Lack of stimulatory activity of a Phytoestrogen-containing soy extract on the growth of breast cancer tumors in mice

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The present study was designed to investigate the effects of a phytoestrogens-containing soy extract (SOYSELECT®, SSE) on the growth of estrogen-dependent (MCF-7) and estrogen-unresponsive (MDA-MB-231) human breast cancer xenografts in athymic mice. Results obtained provided evidence that MCF-7 tumors did not grow over the treatment period (5 weeks) in ovariectomized females receiving 50 or 100 mg/kg/day SSE (oral route); administration of SSE also did not affect the estradiol-sustained growth of MCF-7 tumors in mice. Similarly, no effects on tumor growth were observed in SSE-treated mice bearing MDA-MB-231 xenografts. Data from pS2, progesterone receptor and cyclin D1 mRNA expression in tumors showed that, although SSE was able to induce a moderate estrogenic effect in MCF-7 cells, it did not increase cellular proliferation and tumor growth, in our experimental conditions. Besides, when used in association with 17β-estradiol, it displayed antiestrogenic activity. The expression of other genes involved in tumor progression and angiogenesis, such as Thrombospondin 1, Trasforming Growth Factor β2 and Kallikrein 6 was also evaluated in tumor samples, results showing a decrease in mRNA expression upon SSE treatment. The effect of SSE on angiogenesis in vivo was also evaluated in the Matrigel plug assay; results obtained showed a striking anti-angiogenic activity in mice receiving 100 mg/kg/day SSE, thereby confirming that this extract may interfere with angiogenesis. Collectively, these experimental data suggest that SSE could be not harmful for women with a history of or at high risk for breast cancer, at least for short treatment periods; however, further studies are needed to thoroughly characterize the activity profile of the extract in this specific setting of patients.

Introduction

Worldwide, breast cancer is the most frequent cancer in women (http://www-dep.iarc.fr/; 1). It has estimated that in the year 2002 there were more than one million new breast cancer cases all over the world, with the highest incidence rates observed in North America, Australia, and North and Western Europe, and the lowest risk observed in Asia and Africa (1).

Abbreviations: KLK6, Kallikrein 6; PR, progesterone receptor; TSP, Thrombospondin 1; TGFβ2, Trasforming Growth Factor β2.

Differences in disease incidence have been largely attributed to environmental factors, and, in particular, it has been suggested that there could be a relationship between the eating habits of certain population and a reduced risk of developing this cancer. Asian diet is high in soy and several researchers have suggested that it may exert a protective action on the development of breast cancer, even though other diet features, such as a much lower content of saturated fats and a higher amount of fiber may have a role in reducing cancer risk. Although soy contains a number of putative biologically active compounds that may be relevant in breast cancer (2), initial excitement over the hypothesized anticancer effects of this legume was based largely on the possibility that isoflavones might modulate estrogen action on breast tissue. Besides, consumption of soy-derived products has been encouraged for post-menopausal women as a natural alternative to hormone replacement therapy (HRT), with many women embracing soy products, isoflavone supplements and foods to which isoflavones have been added. One group of women most in need of and who frequently use alternatives consists of breast cancer patients (3,4). Actually, the belief in dietary soy as an HRT alternative is generally stronger among women with a personal history of breast cancer, who are six times as likely to use dietary soy for the relief of menopause symptoms, as are other women (5).

In contrast to correlations of soy consumption and breast cancer development, it has been suggested that exposure of pre-existing estrogen-dependent tumors to estrogenic effects of phytoestrogens may have a negative outcome, by stimulating growth. The estrogenic effects of isoflavones observed in some experimental systems have recently led to considerable controversy among health professionals over the use of soy by breast cancer patients and to confusion among survivors (6). Consequently, it is important to understand whether soy and/or isoflavones do pose a risk for breast cancer patients, and how the consumption of soy supplements may affect a pre-existing breast tumor.

