the vena cava using a syringe with heparin under anesthesia with diethyl ether. The rats were examined for palpable mammary tumors throughout the experimental period. Serum concentration of estradiol-17β was measured by a kit (DPC-E2, DPC, Los Angeles, U.S.A.). Statistical analysis of the tumor incidence was done by using the χ² test, while other data such as body weight, tumor multiplicities, etc. were compared by using Student’s t-test. Results were considered significant at P<0.05.

The percentage of rats with palpable mammary tumors was significantly lower in the rats fed BWPE than those fed casein after 48 d (Fig. 1). There was no difference in final body weight between the casein group and BWPE group (256±8 g and 249±9 g, respectively). Food intake was also unaffected by dietary treatment (data not shown). Fecal wet weight of the BWPE group (4.58±0.20 g/3 d) at d 57–60 was significantly higher than that of the casein group (3.98±0.16 g/3 d). At 61 d, the mean tumor number per tumor-bearing rat and the mean tumor weight per tumor-bearing rat at autopsy were unaffected by dietary manipulation (data not shown). Serum estradiol in BWPE group (89±12 pmol/l) was significantly lower than that in casein group (145±25 pmol/l).

As expected, this study demonstrated that consumption of BWPE retarded the development of DMBA-induced mammary tumor in rats. BWPE intake also resulted in lower concentrations of serum estradiol. These results suggest that BWPE intake suppresses the mammary carcinogenesis by a mechanism involving lowering circulating estradiol. This mechanism of the protective effect of BWPE is similar to that of soy protein isolate since soy protein isolate also reduces circulating estradiol, resulting in the protective effect.10 The tumor number per tumor-bearing-rat and the tumor weight per tumor-bearing rat did not significantly differ between the two groups. This may imply that the chemopreventive effect of BWPE is evident in the early stage of the carcinogenesis, and that the BWPE diet has less effective against the tumor growth.

There might be a possible mechanism causing lower serum estradiol, which is mediated through increasing excretion of fecal estradiol or impairment of intestinal reabsorption of estradiol. Since the consumption of BWPE causes higher excretion of fecal steroids,23 the estradiol-lowering effect of BWPE might be accounted for by this mechanism. Further study is necessary to obtain a conclusion.

Consumption of a large amount of L-arginine (addition of 5% L-arginine to a 15% casein diet) has been reported to suppress the DMBA-induced mammary carcinogenesis in rats,10 although its mechanism is unknown. BWPE has a higher level of arginine when compared to casein.23 The dietary arginine level in the BWPE diet used in this study is equivalent to that in the 23% casein diet supplemented with 1.5% arginine. It is unknown if this amount of arginine in the BWPE diet is related to the suppression in the mammary carcinogenesis. Since BWPE has some lipids and a small amount of fiber as described above,13 the possibility still remains that these minor components are responsible for the preventive effect.

In summary, our study indicated that the consumption of BWPE retarded DMBA-induced mammary carcinogenesis in rats. Chemopreventive effects of soy protein isolate and rice protein isolate against mammary carcinogenesis have been reported.13,15 These facts may imply that consumption of plant proteins is generally beneficial for the prevention of mammary tumor development.

References


