Original Research

Soy Protein Isolate and Protection Against Cancer

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Objective: Results from epidemiological and animal studies suggest that consuming soy-containing diets reduces the incidence of certain cancers. The purpose of this presentation was to evaluate the potential of soy protein to prevent occurrence of prostate, breast and colon cancer.

Methods: Meta-analyses of published epidemiologic studies associating cancer risk with soy intake were performed. The incidence of chemically-induced mammary or colon tumors was determined for rats fed AIN-93G diets made with either casein or soy protein isolate (SPI). Western and Northern blot and microarray analyses were performed on rat mammary and colon tissues to study mechanisms underlying the effects of soy.

Results: Meta-analyses revealed reductions in the mean overall risk estimate for mammary (0.78, $p < 0.001$), colon (0.70, $p < 0.001$) and prostate (0.66, $p < 0.001$) cancer for soy consumers. The incidence of AOM-induced colon tumors and DMBA-induced mammary tumors was reduced ($p < 0.05$) in rats fed SPI-containing diets. Lower incidence of mammary tumors in SPI-fed rats was associated with: 1) reduced terminal end bud numbers ($p < 0.05$), 2) lower expression of the phase I enzyme CYP1B1 ($p < 0.05$) and 3) reduced expression of the Ah Receptor and ARNT ($p < 0.05$).

Conclusions: SPI may protect against cancer via multiple mechanisms, including: 1) increased mammary gland differentiation, 2) decreased activation of procarcinogens to carcinogens and 3) regulation of genes in signal transduction pathways underlying tumor initiation, promotion and/or progression.

INTRODUCTION

Soybeans and soy foods (i.e., tofu, soymilk, and soy flour) are traditional Asian foods that have been consumed for centuries in certain areas of Asia [1]. Americans do not typically consume large amounts of soy, but highly processed soy proteins (soy protein concentrates and isolates) are now used widely to formulate mainstream Western foods and soymilk is rapidly becoming popular in the US.

Results from several studies have suggested that Asian populations have lower incidences of cancer and cardiovascular disease than do Western populations. Although Asians have lower consumption of fat and red meat, higher consumption of fish and rice, and greater physical activity levels than Westerners, much attention has been given to the greater consumption of soy foods in Asia and how this might account for lower incidences of certain diseases.

A meta-analysis of 38 clinical studies suggested an association between increased consumption of soy protein and lower total serum cholesterol, LDL cholesterol and triglyceride concentrations, and with a non-significant increase in serum HDL cholesterol concentrations [2]. Based partly on these results, the FDA in 1999 authorized a health claim concerning the relationship between soy protein and coronary heart disease on labeling of foods containing soy protein. The health claim states that "Diets low in saturated fat and cholesterol and that include 25 grams of soy protein a day may reduce the risk of heart disease" (www.fda.gov/fdac/features/2000/300_soy.html#health). To claim the health effects of soy, a soyfood must contain 6.25 g or more soy protein, less than 3 g of total fat (<1 g of saturated fat) and less than 20 mg of cholesterol.

Recently, a petition was filed with the FDA for a health claim that soy protein as part of a low fat diet may reduce the risk of certain cancers (www.fda.gov). In this petition, evidence

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was presented from epidemiologic and animal studies suggesting that soy protein may reduce the risk of mammary, colon and prostate cancers. Meta-analysis was used to quantitate findings from a set of research studies. Details of this analysis are listed in the health claim petition 2004Q-0151: Qualified Health Claim (QHC): Soy Protein and Cancer (www.fda.gov). Briefly, only published studies with reported odds ratios or relative risks (i.e., risk estimates) and 95% confidence intervals were included in the analysis. A pooled estimate was calculated using a random-effects model in which the weighted effect measures were the log of the odds ratio or relative risk using previously reported methods [3]. This method assigns greater weight in the summary measure to those studies with smaller standard error of estimate. Publication bias was tested by previously reported methods [4]. The statistical program STATA (StataCorp, College Station, TX) was used for the calculation, and Dr. Edward Spitznagel, Professor of Mathematics and Statistics, Washington University, conducted all the analyses.

This paper is a summary of a review presented at the American College of Nutrition 2004 Annual Meeting in Long Beach, California. Herein, we present epidemiological results for breast, prostate and gastro-intestinal cancers used in the above cited FDA petition. In addition, we present data from rodent studies that support the epidemiologic data and in addition, examine potential mechanisms underlying the protective effects of ingested soy protein.