The present study was designed to investigate the effects of a standardized soy extract on the growth of estrogen-dependent (MCF-7) and estrogen-unresponsive (MDA-MB-231) human breast cancer xenografts in athymic mice. The modulation of specific processes governing tumor growth was evaluated as well, in an attempt to clarify the role played by soy products in breast cancer development. Notably, dose selection was based on previous results on this standardized extract, showing that administration of 100 mg/kg/day SSE to ovariectomized rats was effective in preventing experimental osteoporosis without stimulating proliferation in uterus and mammary gland (7,8). Additionally, the treatment schedule was intended to reproduce isoflavones plasma levels comparable to those found in Japanese population on a traditional soy diet (9), and to concentrations detected in a previous clinical trial in menopausal women receiving the tested extract (10).
Materials and methods

Cell lines

MCF-7 and MDA-MB-231 cells were purchased from the European Collection of Cell Cultures (ECACC, Salisbury, UK). According to ECACC suggestions, cells were grown in DMEM supplemented with 10% fetal bovine serum, 1% non-essential amino acids mixture, 1% Kanamycin. Cells, propagated as a monolayer culture, were trypsinized twice weekly and plated at a density of $1 \times 10^5$ cells/ml. All cultures were incubated at 37°C, under 5% CO$_2$ in a high humidity atmosphere. On the day of dosing, cells were trypsinized and a suspension of $8 \times 10^5$ cells was injected subcutaneously in the right flank of each animal (0.2 ml per mice).

Animals

Female athymic mice (HSD: Athymic Nude-nu), 5-6 weeks old and within a weight range of $\sim 18-22$ g, were obtained from Harlan Nossan S.r.l. Correzzana (MI), Italy. Animals were housed in a purpose-built facility with a controlled environment and maintained in an isolator in which control was set to keep temperature and relative humidity at 26 ± 2°C and 50%, respectively. Artificial lighting provided a 24 h cycle of 12 h light and 12 h dark. Sterile water and food were supplied ad libitum during the study. A phytoestrogen-free, semi-purified diet was used (Harlan, Italy). The diet contained the following ingredients: wheat starch, casein, powdered sucrose, dextrin, cellulose, corn oil, sodium chloride, calcium carbonate, magnesium oxide, potassium chloride, DL-methionine, mineral and vitamin premix (proximate analysis: protein 19%, fat 4%, fiber 6%, carbohydrate 53.5% and ash 5.5%). Procedures and facilities followed the requirements of Commission Directive 86/609/EEC concerning the protection of animals used for experimental and other scientific purposes. Italian legislation is defined in the Decreto Legislativo No. 116 of January 27, 1992. The project was approved by the local ethics committee.

Human tumor xenograft growth

SOYSELECT™ (SSE, Indena spa, Milan, Italy) is a standardized extract from soy with a double standardization (13-17% of isoflavone glycosides genistin and daidzin, and <18% of B-group saponins, by HPLC). The product is prepared by extracting ripe whole soy beans or oil-free soy flour with aliphatic alcohols, through an industrial manufacturing proprietary process (patents US 6 280 777 and US 6 607 757). One gram of extract also contains 0.058 g of protein, 0.035 g of fat and 0.023 g of ash, with the remaining matter undefined. The batch used in the study contained 16.8% isoflavone glycosides and 22.3% B-group konin.

For MCF-7 protocol, 1 week after arrival, a total of 60 mice were ovariectomized and allowed a week recovery. There were six experimental groups in the study, each consisting of 10 mice. Groups 2 and 3 received SSE at 50 and 100 mg/kg/day, respectively, by oral gavage, five consecutive days per week, until the end of the study. Groups 4–6 were implanted subcutaneously with 60-day release, 0.18 mg 17b-estradiol-pellets (17b-E2) (Innovative Research of America, Sarasota, FL) 1 week after ovariectomy; these pellets are designed to produce 80–100 pg/ml of serum estradiol (as indicated by the supplier). Mice in groups 5 and 6 also received SSE at the same doses and schedule treatment specified above; group 1 was used as a control and received the vehicle (i.e. sterile water p.o.). Treatments started 1 week after ovariectomy (i.e. the day before tumor inoculation).

