Soybean Phytochemicals Inhibit the Growth of Transplantable Human Prostate Carcinoma and Tumor Angiogenesis in Mice1,2

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ABSTRACT The objectives of our studies are to characterize the ability of dietary soybean components to inhibit the growth of prostate cancer in mice and alter tumor markers associated with angiogenesis. Soy isoflavones (genistein or daidzein) or soy phytochemical concentrate inhibit the growth of prostate cancer cells LNCaP, DU 145 and PC-3 in vitro, but only at supraphysiologic concentrations, i.e., 50% inhibitory concentration (IC50) > 50 μmol/L. G2-M arrest and DNA fragmentation consistent with apoptosis of prostate cancer cells are also observed at concentrations causing growth inhibition. In contrast, the in vitro proliferation of vascular endothelial cells was inhibited by soy phytochemicals at much lower concentrations. We evaluated the ability of dietary soy phytochemical concentrate and soy protein isolate to inhibit the growth of the LNCaP human prostate cancer in severe combined immune-deficient mice. Mice inoculated subcutaneously with LNCaP cells (2 × 106) were randomly assigned to one of the six dietary groups based on the AIN-76A formulation for 3 wk. A 2 × 3 factorial design was employed with two protein sources (20%, casein vs. soy protein) and three levels of soy phytochemical concentrate (0, 0.2 and 1.0% of the diet). Soy components did not alter body weight gain or food intake. Compared with casein-fed controls, the tumor volumes after 3 wk were reduced by 11% (P = 0.45) by soy protein, 19% (P = 0.17) by 0.2% soy phytochemical concentrate, 28% by soy protein with 0.2% soy phytochemical concentrate (P < 0.05), 30% by 1.0% soy phytochemical concentrate (P < 0.05) and 40% by soy protein with 1.0% soy phytochemical concentrate (P < 0.005). Histologic examination of tumor tissue showed that consumption of soy products significantly reduced tumor cell proliferation, increased apoptosis and reduced microvessel density. The angiogenic protein insulin-like growth factor-I was reduced in the circulation of mice fed soy protein and phytochemical concentrate. Our data suggest that dietary soy products may inhibit experimental prostate tumor growth through a combination of direct effects on tumor cells and indirect effects on tumor neovascularization. J. Nutr. 129: 1628–1635, 1999.

KEY WORDS: soy • prostate cancer • apoptosis • angiogenesis • mice

The large geographic differences in the incidence and mortality of prostate cancer between high risk nations such as the United States and low risk countries in Asia may be due to differences in dietary factors (Morton et al. 1996, Parkin and Muir 1992). Among dietary factors, increased consumption of soybean products has been hypothesized to contribute to reduced prostate cancer risk (Hebert et al. 1998, Messina et al. 1994). Epidemiologic, in vitro and laboratory animal studies provide evidence for the hypothesis that phytochemicals in soy products have anticarcinogenic properties (Kennedy 1995, Messina et al. 1994). Much of the attention has focused upon genistein and daidzein, the predominant isoflavones found in soy in amounts of ~1–3 mg/g (Wang and Murphy 1994b). In addition, protease inhibitors, the Bowman-Birk inhibitor, inositol hexaphosphate (phytic acid), lignans, phytoestrogens and saponins found in soy products may also have bioactivities relevant to the inhibition of carcinogenesis (Kennedy 1995, Messina et al. 1994, Rao and Sung 1995, Shamsuddin 1995).

Relatively few animal studies have been conducted to investigate the role of soy components on prostate cancer tumorigenesis, and little is known regarding possible in vivo mechanisms whereby bioactive components in soy may influence the prostate. Earlier studies report that soy-containing diets reduce the severity of prostatitis in rats (Sharma et al. 1992) and prevent the development of dysplastic lesions of the prostate of neonatal diethylstilbestrol-treated mice (Makela et al. 1996). Relatively little is known about the role of soy components in the inhibition of tumor angiogenesis.

