Effects of a phytoestrogen-containing soy extract on the growth-inhibitory activity of ICI 182 780 in an experimental model of estrogen-dependent breast cancer

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Abstract

The study reported here was designed to determine whether a phytoestrogen-containing soy extract (SSE) could negate/overwhelm the inhibitory effects of ICI 182 780 on the growth of estrogen-sustained human breast cancer xenografts (MCF-7), in ovariectomized athymic mice. As expected, estradiol-supplemented tumors did not grow over the study period in ICI 182 780-treated females; concomitant administration of 50 mg/kg per day SSE slightly potentiated the inhibitory activity of the drug, while at 100 mg/kg per day, SSE partially negated ICI 182 780 activity. In keeping with these *in vivo* outcomes, we observed that the level of cyclin D1 (and progesterone receptor) in MCF-7 xenografts was considerably reduced by ICI 182 780, an effect enhanced by concomitant treatment with 50 SSE, but reduced by the higher dosage (i.e. 100 mg/kg per day). Thrombospondin-1 (TSP-1) and kallikrein 6 (KLK6) levels were also reduced following ICI 182 780, although to a lesser degree; again, combined anti-estrogen and SSE produced a dose-dependent regulation in TSP-1 and KLK6 tumor level, with a further reduction in the mRNA gene expression at 50 SSE (compared with ICI 182 780) and a partial reversion of the drug-induced down-regulation at 100 mg/kg per day. No modulation was detected in the serum concentration of IGF-1 (a potent mitogen for estrogen receptor-positive breast cancer cell lines) either upon treatment with ICI 182 780 or concomitant administration of the anti-estrogen with SSE. In conclusion, results from this study raise concerns about the consumption of isoflavone supplements in conjunction with ICI 182 780 therapy, in postmenopausal women with estrogen-dependent breast cancer.

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Introduction

Breast cancer is a major Public Health issue worldwide and among women aged 20–59 years it represents the leading cause of cancer death in the United States (Jemal *et al.* 2006). In postmenopausal women, endocrine therapy is a widely accepted treatment modality for hormone receptor-positive breast cancer, with endocrine agents designed to inhibit the synthesis of estrogen or to block the activity of estrogen at the estrogen receptor (ER; Howell 2005). Tamoxifen, a

selective ER modulator was the first endocrine agent to become widely available; in women with ER-positive breast cancer, 5 years of adjuvant tamoxifen have been shown to reduce disease recurrence rates by almost half and decrease mortality by a third (Early Breast Cancer Trialists' Collaborative Group 2005). However, this drug has been associated with increased risks of potentially life-threatening events, such as endometrial cancer (Bergman *et al.* 2000), uterine sarcoma (Wickerham *et al.* 2002), and thromboembolic events (Decensi *et al.* 2005). These adverse effects have been

largely attributed to the partial estrogen agonist activity of tamoxifen.

Over recent years, as the mechanisms underlying the responses to hormonal interventions were elucidated, a number of new endocrine agents have been developed, including aromatase inhibitors (AIs) and anti-estrogens without partial agonist activity. AIs, which block the enzyme aromatase that catalyses the conversion of androgens into estrogens, are replacing tamoxifen as standard treatment for early and advanced breast cancer. The ER antagonist ICI 182 780 (fulvestrant), which unlike tamoxifen has no estrogen agonist activity (Wakeling *et al.* 1991), is also starting to challenge standard treatments. Fulvestrant (Faslodex) is currently approved in both the United States and Europe for the treatment of hormone receptor-positive metastatic breast cancer in postmenopausal women with disease progression following anti-estrogen therapy. Fulvestrant has a unique mode of action: it binds, blocks, and degrades the ER and consequently lacks cross-resistance with other endocrine agents (Wakeling *et al.* 1991, Dauvois *et al.* 1993, McClelland *et al.* 1996). Hot flushes represent the most common adverse events observed during treatment with the drug.

Soy isoflavones are a class of estrogen-like compounds that have become widely used among postmenopausal women as a 'natural' alternative to hormone replacement therapy (Duncan *et al.* 2003). Since the use of isoflavone supplements to relieve menopausal symptoms (and to enhance general well-being) is more frequent among breast cancer patients (Newton *et al.* 2002), their use in this specific setting of patients is emerging as an area of increasing interest, relevant to the safety of isoflavones (Messina & Loprini 2001, Messina *et al.* 2006). Much of the concern arises from studies of ovariectomized, athymic mice that have demonstrated the ability of genistein and soy protein to stimulate the growth of breast cancer cells in a dose-dependent manner (Allred *et al.* 2001, Ju *et al.* 2001), as well as the ability of genistein to antagonize the inhibitory effects of tamoxifen on tumor growth (Ju *et al.* 2002). However, Ju *et al.* (2006) recently reported that dietary daidzein only slightly stimulated the *in vivo* growth of estrogen-dependent human breast tumor (MCF-7), while ($\pm$)-equol did not induce any stimulatory activity. Overall findings from these and our previous study (Gallo *et al.* 2006a) support the concept that the type, combination, and doses of isoflavones are critical factors and are likely modifiers of the different biological effects observed.