For the MDA-MB-231 protocol, a total of three experimental groups was used, each consisting of 10 mice; treatment (vehicle, SSE 50 or SSE 100 mg/kg/day) started the day of cell inoculation, at the same schedule treatment indicated above. During the study, mice were checked daily for any adverse clinical reactions. Tumor dimensions were measured with a caliper three times per week. Solid tumor weight was estimated from two-dimensional measurements (mm): Tumor weight = length $\times$ width$^2$/2 (11). Body weight was also recorded three times per week; food intake was determined over several 24 h periods randomly throughout the study.

At the end of the study animals were killed by carbon dioxide; uteri were rapidly removed, free of fat and weighted. All tumors were also removed, immediately placed in liquid nitrogen and stored at −80°C for subsequent analysis.

Angiogenesis assay

The method described by Passantini et al. (12) was used, with some modifications. Briefly, basic Fibroblast Growth Factor (bFGF, 300 ng/ml) was incorporated into liquid Matrigel (12.5 mg/ml) and a plug of 0.45 ml Matrigel was injected subcutaneously into the ventral abdominal wall of each animal. Mice (10 per group) received SSE (50 or 100 mg/kg o.s.) daily for 6 days from the day of Matrigel injection. Control mice received the same volume of vehicle. At day 7, the pellet was removed, and the hemoglobin content was measured by the Drabkin’s procedure (Drabkin reagent kit; Sigma Chemical Co., St Louis, MO). Matrigel and bFGF were obtained from Becton Dickinson (Bedford, MA).

Real-time quantitative PCR

Six mRNA targets were selected to evaluate the ability of SSE to modulate these targets. Analysis was done on P25, progestrone receptor (PR), cyclin D1, Thrombospondin 1 (TSP), Trasforming Growth Factor β2 (TGFβ2) and Kallikrein 6 (KLK6). Analyses were carried out on MCF-7 tumors excised at the end of the study. A real-time quantitative RT–PCR was performed using the iCycler iQ system (Bio-Rad, Hercules, CA). cDNA was prepared starting from 1 μg of total RNA using the iScript cDNA Synthesis Kit, according to the manufacture’s instructions. For those groups not receiving 17b-estradiol supplementation, the yield of RNA extraction from xenografts was low and, for this reason, RNA was pooled for each group of animals; specifically, after extraction, equal amounts of RNA were taken from each tumor and pooled per experimental group. To normalize gene expression data, we tried a panel of housekeeping genes, including GAPDH, tubulin, RNA polymerase II and 18S: none of these genes resulted equally expressed among samples in treatment groups, with considerable differences of expression, and thus preventing an inter-group data normalization. Actually, the expression of housekeeping genes was comparable only within two different sets of data, i.e. tumors with or without 17b-estradiol supplementation. It was therefore decided to analyze the data by independently normalizing the two sets for GAPDH expression; the effects of SSE on gene expression were thus evaluated as SSE-treatment versus non-treatment ratio, separately in the two sets of data (i.e. with or without concomitant 17b-estradiol supplementation). Each analysis was repeated in triplicate, at least three times for each condition, and results were then averaged. Amplifications were carried out using the primers reported in Table I. To each primer, iQ SYBR Green Supermix (Bio-Rad) was used in a final volume of 25 μl, starting with a 3 min template denaturation step at 95°C, followed by 40 cycles of 15 s at 95°C, and 1 min at 60°C. Standard curves were generated using a serial dilution of the initial amount of control cDNA to determine the range of template concentrations, which showed a good linearity and efficiency for the different reactions. Melt curves of the reaction products were also generated to assess the specificity of the measured fluorescence. The mean of threshold cycles ($C_t$) for each specimen was used to obtain the fold change expression level applying the following equation:

$\text{Fold change} = 2^{-\Delta C_{\text{t}}}$, where $\Delta C_{\text{t}} = C_{\text{t}}$ specific gene $- C_{\text{t}}$ GAPDH and $\Delta C = C_{\text{t}}$ specimen $- C_{\text{t}}$ control. A fold change equal to 1 represents a sample with an expression level equal to the control cell line. This operation was done using the Excel spreadsheet RelQuant (Bio-Rad).