References

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2 Supported in part by National Institutes of Health grants F32 CA71161 and Harvard Clinical Nutrition Research Center, NIH Grant #P30DK40561 to J.-R.Z., and K07 CA01680 and RO1 CA72482 to S.K.C.
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al. 1995). Rats consuming a soy flour–containing diet exhibited reduced growth of well-differentiated transplantable Dunning R3327 prostatic adenocarcinoma compared with those fed a casein-based control diet (Landstrom et al. 1998, Zhang et al. 1997). In contrast, a small study with genistein added to the drinking water (intake not quantitated) or administered via intraperitoneal injection of 0.143–0.428 mg genistein/kg body weight had no effect on the growth of the subcutaneously implanted MAT-LYLu prostate carcinoma in rats (Naik et al. 1994). Overall, additional in vivo studies are required to allow definitive conclusions regarding soy products or isoflavones on prostate carcinogenesis, tumor progression and mechanism of action.

We report that concentrations of soy isoflavones exceeding those typically observed in vivo are necessary to inhibit the growth of human prostate cancer cell lines in vitro. In contrast, the proliferation of vascular endothelial cells is significantly inhibited at concentrations ≤25 μM/L. Feeding soy phytochemicals to rodents also inhibits the growth of the human LNCaP prostate cancer cell line in vivo. The inhibition of tumor growth in vivo is correlated with alterations in tumor biomarkers, including reduced proliferating cell nuclear antigen (PCNA) labeling as a marker of proliferation, increased apoptosis and reduced microvessel density. Our observations suggest that dietary soy products inhibit prostate cancer progression in vivo via multiple interacting mechanisms.

MATERIALS AND METHODS

Soy isoflavones, soy protein, and soy phytochemical extracts. Soy isoflavones (genistein, daidzein) were purchased from Sigma Chemical (St. Louis, MO). The soy protein isolate (SPI, Supro 670HG, Lot#CSC-XPC-001, Protein Technology International, St. Louis, MO) contains 2.07 mg isoflavone aglycone equivalents (each isoflavone was calculated to reflect only the weight of the aglycone because mixtures of free isoflavones and glycosylated forms are found in the product), including 1.22 mg genistein equivalents, 0.64 mg daidzein equivalents and 0.21 mg glycitein equivalents per gram. A soy phytochemical concentrate (SPC) was provided by Archer Daniels Midland Company (Decatur, IL). Soy phytochemical concentrate was prepared as follows: soybeans were cracked, dehulled and flaked by standard procedures followed by a hexane extraction to remove the majority of lipid. The resulting defatted soy flour was extracted with aqueous ethanol (60%, v/v) to produce a mixture containing carbohydrates (0.6–0.7 g/g material), isoflavones (0.02 g/g), fat (0.12 g/g), ash (0.04 g/g) and protein (0.05 g/g). A proprietary extraction procedure was then employed to remove the carbohydrates; the remaining material was spray-dried to form a powder called SPC and analyzed for isoflavones according to published methods (Wang and Murphy 1994a). The final SPC employed in our studies contains 170 mg isoflavone aglycone equivalents per gram of material, which is 79.2% of the genistein equivalents, 20.4 mg of daidzein equivalents and 20.4 mg of glycitein equivalents. One gram of soy phytochemical concentrate also contains 0.14 g of protein, 0.055 g of fat, 0.027 g of ash and 0.065 g of moisture, with the remaining matter undefined but apparently rich in saponins.

Prostate cancer cell culture studies. Three human prostate cancer cell lines, LNCaP, PC-3 and DU 145 (American Type Culture Collection, Rockville, MD), were used for the studies. Human prostate cancer cell lines were maintained as monolayer cultures in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum, 2 mmol l-glutamine/L, 1 × 10^6 U penicillin/L and 100 g streptomycin/L in a 95% air, 5% CO2, and water-saturated atmosphere. The in vitro growth studies were completed with 5 × 10^5 cells/well, plated into 96-well microplates, treated with soy isoflavones or soy phytochemical concentrate dissolved in dimethyl sulfoxide (final dimethyl sulfoxide concentration ≤ 0.1% by volume) and incubated for 72 h. Dimethyl sulfoxide vehicle controls were used in all studies. Cell numbers were quantitated by the XTT (sodium 3-[(phenylamino)-carbonyl]-3,4-tetrazolium]-bis (4-methoxy-6-nitro) benzene-sulfonic acid hydrate) assay (Roehm et al. 1991). All assays were completed in triplicate, experiments were replicated at least once and results were confirmed by direct cell counting using a hemocytometer.