The present study was designed to investigate the potential interaction between a phytoestrogen-containing soy extract (SSE) and the anti-estrogen ICI 182 780 in a well established preclinical model of estrogen-dependent

breast cancer. The primary endpoint was tumor proliferation as determined by tumor growth curves; secondary endpoints included markers of ER expression and activation (i.e. Cyclin D1, progesterone receptor, PR) as well as regulation of molecules related to angiogenesis and cell cycle control (i.e. thrombospondin-1, TSP-1 and kallikrein 6, KLK6). Finally, since there is considerable evidence for a crosstalk between the insulin-like growth factor-1 (IGF-I) and estrogen signaling pathways in the regulation of breast cancer cell proliferation, IGF-I level were measured in serum samples from all animals.

Materials and methods

Cell line

MCF-7 cells were purchased from the European Collection of Cell Cultures (ECACC, Salisbury, UK). According to ECACC suggestions, cells were grown in Dulbecco's modified eagle medium supplemented with 10% fetal bovine serum, 1% non-essential amino acids mixture, and 1% kanamycin. Cells, propagated as a monolayer culture, were trypsinized twice weekly and plated at a density of 1×10^5 cells/ml. All cultures were incubated at 37 °C, under 5% CO₂, in a high humidity atmosphere. On the day of dosing, cells were trypsinized and a suspension of 8×10^6 cells was injected subcutaneously in the right flank of each animal (0.2 ml/mice).

Animals

Female athymic mice (homozygous Athymic Nude-nu), 5-weeks old and within a weight range of ~18–22 g, were obtained from Charles River (Calco, Lecco, 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:12 h dark. Sterile water and food were supplied *ad libitum* during the study. A phytoestrogen-free semi-purified diet was used (Harlan, Carrezzana, Milano, Italy). The diet contained the following ingredients: wheat starch (42%), casein (20%), dextrin (13%), powdered sucrose (10%), cellulose (6%), corn oil (4%), mineral and vitamin premix (3%), DL-methionine (0.45%), sodium chloride, calcium carbonate, magnesium oxide, and potassium chloride. Proximate analysis: protein 19%, fat 4%, fiber 6%, carbohydrate 53.5%, ash 5.5%, and moisture 12%. 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 27 January 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% isoflavone glycosides genistin and daidzin, and not <18% 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 protein, 0.035 g fat, and 0.023 g ash, with the remaining matter undefined. The batch used in the study contained 14.7% isoflavone glycosides and 21.2% B-group saponins. SSE was dissolved in sterile water at the concentrations required for dosing. ICI 182 780, purchased from Tocris (Bristol, UK), was dissolved in peanut oil.

One week after the arrival, a total of 40 mice were ovariectomized and allowed a week recovery. There were five experimental groups in the study, each consisting of eight mice. Groups 2 (referred to as 17 β -estradiol (17 β -E2) group), 3 (ICI 182 780 group), 4 (SSE 50 group), and 5 (SSE 100 group) were implanted subcutaneously with 60-day release, 0.18 mg 17 β -E2 pellets (Innovative Research of America, Sarasota, FL, USA) one week after ovariectomy (i.e. the day before tumor inoculation); these pellets are designed to produce 80–100 pg/ml of serum estradiol (as indicated by the supplier). Mice in groups 3, 4, and 5 also received ICI 182 780 via the s.c. route, at 5 mg/mouse, once a week for 4 weeks. Groups 4 and 5 also received SSE at 50 and 100 mg/kg per day respectively, by oral gavage, 5 consecutive days per week, until the end of the study (i.e. day 51). Group 1 was used as negative control (NC, i.e. no estrogen supplementation). Groups 1, 2, and 3 received the vehicle (sterile water) by oral gavage. Treatments started one day after tumor inoculation.

Dosages of SSE were selected on the basis of previous studies carried out in our laboratory (Gallo *et al.* 2005, 2006a,b); these dosages were seen to produce physiological plasma levels of isoflavones. In fact, a previous pilot study carried out in our laboratory showed that following administration of 50 and 100 mg/kg per 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 μ M, total genistein 0.8 ± 0.1 and 0.9 ± 0.2 μ M (mean $\pm$ s.d.), for the low- and high-dose groups respectively

(unpublished data). Actually, these isoflavone blood levels are in the range of those found in Japanese women on a traditional soy diet: daidzein mean concentrations, 246.8 nmol/l (range 0–2407); genistein mean concentrations, 501.9 nmol/l (range 0–4192; Morton *et al.* 2002). In addition, similar blood concentrations of isoflavones were detected in a previous clinical trial in menopausal women receiving the tested extract (Scambia *et al.* 2000). During the study, mice were checked daily for any adverse clinical reactions. Body weight was also recorded twice 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; blood was collected and serum frozen at -20°C for analysis; uteri were rapidly removed, free of fat and weighed. All tumors were also removed, immediately placed in liquid nitrogen and stored at -80°C for analysis.