Statistics

All the data were analyzed for homogeneity of variance using Bartlett’s test. If the group variance appeared homogeneous, a parametric ANOVA was used, followed by Dunnett’s multiple comparison test. If the variances were heterogeneous, log or reciprocal transformations were made in an attempt to stabilize the variances. If the variances remained heterogeneous non-parametric tests such as the Kruskall–Wallis test, followed by Steel multiple comparison test, were used. P-values were considered to be significant when <0.05. Statistical analysis was achieved using the Kypplot freeware package (Kyens Lab. Inc., Tokyo, Japan).

Table I. Primers utilized for real time PCR analysis

<table>
<thead>
<tr>
<th>Genes</th>
<th>Primer sequence</th>
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<tbody>
<tr>
<td>Cyclin D1 Forward</td>
<td>CCGAAGCAGACGACGCAAGCAAGA</td>
</tr>
<tr>
<td>Cyclin D1 Reverse</td>
<td>GATCCGGAGAGAGAGAGAGAGAAG</td>
</tr>
<tr>
<td>pS2 Forward</td>
<td>GTCTCTGCAAACTCAGACGCCC</td>
</tr>
<tr>
<td>pS2 Reverse</td>
<td>GCTTGGATGCTTGGAGAG</td>
</tr>
<tr>
<td>PR Forward</td>
<td>TCTGGTGTTTGGCAGTCCT</td>
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<tr>
<td>PR Reverse</td>
<td>CTTGCTTTTGGGTCGCGTTT</td>
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<tr>
<td>KLK6 Forward</td>
<td>CGCAGGCTCGAGCAGCGAAC</td>
</tr>
<tr>
<td>KLK6 Reverse</td>
<td>CTTGAGGCAGCCAGCAG</td>
</tr>
<tr>
<td>TGFβ2 Forward</td>
<td>CGGAGGAGGAGGAGCAGAGAAG</td>
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<tr>
<td>TGFβ2 Reverse</td>
<td>GTTGGTTAAGGCTGCGGG</td>
</tr>
<tr>
<td>TSP Forward</td>
<td>GAGAACCTGCAGGCGGATG</td>
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<td>TSP Reverse</td>
<td>ATCCGGCGGAAAATCCGTTCC</td>
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Results

Effect of SSE on the growth of MCF-7 in ovariectomized athymic female mice

Ovariectomized athymic mice implanted with MCF-7 cells were divided into six treatment groups. As expected, MCF7 xenografts did not form proliferating tumors in ovariectomized hosts without estrogenic supplementation (negative controls) (Figure 1). No growth was also observed in tumor-bearing mice treated with 50 or 100 mg/kg/day SSE (daily, weekend off, for a total of 5 weeks). As expected, tumors in the positive control group (17β-estradiol) grew rapidly, statistical analysis showing significant differences when compared with negative controls (P < 0.01). Treatment with 50 or 100 mg/kg/day SSE did not affect the development of 17β-estradiol-sustained tumors, growth curves being significantly different from the negative control group (P < 0.01), but not from the positive one.