Analysis of prostate cancer cell cycle progression and DNA fragmentation. Cells were grown under conditions as described above, harvested by trypsinization and centrifugation at 1500 × g for 5 min, washed with PBS and fixed with 80% ethanol. Cells were then washed with PBS, resuspended, stained by adding propidium iodide (at a final concentration of 50 μg/L) and RNase (at a final concentration of 50 μg/L), and incubated at 37°C for 30 min. Stained cells were analyzed by FACScans (Becton Dickinson, San Jose, CA) for DNA fragmentation and cell cycle using programs provided by Becton-Dickinson.

Endothelial cell proliferation studies. Endothelial cell proliferation studies employed methods previously described (Tanaka et al. 1997). In brief, human umbilical vein endothelial cells (American Type Culture Collection) were maintained in Medium 199 containing 10% fetal bovine serum, 100 μg/mL heparin, and 30 μg/mL endothelial cell growth supplement (Collaborative Biomedical Products, Bedford, MA). For assays, human umbilical vein endothelial cells (5 × 10^5) were plated in 12-well plates; genistein or SPI was added at predefined concentrations (0, 25 and 50 μM/L). After 72 h, cells were labeled with 3.7 × 10^4 Bq of ³H-thymidine, and incorporation into DNA was quantitated in a scintillation counter 24 h later. Studies were completed in quiescent conditions (no heparin) and with heparin stimulation.

Determination of serum insulin-like growth factor-1 (IGF-I). Serum IGF-I was extracted and quantitated by RIA following the procedures provided by Nichols Institute Diagnostics (San Juan Capistrano, CA).

Diet formulations and treatment groups. SPI and SPC were used to prepare the following six semipurified experimental diets according to our formulation (Table 1) by Research Diets (New Brunswick, NJ): 1) AIN-76 diet as the control; 2) AIN-76 with casein replaced by SPI, 20% by weight, providing 415 mg isoflavone equivalents/kg diet; 3) AIN-76 with SPI at 0.2% of the diet providing 341 mg isoflavone equivalents/kg; 4) AIN-76 with casein replaced by SPI (20% of the diet) with addition of SPC (0.2% of the diet), providing 756 mg isoflavone equivalents/kg; 5) AIN-76 with SPI at 1.0% of the diet, providing 1705 mg isoflavone equivalents/kg; and 6) AIN-76 without casein replaced by SPI (20%) with addition of SPC (1.0% of the diet), providing 2120 mg isoflavone equivalents/kg. Isoflavone levels were confirmed by HPLC analysis.

Animal studies. Forty-eight male SCID mice (8 wk old) were purchased from Harlan Sprague Dawley (Indianapolis, IN). After 12 wk of adaptation to the AIN-76 diet, mice were inoculated subcutaneously on the right flank with a suspension of 2 × 10^6 LNCaP cells isolated from subcutaneously grown LNCaP tumors from donor SCID mice. Recipient mice were then randomly assigned into six groups (n = 8) and fed one of the experimental diets. Food intake, body weight and tumor diameters were measured three times weekly. Tumor volumes were calculated by the following formula: tumor volume (cm³) = 0.523 × [length (cm) × width² (cm²)]. The experiment was terminated at 21 weeks after inoculation. Analysis of serum IGF-I. Serum IGF-I was extracted and quantitated by RIA following the procedures provided by Nichols Institute Diagnostics (San Juan Capistrano, CA).

Immunohistochemical determination of angiogenesis (microvessel density). Immunohistochemical quantitation of microvessel...
density was used as a marker for tumor angiogenesis following a previously described method (Zhou et al. 1998). In brief, after deparaffinization, rehydration and washing in PBS, tissue sections were incubated with trypsin at 37°C for 30 min, quenched with 8.8 mmol/1 buffer (1.0 g bovine serum albumin and 0.1 mL Tween 20 in 100 mL PBS). The sections were then immunoreacted with a rabbit polyclonal antibody directed against human Factor VIII related antigen (DAKO, Carpinteria, CA, 1:100 dilution), and a biotinylated “universal” horse anti-mouse/rabbit immunoglobulin (Ig)G (Vector Laboratories, Burlingame, CA), followed by treatment with avidin-biotin complex (Vector Laboratories) and 3-3′ diaminobenzidine.