Evaluation of anti-tumor activity

Tumor dimensions were measured twice per week using a caliper. The tumor weight was calculated from two-dimensional measurements (mm; Corbett *et al.* 1997): Tumor weight = length $\times$ width²/2. Differences in efficacy between treatment groups were expressed as the percentage of maximum tumor weight inhibition (TWI%). The ratio between the median tumor weights of treated tumors and that of control tumors $\times 100$ (T/C%) was assessed on each day of measurement and used to calculate the TWI%: TWI% = $100 - \text{T/C}\%$.

Real-time quantitative PCR

Four mRNA targets were selected to evaluate the ability of SSE to modulate these targets. Analysis was done on cyclin D1, PR, TSP-1, and KLK6. Analysis was 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). cDNA was prepared starting from 1 μ g total RNA using the iScript cDNA Synthesis Kit, according to the manufacture's instructions. For those groups receiving ICI 182 780 treatment, 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. RNA polymerase II was used to normalize gene expression data. Each analysis was repeated in triplicate, at least thrice for each condition, and results were then averaged. Amplifications were carried out using the primers reported in Table 1. To each primer, iQ SYBR

Table 1 Primers utilized for real-time PCR analysis

Genes	Primer sequence
<i>Cyclin D1</i> forward	CCATGCTGAAGGCGGAGGAG
<i>Cyclin D1</i> reverse	CCAGGTGGCGACGATCTTCC
<i>PR</i> forward	GGCTGGCATGGTCCTTGGAG
<i>PR</i> reverse	TCTGGCTTAGGGCTTGGCTTTC
<i>KLK6</i> forward	CGCCAGCCATGACCAGGAC
<i>KLK6</i> reverse	CTTGCCCCAGCCCAGGATG
<i>TSP-1</i> forward	GAAGGACTCTGACGGCGATGG
<i>TSP-1</i> reverse	ATCGGCGGAAATCGGTCTCAC

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 (Ct) for each specimen was used to obtain the fold change expression level applying the following equation: $\text{Fold change} = 2^{-\Delta(\Delta C_t)}$, where $\Delta C_t = C_t$ specific gene- C_t housekeeping gene and $\Delta(\Delta C_t) = \Delta C_t$ specimen - ΔC_t control. A fold change equal to 1 represents a sample with an expression level equal to the selected control condition (i.e. 17β-E2). This operation was done using the Excel spreadsheet RelQuant (Bio-Rad).

Determination of serum IGF-I.

Circulating levels of IGF-I were determined by RIA following the instructions provided by the manufacturer (product code IGF-R22, Mediagnost, Reutlingen, Germany).

Statistical analysis

The Steel–Dwass test was used to detect the statistical significance of the differences between treatments on tumor weight growth curves. Data from plasma IGF-I analysis and uterus weight were analyzed by one-way ANOVA followed by Dunnett’s or Tukey–Kramer multiple comparison test. *P*-values were considered to be significant when <0.05. Statistical analysis was performed using the Kyplot freeware package (Kyens Lab. Inc., Tokyo, Japan).

Results

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

Ovariectomized athymic mice implanted with MCF-7 cells were divided into five treatment groups. As expected, MCF-7 xenografts did not form proliferating tumors in the ovariectomized hosts without estrogenic supplementation (negative controls; Fig. 1). Tumors in the positive control group (17β-E2) grew rapidly, statistical analysis showing significant differences when compared with negative controls (*P*<0.01). Treatment with ICI 182 780 (5 mg/mouse, once weekly, for 4 weeks) significantly inhibited (*P*<0.01) the estradiol-sustained growth of MCF-7 xenografts in athymic mice. Concomitant administration of 50 mg/kg per day SSE slightly potentiated the growth inhibitory activity of ICI 182 780, being the group mean tumor weight consistently lower than ICI 182 780 group throughout the study; this difference, however, did not achieve statistical significance. Conversely, treatment with 100 mg/kg per day SSE partially negated ICI activity, growth curves being significantly different from both ICI 182 780- and SSE 50-treated group (*P*<0.01); the tumor growth remained, however, significantly lower when