A reduction in body weight gain was observed in all groups receiving 17β-estradiol when compared with negative control, though the statistical significance was achieved only for mice upon 17β-estradiol treatment alone (P < 0.05) (Figure 2). In the absence of estrogenic supplementation, no effect on body weight was noticed in SSE-treated animals (low and high dose). A moderate reduction in food consumption was recorded in mice upon 17β-estradiol treatment alone when compared with vehicle controls (2.3 ± 0.2 versus 2.9 ± 0.7 g/mouse/day, respectively); food consumption in animal receiving 50 or 100 mg/kg/day SSE were 3.3 ± 0.1 and 3.2 ± 0.1 g/mouse/day, respectively; finally, values in groups receiving 17β-estradiol and SSE in association were 2.8 ± 0.2 and 2.9 ± 0.3, for the low and the high-dose groups, respectively.

Effect of SSE on the growth of MDA-MB-231 in athymic female mice

No differences in tumor growth were observed in animals receiving 50 or 100 mg/kg/day SSE when compared with vehicle controls (Figure 3). In addition, test substance administration did not affect body weight of treated mice (data not shown).

Regulation of the mRNA expression by SSE treatment

To evaluate the ability of SSE to modulate mRNA expression of selected genes, we conducted quantitative RT–PCR analysis using mRNA isolated from MCF-7 tumors in negative controls, 100 mg/kg/day SSE-treated mice, positive controls (17β-estradiol) and mice receiving both treatments. Specifically, the following genes were evaluated: pS2, PR, cyclin D1, TGFβ2, TSP and KLK6. Results obtained are shown in Figure 4; results are expressed as ratio of gene expression in SSE-treated over non-treated tumors, calculated after independent normalization in two different sets of data (i.e. with or without concomitant 17β-estradiol supplementation). In the absence of 17β-estradiol supplementation, the expression of pS2 and PR was significantly higher in SSE treated animals (P < 0.05) compared with tumors from negative controls; conversely, in 17β-estradiol-supplemented tumors, SSE induced a statistically significant down-regulation of both genes (P < 0.01 versus positive control). The expression of both Cyclin
D1 and KLK6 was seen to be reduced by SSE without ($P < 0.05$ and $P < 0.01$ for Cyclin D1 and KLK6, respectively) or with $17\beta$-estradiol supplementation ($P < 0.01$ for both Cyclin D1 and KLK6), where the effect was more consistent in the degree of inhibition. On the other hand, a comparable modulation of TGFb2 expression in tumor samples was seen either in the presence or in the absence of $17\beta$-estradiol ($P < 0.05$ in both cases). Finally, $17\beta$-estradiol-supplemented tumors from animals receiving SSE had significantly reduced levels of TSP expression with $17\beta$-estradiol supplementation ($P < 0.01$); in the absence of estrogene supplementation, no significant changes were observed in SSE-treated tumors with respect to negative controls.

**Effects of SSE on uterus weight in ovariectomized athymic mice**

At both doses tested, SSE did not induce any significant change in the relative uterine weight (mg/g body weight) compared with negative controls ovariectomized mice (Figure 5). On the other hand, treatment with $17\beta$-estradiol, 0.18 mg/pellet, significantly increased the relative uterus weight ($P < 0.05$ versus controls); similar changes were observed in mice receiving $17\beta$-estradiol and SSE in association ($P < 0.01$ versus negative controls, at both dosages).

**The effect of SSE on angiogenesis in vivo**

The effect of SSE on angiogenesis in vivo was evaluated in the Matrigel plug assay, where angiogenesis is induced by bFGF embedded in a pellet of Matrigel implanted subcutaneously in athymic mice. Daily oral doses of 100 mg/kg SSE significantly reduced the angiogenic response induced by bFGF (Figure 6). The mean hemoglobin content of bFGF-containing pellets was significantly lower ($P < 0.05$) in SSE-treated mice (0.05 ± 0.02 g/dl, mean ± SD) than in vehicle-treated mice (0.11 ± 0.05 g/dl, mean ± SD). No significant effects were observed at 50 mg/kg/day SSE (0.09 ± 0.03 g/dl, mean ± SD).

**Discussion**

The present study provided evidence that MCF-7 tumors did not grow over the study period in animals receiving 50 or 100 mg/kg/day of SSE, treatment also not affecting the $17\beta$-estradiol-sustained growth of MCF-7. Similarly, no significant effects on tumor growth were observed in MDA-MB-231-bearing mice receiving both doses of SSE.