MATERIALS AND METHODS

Pregnant Sprague-Dawley rats (Harlan Industries) were fed AIN-93G diets from day 4 of gestation through weaning. The diets were formulated as described previously [5] to contain either casein or soy protein isolate (SPI) as the sole source of protein. The offspring were fed the same diets as their dams. At age 50 days, the females were orally gavaged with DMBA (50 mg/kg) [Aldrich Chemical] and at age 90 and 96 days the males were injected s.c. with 15 mg/kg azoxymethane (AOM) [Midwest Research Institute]. Female rats were euthanized when 100% of the control group had at least one palpable tumor and male rats were euthanized at 48 weeks post-AOM treatment.

RESULTS AND DISCUSSION

Pooled risk estimates from three meta-analyses indicate significant reductions in the risk of developing breast, prostate and gastro-intestinal cancers among those who consumed foods containing soy protein (Table 1). These data were extracted directly from the health claim petition 2004Q-0151: Qualified Health Claim (QHC): Soy Protein and Cancer (www.fda.gov).

Based upon the review and evaluation of published data presented in Table 1 and results from published animal studies, strong inverse relationships exist between soy food intake and the risks of breast, prostate and gastrointestinal cancers. Using the estimated soy protein consumption of subjects in these epidemiological studies, 5 g of soy protein was found to be associated with significant reduction in the risks of the stated cancers. Thus, the petition suggests inclusion of 5 g of soy protein to qualify a food or beverage to carry the claim. The data further suggest that those subjects consuming the greatest amount of soy protein had the lowest risk of these cancers.

While the results of the meta-analyses presented in Table 1 strongly suggest that consumption of soy protein may reduce the risk of some cancers, they do not provide information on the mechanisms by which these protective effects occur. Therefore, we have studied chemically-induced mammary and colon cancers in rats fed soy protein isolate (SPI) in an attempt to identify the mechanisms underlying the protective effects of soy.

Colon Cancer

We have found that adult male Sprague-Dawley rats fed SPI-containing diets had significantly lower ($p < 0.05$) incidence of azoxymethane (AOM)-induced colon cancers than control rats fed casein diets [6]. The rats in this study were offspring of dams that were fed soy from gestation day 4 (GD4) until weaning on PND21. The offspring were weaned to the same diet and, therefore, these rats were exposed to dietary factors associated with SPI intake throughout their entire lives. This is essentially the same pattern of soy exposure as Asians who consume large daily amounts of soy.

Recently, we addressed the question of whether protective effects could be attained with shorter duration of SPI exposure,

Table 1. Pooled risk estimates from three meta-analyses

Cancer	Population	Pooled Risk Estimate[1]	*p* value
Breast Cancer[2,3]	Women (all ages)	0.78 (0.68–0.91)	<0.001
	Postmenopausal Women	0.64 (0.47–0.88)	<0.005
Prostate Cancer[4]	Men	0.66 (0.54–0.81)	<0.001
Gastro-intestinal Cancer[5]	Men/Women	0.70 (0.61–0.80)	<0.001

[1] PRE (95% confidence interval).

The number of studies meeting the stated criteria for inclusion were:

[2] 14 breast cancer studies in women (all ages),

[3] 6 for breast cancer studies in postmenopausal women,

[4] 6 prostate cancer studies,

[5] 15 gastro-intestinal cancer studies.

Table 2. Soy protein isolate diets

	Casein	SPI		
		GD4—PD21	PD21—PD138	GD4—PD138
ACF >4 Crypts[1]	7.9 ± 1.3	3.9 ± 0.8*	3.6 ± 0.9*	3.2 ± 1.0*

[1] ACF containing more than 4 crypts.

* $p < 0.05$.

GD4 = gestational day 4, PD12 = postnatal day 12.

with a focus on early exposure. Early exposure to SPI is more reflective of American infants fed soy formula. We studied aberrant crypt foci (ACF) in the colon of offspring at age 138 days after exposure to soy over the following periods: GD4-PD21 (postnatal day 21); PD21-PD138; GD4-PD138 [7]. Although somewhat controversial, large multi-cryptal ACF are generally correlated with tumor incidence in rodents and humans [8,9]. We found that SPI diets reduced the incidence of the largest size classes of ACF in AOM-treated, adult Sprague-Dawley male rats, irregardless of the developmental stage of dietary exposure to SPI (Table 2). These ACF data confirm earlier results of colon cancer protection by life-time SPI exposure using tumor incidence as the end point [6] and suggest that a shorter exposure to SPI early in life also may protect against colon cancer. These results may have implications for infants fed soy formula, since they too are exposed to SPI for a relatively short period of their early life.