**Immunohistochemical determination of proliferation.** The proliferation index was evaluated by counting the proportion of cells with PCNA staining (Zhou et al. 1998). In brief, after deparaffinization, rehydration and washing in PBS, tumor sections were soaked in 10 mmol citrate buffer/L and heat-treated for 5 min in a microwave oven. Sections were then stained following the procedures as described for factor VIII staining, using horse serum at 100 mL/L of the buffer (1.0 g bovine serum albumin and 0.1 mL Tween 20 in 100 mL PBS) for blocking and a PCNA mouse monoclonal antibody (DAKO) as a primary antibody. Both PCNA-positive proliferating cells and total tumor cells were counted under light microscopy at 1000-fold magnification as three representative areas that did not contain necrosis.

**In situ apoptotic cell detection.** Apoptotic cells were determined by a terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) assay using the ApopTag in situ detection kit (Oncor, Gaithersburg, MD), following the manufacture’s procedures with modification (Zhou et al. 1998). In brief, after deparaffinization, rehydration and washing in PBS, sections were treated with 20 mg/L proteinase K for 20 min at room temperature and washed. Endogenous peroxidase activities in sections were quenched with 0.88 mol/L H2O2 of PBS for 5 min. Sections were applied with terminal deoxynucleotidyl transferase labeled with digoxigenin peroxidase and incubated for 1 h at 37°C; the reaction was stopped by stop and wash buffer. Sections were then incubated with antidigoxigenin peroxidase for 30 min at room temperature, washed, stained with 3–3′ diaminobenzidine substrate, counterstained with methyl green and mounted. Known positive and negative control slides were used for comparison. Three representative areas of each section without necrosis were selected, and both apoptotic cells and total nuclei cells were counted under light microscopy at 400-fold magnification. The apoptotic index was expressed as the percentage of apoptotic nuclei to total nuclei.

**Statistical analysis.** Results from cell culture studies, tumor volume, apoptotic index, proliferation index and microvessel density were initially evaluated by ANOVA followed by Fisher’s protected least significant difference test (Steel and Torrie 1980) to evaluate pairwise comparisons among treatment groups using the Statview 4.5 (Abacus Concepts, Berkeley, CA) program. A probability level of P < 0.05 was considered significant.

## RESULTS

**Soy isoflavones and soy phytochemical concentrate modulation of prostate cancer cell lines in vitro.** Genistein at 2.5 and 50 μmol/L significantly inhibited LNCaP cell growth by 33% (P < 0.001) and 50% (P < 0.001), respectively (Fig. 1A). Genistein at 50 μmol/L also significantly inhibited the growth of DU 145 cells by 23% (P < 0.01, Fig. 1B) and PC-3 cells by 34% (P < 0.005, Fig. 1C). In comparison to genistein, daidzein had weaker effects on human prostate cancer cell lines in vitro. Daidzein at 50 μmol/L significantly inhibited the growth of LNCaP cells by 40% (P < 0.05, Fig. 1A). At lower concentrations (5 or 10 μmol/L), daidzein tended (P = 0.15) to increase human prostate cancer cell numbers. SPC also demonstrated a dose-dependent inhibition of prostate cancer cell growth (Fig. 1D) at concentrations between 5 μmol/L (18 μg/mL) and 100 μmol/L (360 μg/mL) of total aglycone isoflavone equivalents (2.3–46 μmol/L of genistein equivalents and 2.0–40 μmol/L of daidzein equivalents). At 100 μmol/L (46 μmol/L genistein equivalents and 40 μmol/L daidzein equivalents), SPC inhibited LNCaP cells by 80% (P < 0.0001), DU 145 cells by 50% (P < 0.001) and PC-3 cells by 25% (P < 0.05). These data suggest that soy isoflavones probably account for much of the inhibitory effects of SPC on prostate cancer cell growth in vitro.
Cell cycle progression and DNA fragmentation of LNCaP cells treated with genistein and SPC in vitro. Genistein at 50 μmol/L, but not at 10 μmol/L, significantly affected cell cycle progression by arresting LNCaP cells at G2-M phases (Table 2, P < 0.005). Genistein at 50 μmol/L induced DNA fragmentation, a marker for apoptosis, of LNCaP cells by twofold (P = 0.45). Parallel studies (data not shown) with SPC (0, 10, or 50 μmol/L) also showed DNA fragmentation and cell cycle arrest in G2-M phases for LNCaP cells although the magnitude of the response was attenuated compared with that of pure genistein. Additional studies with PC-3 and DU 145 cells showed statistically significant (P < 0.05) dose-dependent G2-M arrest and enhanced DNA fragmentation at >50 μmol/L concentrations (data not shown).