Conflicting data exist on the effects of soy-based products or pure phytoestrogens on breast tumor progression [reviewed in (6)]. In *in vitro* systems a biphasic effect of genistein on the growth of MCF-7 cells (i.e. stimulation at low and cytotoxicity at high concentrations) has been consistently observed (13–16), while, in *in vivo* studies, where high isoflavone plasma levels...
are not usually achieved, stimulatory effects on MCF-7 tumors have been generally reported (14,17–19). In particular, these latter studies from Helferich’s laboratory showed that dietary treatment with genistein/genistin dose dependently enhanced the growth of MCF-7 tumors *in vivo*, increasing cellular proliferation and inducing pS2 gene expression; it is worthy to note, however, that at low dietary genistein levels (i.e. ≤125 μg/g diet as aglycone) stimulation of tumor growth was not observed (17,18). Isoflavone dosages achieved in the present experiment were ~8 and 17 mg/kg mouse/day, as glycoside (low- and high-dose group, respectively); thus, exposure levels were in the range of those found inactive in the above reported studies (if extrapolating dosages from dietary isoflavone concentrations) (17,18). In a later study by Allred et al. (20), not only the dosage, but also the degree of soy processing was seen to be a critical factor, affecting the ultimate impact of phytoestrogens-containing products on the growth of estrogen-dependent breast tumors. Results from this study showed that even a same dosage of genistein (750 p.p.m., showed previously to enhance growth), administered in different soy products or as a pure compound, produced different effects in tumor-bearing mice; specifically, a different modulation was observed not only in MCF-7 growth (with activity ranging from lack to significant stimulation), but also in the expression of target genes. On the other hand, Shao et al. (21) demonstrated that subcutaneous injection of pure genistein (0.1–0.5 mg/kg every other day for 2 weeks) inhibited the estradiol-sustained growth of MCF-7 in nude mice, stimulating apoptosis and increasing p21WAF1/CIP1 expression; they also reported similar effects in nude mouse xenografts of MDA-MB-231.

Collectively, our results are barely comparable with the above mentioned literature data when considering that different products with different schedule treatment were tested, and it is generally accepted that the type, combination and doses of isoflavones are critical factors, likely modifiers of the different biological effects. In particular, as pointed out recently by Jeffery (22), the evaluation of specific isoflavones alone (e.g. genistein), a combination of isoflavones, or isoflavones together with saponins or other components within soy protein, may provide very different patterns of activity and, in this context, the pharmaco-toxicological characterization of each single product appears essentially. Finally, also the duration of treatment could be crucial in determining the stimulatory effects of soy or pure genistein on estrogen-dependent breast cancer, as shown by previous preclinical studies (17,20) and, in the light of this, the activity profile of SSE following prolonged administration deserves further investigations.

It is worth of note that the findings observed in the present study occurred at physiological plasma levels of phytoestrogens. In fact, a previous pilot study carried out in our laboratory showed that following administration of 50 and 100 mg/kg/day SSE to healthy athymic mice, plasma concentrations of total daidzein and genistein were as follows: total daidzein 1.1 ± 0.3 and 1.6 ± 0.7 μM, total genistein 0.8 ± 0.1 and 0.9 ± 0.2 μM (mean ± SD), for the low and the high-dose groups, respectively (D. Gallo, unpublished data). Actually, these isoflavones blood levels are comparable with those found in Japanese women on a traditional soy diet (9), and to concentrations detected in a previous clinical trial in menopausal women receiving the tested extract (10). However, although isoflavones have been increasingly regarded as the biologically active compounds relevant in breast cancer, it is also possible that other components in the extract (e.g. soysaponins) could be responsible for or could contribute to the observed effects.