Breast Cancer

Chemically-induced animal cancer models have been employed widely to study mechanisms by which cancers develop, as well as to study prevention or treatment modalities. These models employ either direct carcinogens or procarcinogens. There are many procarcinogens to which people can be exposed and these must be metabolically activated to carcinogens before they can form gene mutations that lead to cancer. 7,12-dimethyl-benz(a) anthrocene (DMBA) is the most widely studied procarcinogen in animal models of human breast cancer. When treated with DMBA, 100% of female rats fed casein diets developed mammary tumors, while only 80% of those fed SPI diets developed tumors (Table 3 [5]).

We have studied the mechanisms by which SPI reduces the risk of mammary tumors in this model. The major pathway whereby DMBA is activated to the proximate carcinogen (DMBA 1,2 epoxide, 3,4 dihydrodiol) in the mammary gland is primarily by the enzyme, cytochrome P450 1B1 (CYP1B1). CYP1B1 gene expression is induced by its substrate, DMBA, indicating DMBA auto-regulation of its own activation. This process involves DMBA binding to the Ah receptor in the cytosol of mammary epithelial cells, translocation of the DMBA-Ah Receptor complex to the nucleus and subsequent binding to the ARNT transcription factor to form a heterodimer that binds to xenobiotic response elements in the promoter region of the CYP1B1 gene.

We studied the expression of CYP1B1, Ah Receptor and ARNT in the mammary glands of female rats fed AIN-93G diets made with either casein or SPI as the sole protein source (Table 3 [10]). Adult female Sprague-Dawley rats fed SPI expressed less CYP1B1, Ah-receptor and ARNT than did casein-fed rats ($p < 0.05$), suggesting that SPI reduces the incidence of mammary gland cancer in part by preventing the activation of procarcinogens to carcinogens.

Breast cancer develops in the undifferentiated epithelial cells (terminal end buds) of the mammary gland. With the developmental maturation that comes with age, puberty, pregnancy and lactation, the terminal end buds differentiate into alveolar buds and then into lobules. Cancer risk drops as the differentiation process continues. This is likely to be the basis for the reduced breast cancer risk in women who had early pregnancy, multiple pregnancies and/or breast fed their infants. All of these factors drive the undifferentiated terminal end bud towards maturation, thereby reducing the number of terminal end buds that can become cancerous. Thus, any factor that induces the mammary gland differentiation has the potential to also reduce mammary gland cancer incidence. We examined mammary glands of female rats fed SPI-containing diets and compared the terminal end bud numbers with those for casein-fed rats (Table 3 [11]). We found that SPI reduced ($p < 0.05$)

Table 3. Casein *vs.* soy protein isolate diets

Diet	Tumor Incidence[1]	CYP1B1[2]	Ah-Receptor[3]	ARNT[3]	TEB[4]
Casein	100%	1.5 ± 0.2	8.8 ± 1.1	15 ± 3.2	0.24 ± 0.08
SPI	80%*	0.1 ± 0.05*	3.9 ± 1.0*	7.1 ± 1.4*	0.06 ± 0.03*

[1] % of Rats with mammary tumors (n = 40–50/group).

[2] DMBA-induced CYP1B1 protein expression relative to vehicle-treated control (arbitrary density units) [n = 8/12 rats/group].

[3] Arbitrary Density Units from Western blots (n = 8–12 rats/group).

[4] Terminal End Buds (n = 8/12 rats/group).

* $p < 0.05$.

the numbers of terminal end buds, suggesting that accelerated mammary gland development by SPI can lead to reduction in the risk of mammary gland cancer.

The results from experimentally-induced colon and mammary cancer in rats fed diets made with either casein or soy protein isolate are consistent with epidemiological data that was discussed above and which demonstrate lower tumor incidence in populations who regularly consume soyfoods. This provides some confidence that further research in these models will reveal the mechanisms by which soy protein acts to reduce cancer incidence and lead to improved protection through diet.

CONCLUSIONS

Both epidemiological and animal studies suggest that consumption of soy protein is associated with lower incidence of breast, colon and prostate cancer. A limited number of studies have been conducted on mechanisms underlying these effects. It is clear that the gene expression profile of animals fed SPI differs substantially from that of control animals fed casein [12]. This is likely to occur in humans as well. We have shown that expression of proteins necessary to activate carcinogens can be suppressed by SPI and this is likely responsible, in part, for reduced mammary and colon cancer incidence. In addition, SPI advances mammary gland differentiation to a significant degree and thereby essentially reduces the number of breast cancer target cells. It is likely that this too will occur in children. Future studies should be directed to elucidate the bioactive molecules involved and expand our understanding of the relevant molecular mechanisms underlying the cancer protection of SPI.

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