Short Communication

Diets Containing Whey Proteins or Soy Protein Isolate Protect against 7,12-Dimethylbenz(*a*)anthracene-induced Mammary Tumors in Female Rats[1]

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Abstract

A study was conducted to determine the protective effects of two common dietary proteins, soy protein isolate (soy) and bovine whey, against chemically induced mammary tumors in female Sprague Dawley rats. Rats were fed AIN-93G diets having casein, soy, or whey as the sole protein source. Rats within the same dietary groups were mated to obtain the F_1 and F_2 generations. At age 50 days, F_1 (experiment A) or F_2 (experiment B) female offspring (≥19 rats/group) were p.o. gavaged with 80 mg/kg 7,12-dimethylbenz(*a*)anthracene, and mammary glands were evaluated when 100% of the casein-fed group developed at least one palpatable tumor. Rats grew well on all three diets, but casein-fed rats gained slightly more body weight than soy- or whey-fed rats ($P < 0.05$). Vaginal opening occurred 1 day earlier in soy-fed rats than in casein- or whey-fed rats, but no other differences in reproductive and developmental parameters were observed between groups. When 50% of the casein-fed rats had at least one mammary tumor, lower tumor incidences (24–34%) were observed in the soy-fed ($P < 0.009$) and whey-fed groups ($P < 0.001$). When 100% of the casein-fed rats had at least one tumor, soy-fed rats had a lower tumor incidence (77%) in experiment B ($P < 0.002$), but not in experiment A ($P < 0.12$), and there were no differences in tumor multiplicity. Whey-fed rats had lower mammary tumor incidence (54–62%; $P < 0.002$) and multiplicity ($P < 0.007$) than casein-fed rats in both experiments. Our results indicate that diets rich in soy reduce the incidence of chemically induced mammary tumors by approximately 20%. Furthermore, whey appears to be at least twice as effective as soy in reducing both tumor incidence and multiplicity.

Introduction

Of all environmental factors known to influence cancer, diet appears to be one of the most significant (1). A wide variety of dietary factors are thought to be important in altering cancer initiation, promotion, and progression, as well as in the prevention of cancer. Breast cancer is the most common malignant tumor among women and is the number two killer of women in the United States. Breast cancer incidence in women consuming traditional Asian diets is approximately 10% that of the general female population of the United States. Asian diets are low in red meat and fats but rich in grains such as rice and high in soybean products such as tufu and miso. Although such diets contain many components other than soy, factors found in soybeans have been reported to provide important protection against initiation, promotion, or progression of breast cancer in animal models. In addition to the protective effects of certain phytochemicals, epidemiological and experimental studies suggest that dietary bovine milk products may exert inhibitory effects on the growth of several tumor types (for a review, see Ref. 2). The antitumor activity of these dairy products has been attributed to a class of proteins that comprise approximately 20% of the total milk protein, the whey fraction (2, 3).

The present study was conducted to determine the possible preventive effects of diets containing soy protein isolate or bovine whey proteins on DMBA[3]-induced breast tumors in rats. The experiments were designed to determine the effects of long-term consumption of these proteins in diets that were formulated to meet the allowances recommended by the American Institute of Nutrition for the rat.

Materials and Methods

Experimental Design. Adult breeder female and male Sprague Dawley rats were purchased from Harlan Industries (Indianapolis, IN). They were housed individually in polycarbonate cages and allowed *ad libitum* access to water and pelleted food. Rats were randomly assigned to three groups and fed one of three semipurified diets made according to the AIN-93G diet formula (4), except that corn oil replaced soybean oil, and the protein source was either casein (New Zealand Milk Products, Santa Rosa, CA), whey (New Zealand Milk Products), or soy protein isolate (Protein Technologies International, Inc., St. Louis, MO). Diets containing soy protein isolate had 430 mg total isoflavones/kg diet, including 276 mg/kg genistein and 132 mg/kg diadzein. Amino acids were added to each diet to equalize the essential amino acids among diets.

Rats were allowed to breed, and the offspring were weaned to the same diet as their mothers. Offspring from

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[3] The abbreviations used are: DMBA, 7,12-dimethylbenz(*a*)anthracene; GSH, glutathione.

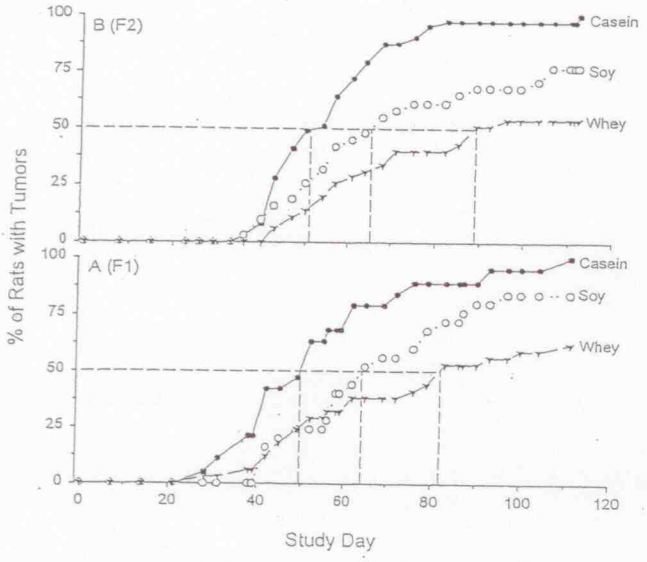

Fig. 2. Mammary tumor incidence (percentage of rats with tumors) of female rats depicted in Fig. 1. *Dashed line* indicates the post-DMBA day at which 50% of the casein-fed rats developed at least one mammary tumor. Statistical analyses of these data appear in Table 1.