Soy isoflavones and SPC on endothelial cell growth. The effects of genistein or SPC on cell proliferation were examined under quiescent (growth suppressed) conditions and after stimulation by heparin (Fig. 2). Quiescent cells showed a significant inhibition of incorporation of 3H-thymidine at 25 μmol/L genistein (P < 0.001) or SPC (P < 0.05). Heparin stimulation increased 3H-thymidine incorporation ~10-fold. Incorporation of label was reduced by >50% by 25 μmol/L genistein (P < 0.001) or SPC (P < 0.001) and by >80% (P < 0.001) by either of these soy products at a concentration of 50 μmol/L.

The growth of transplanted human prostate tumors in mice fed soy products. Subcutaneous growth of the human prostate cancer cell line LNCaP in male SCID mice was used as an in vivo model to evaluate the effects of dietary soy products SPC and SPI on prostate tumor growth. The experiment was terminated when mean tumor volume of the control group reached 2.3 ± 0.3 cm³. Dietary soy products did not significantly alter food intake or body weight (Table 3). Tumor volumes at d 21 (Fig. 3 and Table 4) from mice treated with diets containing SPI (20% of the diet) alone, SPC (0.2%) alone, SPC (0.2%) and SPI (2%) and SPC (0.2%) alone and SPI (20%) with SPC (1.0%) were reduced by 11% (P = 0.45), 19% (P = 0.17), 28% (P < 0.05), 30% (P < 0.05) and 40% (P < 0.005), respectively, compared with those of casein-fed control mice. Factorial analysis indicated that there was a significant main effect of SPI on prostate tumor growth (P < 0.05), whereas there was no significant main effect of soy protein as protein source (P = 0.09).

Effects of soy products on tumor proliferation, apoptosis, and angiogenesis. Formalin-fixed tumor tissues were processed to prepare tissue slides and used for in situ histochemical detection of apoptosis by TUNEL assay, proliferation by PCNA staining, and angiogenesis by microvessel density quantitation (factor VIII staining). The results are presented in Table 4. Compared with controls, tumors from mice treated with the diets containing SPI (20%) alone, SPC (0.2%) alone, SPI with SPC (0.2%), SPC (1.0%) alone and SPI with SPC (1.0%) showed a lower proliferation index by 6% (P = 0.24), 14% (P < 0.01), 15% (P < 0.005), 18% (P < 0.001) and 21% (P < 0.0001) respectively (Table 4). In contrast, tumor cell apoptosis rates were greater in mice fed the above diets by 33% (P = 0.28), 84% (P < 0.05), 80% (P < 0.01), 60% (P < 0.05) and 136% (P < 0.0001) respectively, compared with controls. The microvessel densities of tumors derived from mice fed the above diets were reduced by 43% (P < 0.01), 29% (P = 0.07), 49% (P < 0.005), 51% (P < 0.005), and 61% (P < 0.001) respectively, compared with that of control group.

Multiple linear regression analysis was applied to determine the correlations between tumor biomarkers and tumor volumes. The analysis resulted in the following correlation equation: tumor volume (cm³) = 2.52 – 0.09(apoptotic index) + 0.138(microvessel density) – 0.008(proliferation index) (R = 0.673, P < 0.001). Using this model, the lower tumor volume in soy-fed mice was associated with increased tumor cell apoptosis (P < 0.05) and reduced tumor microvessel density (P < 0.0001), but not significantly associated with tumor proliferation (P = 0.64). These results suggest that both apoptotic index and microvessel density may serve as biomarkers in evaluating the effects of soy products on prostate tumor growth, whereas proliferation index may be a dependent biomarker.