In order to better understand the specific molecular mechanisms by which the extract could exert its effect on MCF-7 tumors, a number of genes were identified and used to evaluate SSE (high dose) for its ability to produce estrogenic effects or to influence tumor suppression or progression. Collectively, our data showed that, in the absence of estrogenic supplementation, SSE was able to induce a moderate estrogenic effect in MCF-7 tumors, by enhancing relative levels of pS2 and PR expression, but it did not increase cellular proliferation (cyclin D1) and tumor growth. In addition, when used in association with 17β-estradiol, it displayed anti-estrogenic activity, as consistently indicated by the reduction of pS2, PR and cyclin D1 mRNA expression, all of them being markers of estrogenic activity in MCF-7 cells (23–25). Li and Sarkar (26) identified recently, by cDNA microarray and RT–PCR analysis, a number of invasion and angiogenesis-related genes that are down-regulated in PC3 prostate cancer cells by genistein; among these, a significant decrease in the mRNA expression of TSP, TGFβ2 and KLK6 was found. In keeping with these findings, our results showed that SSE down-regulated the mRNA expression of both TSP and KLK6 (two genes showed recently to be estrogen-responsive) (27,28), with a more pronounced effect in 17β-estradiol-supplemented tumors; the effect on TGFβ2 was instead independent on 17β-estradiol. Considering the complex role played by TSP (29), TGFβ signaling (30,31) and tissue kallikreins (28) in tumor suppression and progression, as well as in angiogenesis, invasion and metastasis, which are context and stage-dependent, any attempt to draw conclusion from the changes observed is fraught with difficulties; further in depth studies are thus required to investigate the influence of this soy extract on the regulation of important cellular molecules at the protein levels and to elucidate any interaction with specific cellular functions. These findings, however, prompted us to investigate whether SSE could be able to modulate angiogenesis in a well-established pre-clinical *in vivo* model. Results obtained showed a striking anti-angiogenic activity in mice receiving 100 mg/kg/day SSE, thereby confirming that this extract may interfere with angiogenesis, and suggesting that this activity may contribute to the chemopreventive potential of SSE, reported previously in our laboratory (32). Notably, other authors also showed inhibition of angiogenesis *in vivo* at relatively low genistein dosages (21), although previous *in vitro* studies demonstrated that inhibition of angiogenesis occurs at higher concentrations (33); collectively, results from these studies suggest that the ability of soy products to modulate tumor angiogenesis could be more profound *in vivo* than *in vitro*, this possibly owing to profound differences between conditions employed in the *in vitro* studies and complex and severe *in vivo* conditions in the tumor microenvironment. Finally, the contribution of other compounds (e.g. daidzein, equol) can not be ruled out.

In conclusion, using well-established pre-clinical cancer models we have demonstrated that consumption of SSE did not have deleterious effects on the growth of estrogen-dependent (MCF-7) and estrogen-unresponsive (MDA-MB-231) human breast cancer xenografts. Analysis of mRNA expression of selected genes in MCF-7 tumors also indicated that, although endowed with a weak estrogenic activity, SSE did not enhance cellular proliferation, and when used in combination with 17β-estradiol it could exert a favorable modulation of specific processes.
governing tumor growth. Importantly, no increases in uterine weight were recorded in ovariectomized treated mice; this latter finding is in keeping with our previous data in ovariectomized rats, showing that administration of SSE (at the same dosages used in the present study) did not cause stimulation in uterus and mammary gland at doses that were effective in preventing experimental osteoporosis (7,8). Collectively, these experimental data suggest that SSE could be not harmful for women with a history of or at high risk for breast cancer, at least for short treatment periods; however, also considering the existing literature data, further studies are warranted to thoroughly characterize the activity profile of the extract, particularly in terms of safety of use in this specific setting of patients. Notably, SSE has already been tested in clinical studies and proved to be a safe and efficacious therapy for relief of vasomotor symptoms (10,34) and to have favorable effects on cognitive function, particularly verbal memory, in postmenopausal women (35).

References