Tumor Multiplicity and Location. Of the tumor-bearing rats, the median number of mammary tumors/tumor-bearing rat (*i.e.*, multiplicity) was lower ($P < 0.007$) in each experiment for whey-fed rats than for casein-fed rats (Table 1), whereas multiplicity for soy-fed rats did not differ significantly from that of the casein-fed group. Tumor location was studied, and cervical and thoracic regions were at higher risk of developing tumors (data not shown).

Discussion

Cancer rates in countries with high consumption of soybeans are lower than those in the United States, where less soy products are consumed (1), and cancer rates increase in the second generation of families that migrate to the United States from these countries as their diet becomes Westernized (11). Data from the present study substantiate the breast cancer prevention claims for experimental diets containing soy protein isolate as reported by others (5, 12). Furthermore, these data extend our knowledge on soy protein-containing diets and add new information on another dietary factor of animal origin, whey protein, with respect to chemically induced breast cancer. Results from our work demonstrate clearly that diets containing isolated soy protein or whey protein can significantly increase the age of onset of DMBA-induced tumors and reduce the percentages of rats that develop tumors. Moreover, rats fed diets containing whey also had decreased tumor multiplicity, whereas soy treatment did not appear to alter multiplicity. There were no detectable dietary effects on tumor volume.

To our knowledge, this is the first demonstration that rats fed whey protein-containing diets develop fewer DMBA-induced breast tumors than rats fed either casein- or soy-based diets. The whey protein diet delayed the age of onset of DMBA-induced tumors, reduced the percentages of rats that develop tumors, and attenuated tumor multiplicity compared with soy protein isolate or casein protein diets.

The mechanisms by which whey protein could alter carcinogenesis are unknown. Speculation has focused primarily on increases in tissue GSH levels observed during whey consumption (2). Greater GSH concentrations would tend to be protective because of: (*a*) the well-known xenobiotic detoxification pathway involving GSH and glutathione *S*-transferases; (*b*) GSH free radical detoxification; and (*c*) improved immune responses (13). Other possible mechanisms could include bioactive peptides that are either fragments of whey hydrolysis or contaminants of whey protein, such as insulin-like growth factor I, which could act on one of many cellular processes to reduce tumor incidence. Studies are currently underway in our laboratory to determine the mechanisms by which whey protein consumption prevents DMBA-induced breast cancer.

Although the mechanisms by which soy protein isolate prevents such cancers are still unknown, factors that are physically bound to or associated with the isolated protein, especially the isoflavones, have been implicated as being important (5). Genistein is one such isoflavone present in soybeans as genistein glucoside or the 6′-*O*-malonylglucoside. Lamartiniere *et al.* (6) demonstrated that rat pups receiving high doses of pure genistein aglycone at ages 2, 4, and 6 days developed fewer DMBA-induced breast tumors. This work has been substantiated in unpublished studies in our laboratory.[4] However, it should be noted that isoflavones have not been reported to be uniformly protective against chemically induced mammary tumor models in animals. For example, Hilakivi-Clarke *et al.* (14) reported a doubling of chemically induced mammary tumors in the offspring of mothers treated with genistein during pregnancy. Hsieh *et al.* (15) reported that dietary genistein enhanced the growth of MCF-7 tumors that were implanted s.c. in ovariectomized athymic mice. It should be pointed out that these two studies used genistein aglycone rather than diets made with soybean meal, soybean flour, or soy protein isolates. The disparity between laboratories using various animal models may reflect differences in species, developmental timing of genistein treatment, differences in endocrine status (*e.g.*, intact *versus* ovariectomized athymic females), or the chemical form of the isoflavone (pure aglycone, glucoside, or protein-bound form) and points to the need for further careful research on the conditions under which dietary factors affect cancer risk.

In the current study, soy-fed rats received a diet in which the entire protein source was the soy protein isolate used in the majority of commercially available soy-based infant formulas (Protein Technologies International, Inc.). A 333-g rat in our study consumed approximately 25 g/day of diet formulated with 20% soy protein (w/w). Because the genistein content was 1.36 mg/g protein, the genistein intake was approximately 6.8 mg/day (20.4 mg/kg/day). This is the highest dose of isolated soy protein possible within the AIN-93G diet formula. This genistein intake compares with the approximately 11 mg/day reported for 4-month-old infants who consume soy-based infant formula (16). Dose-response and time course studies for soy isolated protein effects and its cancer-preventive actions remain to be determined.

Several mechanisms have been proposed for the anticarcinogenic activity of isoflavones, including: (*a*) inhibition of proteases; (*b*) antioxidant activity; (*c*) increased synthesis and decreased degradation of steroid hormone binding globulin synthesis; (*d*) weak estrogenic agonist/antagonist activity through estrogen receptor α; (*e*) estrogen receptor β-mediated actions; (*f*) altered hormone production, metabolism, or action;

[4] T. M. Badger, unpublished observations.