Effects of soy products on serum IGF-I levels. To explore the possible effects of dietary soy products on IGF-I, we quan-
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Effects of genistein on the cell cycle of human prostate cancer cell line LNCaP¹

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Effects of genistein and soy phytochemical concentration (SPC) on endothelial cell proliferation in vitro. Quiescent cells showed a significant inhibition of incorporation of ³H-thymidine at 25 μmol/L genistein (P < 0.001) or SPC (P < 0.05). Heparin stimulation increased ³H-thymidine incorporation ~10-fold. Incorporation of label was reduced by >50% by 25 μmol/L genistein (P < 0.001) or SPC (P < 0.001) and by >80% (P < 0.001) by either of these soy products at a concentration of 50 μmol/L.

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Effects of genistein and soy phytochemical concentration (SPC) on endothelial cell proliferation in vitro. Quiescent cells showed a significant inhibition of incorporation of ³H-thymidine at 25 μmol/L genistein (P < 0.001) or SPC (P < 0.05). Heparin stimulation increased ³H-thymidine incorporation ~10-fold. Incorporation of label was reduced by >50% by 25 μmol/L genistein (P < 0.001) or SPC (P < 0.001) and by >80% (P < 0.001) by either of these soy products at a concentration of 50 μmol/L.

FIGURE 2  Effects of genistein and soy phytochemical concentrate (SPC) on endothelial cell proliferation in vitro. Quiescent cells showed a significant inhibition of incorporation of ³H-thymidine at 25 μmol/L genistein (P < 0.001) or SPC (P < 0.05). Heparin stimulation increased ³H-thymidine incorporation ~10-fold. Incorporation of label was reduced by >50% by 25 μmol/L genistein (P < 0.001) or SPC (P < 0.001) and by >80% (P < 0.001) by either of these soy products at a concentration of 50 μmol/L.
with carefully controlled media conditions, temperature and oxygenation are not predictive of the complex and harsh in vivo conditions in the tumor microenvironment, in which hypoxia, necrosis, and suboptimal perfusion and diffusion limit nutrient availability and removal of metabolic waste. Our studies indicate that the ability of soy products to modulate prostate tumor cell function is more profound in vivo than in vitro.

In addition to direct effects on tumor cells, soy products may modulate a number of other host processes, indirectly influencing prostate tumor growth. For example, others have reported that genistein inhibits endothelial cell proliferation in response to growth factors in vitro. Our studies show that pure genistein and SPC inhibit heparin-stimulated endothelial cell proliferation in vitro by >50% with concentrations ≤ 25 μmol/L. The in vivo assessment of prostate tumor microvessel density as a biomarker of tumor angiogenesis shows a reduced vascularity in mice fed soy products. The inhibition of tumor angiogenesis is typically associated with enhanced apoptosis and has little effect on proliferation index (Folkman 1995). The significant increase in tumor apoptosis may represent a combined direct effect on tumor cell function and a secondary indirect effect related to inhibition of tumor angiogenesis.

The antiangiogenesis mechanisms influenced by soy isoflavones may be multiple. First, soy isoflavones may inhibit the production and/or bioactivity of angiogenic factors. Angiogenesis factors control vascular endothelial cell proliferation and migration within the growing tumor matrix. Among them, vascular endothelial growth factor (VEGF) is believed to play an important role in angiogenesis. Genistein inhibits VEGF level by post-transcriptional regulation of its expression (Levy et al. 1996) in vitro. Soy isoflavones may also directly inhibit endothelial cell proliferation. Genistein has been shown to be more potent than other isoflavones in inhibiting endothelial cell proliferation in vitro (Fotsis et al. 1993, Xia et al. 1996).

IGF-I is also a growth factor associated with enhanced angiogenesis (Nakao-Hayashi et al. 1992). Furthermore, circulating IGF-I concentrations are positively associated with prostate cancer risk in human studies (Chan et al. 1998, Mantzoros et al. 1997, Wolk et al. 1998). Our studies provide the foundation for the hypothesis that soy may inhibit prostate angiogenesis both by direct effects on endothelial cells and by reducing circulating concentrations of critical growth factors.

We chose SPC as the major source of soybean bioactive components for our studies because it contains a diverse array of biologically active compounds that could potentially interfere to provide more potent anti-prostate cancer activity. These hypothetical benefits would not be appreciated in studies of pure compounds. The in vivo inhibition of cancer incidence or progression by soy products or pure isoflavones has been reported for gastric cancer (Yanagihara et al. 1993), leukemia cells (Jing et al. 1993), breast cancer (Hawrylewicz et al. 1991, and 1995) and others (Messina et al. 1994). In contrast, some studies have not found in vivo inhibitory effects of soy on tumorigenesis (Clinton et al. 1979, Messina et al. 1994, Naik et al. 1994). Of concern, some studies report that soy-based dietary treatments had tumor-promoting effects (McIntosh et al. 1995, Rao et al. 1997). In addition, it has been hypothesized that the estrogenic properties of soy isoflavones may stimulate breast tumor growth under some conditions (Hsieh et al. 1998). Investigators, clinicians and commercial enterprises should use caution in universally recommending soy supplements enriched in isoflavones for cancer prevention or therapy except in the context of clinical studies.

In summary, we observed that dietary soy phytochemicals

### TABLE 3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Protein</th>
<th>SPC</th>
<th>n</th>
<th>Body weight²</th>
<th>Food intake</th>
<th>Isoflavone intake</th>
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<tr>
<td></td>
<td>g/100 g</td>
<td></td>
<td></td>
<td>g</td>
<td>g/d</td>
<td>mg/d</td>
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<tr>
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<td>22.4 ± 0.5a</td>
<td>2.2 ± 0.1a</td>
<td>0.0 ± 0.0a</td>
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</tr>
<tr>
<td>Casein</td>
<td>0.2</td>
<td>5</td>
<td>21.4 ± 0.7a</td>
<td>2.1 ± 0.1a</td>
<td>0.91 ± 0.04ad</td>
<td></td>
</tr>
<tr>
<td>SPI</td>
<td>0.2</td>
<td>6</td>
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<td>2.4 ± 0.2a</td>
<td>0.68 ± 0.07aee</td>
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</tr>
<tr>
<td>Casein</td>
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<td>8</td>
<td>22.2 ± 0.7a</td>
<td>2.1 ± 0.3a</td>
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</tr>
<tr>
<td>SPI</td>
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<td>7</td>
<td>22.0 ± 0.6a</td>
<td>2.3 ± 0.1a</td>
<td>4.88 ± 0.21c</td>
<td></td>
</tr>
</tbody>
</table>

1 Values are means ± SEM. Within a column, means not having a common superscript letter are significantly different, P < 0.05.

FIGURE 3  Effects of dietary treatment on the growth of LNCaP tumors in SCID mice. SCID mice were inoculated subcutaneously with 2 × 10⁶ LNCaP cells isolated from LNCaP tumors grown in host SCID mice, randomized and fed with free access one of the six experimental diets (n = 8). The experiment was terminated when mean tumor volume of control group reached 2.3 ± 0.3 cm³. The final tumor volumes from mice treated with diets containing 20% of SPI, 0.2% of SPC, 20% of SPI with 0.2% of SPC, 1.0% of SPC, and 20% of SPI with 1.0% of SPC were reduced by 11% (P < 0.05), 19% (P = 0.17), 28% (P < 0.05), 30% (P < 0.05) or 40% (P < 0.005), respectively, compared with those of casein-fed control mice. Data represent mean tumor volumes. SEM range from 0.22 to 0.35 at d 21.
inhibited the growth of LNCaP tumor in mice associated with reduced proliferation, enhanced tumor cell apoptosis and reduced tumor angiogenesis. These observations were supported by in vitro studies showing that soy isoflavones or soy phytochemicals inhibited LNCaP cell growth, blocked cell cycle progression at G2-M phases and enhanced DNA fragmentation, a marker for apoptosis. Our studies provide evidence that dietary soy phytochemical-containing soybean products should be developed further as agents for the prevention and treatment of prostate cancer.

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