Figure 1 Effect of treatments on MCF7-tumor growth in ovariectomized athymic mice (mean ± s.d., n=8 mice/group). One day before tumor inoculation, mice were implanted with 17β-estradiol pellet (17β-E2, 0.18 mg/pellet). Starting from the day after tumor cell implantation, mice were treated with 0 (positive control) or ICI 182 780 (5 mg/mouse, once weekly, 4 consecutive weeks); the SSE 50 and SSE 100 groups also received either 50 or 100 mg/kg per day SSE, by oral gavage, 5 consecutive days per week, until the end of the study. A negative control group (NC), without 17β-estradiol supplementation, was also included in the study. Groups with different letters are significantly different (*P*<0.01, by Steel–Dwass test).

compared with the positive control ($P<0.01$). At the end of the study, a TWI% of 93, 93, and 76% was calculated for groups 3 (ICI 182 780), 4 (SSE 50), and 5 (SSE 100) respectively. Body weight and food consumption did not significantly differ among treatment groups (data not shown).

Regulation of the mRNA expression by treatments

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 positive controls (17β-E2), ICI 182 780-treated mice, 50 and 100 mg/kg per day SSE-treated mice. Specifically, the following genes were evaluated: cyclin D1, PR, TSP-1, and KLK6. The analysis was performed through direct quantification of the expression of the selected genes versus the positive control (i.e. 17β-E2 group). Results obtained are shown in Table 2. In ICI 182 780-treated tumors, the expression of cyclin D1 (0.52) and PR (0.05) was considerably reduced; likewise, mRNA expressions of TSP-1 (0.6) and KLK6 (0.34) were down-regulated in this group, although to a lesser degree. These findings are in keeping with the ability of pure anti-estrogens to affect the expression of direct targets of 17β-E2. In SSE-treated mice, the modulation of the selected genes was seen to be dose-dependent. Specifically, at a low dosage (SSE 50) there was a tendency towards an increase of the anti-estrogen effect, with a further reduction in the mRNA expression of cyclin D1, PR, TSP-1, and KLK6 (compared with ICI 182 780); on the other hand, at the higher dosages (SSE 100), the enhancement of the anti-estrogen effect disappeared and, instead, there was a tendency towards increased expression of the 17β-E2 targets, particularly evident for TSP-1 and KLK6.

Table 2 Results of qRT-PCR analysis in tumor samples

	17β-E2 + ICI		
	17β-E2 + ICI 182 780	182 780 + SSE 50	17β-E2 + ICI 182 780 + SSE 100
CYCLIN D1	0.52	0.02	0.63
PR	0.05	0.03	0.07
TSP-1	0.60	0.10	1.13
KLK6	0.34	0.13	0.73

A value of 1 represents a sample with an expression level equal to 17β-estradiol group. Data were generated from a single animal experiment, with 8 tumors pooled for each group.

Effects of treatments on serum IGF-I levels

To explore the possible effects of treatments on IGF-I, we quantitated IGF-I concentrations in the serums derived from mice at the end of the study. Results obtained (Table 3) did not show any significant difference among treatment groups.

Effects of treatments on uterus weight in ovariectomized athymic mice

Treatment with 17β-E2, 0.18 mg/pellet, significantly increased the relative uterus weight (mg/g body weight) when compared with ovariectomized controls ($P<0.001$; Fig. 2). Administration of ICI 182 780 (5 mg/mouse, once weekly, for 4 weeks) significantly decreased uterus weight with respect to mice receiving 17β-E2 ($P<0.001$). At both doses tested, SSE did not induce any significant change in the relative uterine weight compared with ICI 182 780-treated mice: in each group, weight means were significantly lower than 17β-E2-treated mice and not significantly different from negative controls.

Discussion

The study reported here was designed to determine whether a phytoestrogen-containing soy extract could negate/overwhelm the inhibitory effects of ICI 182 780 on estradiol-dependent tumor growth *in vivo*. To address this important issue, we evaluated the interaction between SSE and the anti-estrogen on MCF-7 tumor growth. The estradiol implants produced in these mice plasma levels sufficient to stimulate MCF-7 tumor growth (Gallo *et al.* 2006a), and the ICI 182 780 dosage was selected on the basis of previous studies showing an antagonism on the stimulatory effect of 17β-E2 on MCF-7 proliferation (Osborne *et al.* 1995). As expected, tumor growth ceased in ICI 182 780-treated animals, but no significant involution was seen, an effect consistent with previous observation in other laboratories, where ICI 182 780-treatment failed to precipitate

Table 3 Effects of various treatments on IGF-1 serum levels

Treatment	IGF-1 (ng/ml; mean ± SD, n=8)
NC	206.1 ± 32.4 ^a
17β-E2	198.1 ± 45.8 ^a
17β-E2 + ICI 182 780	184.8 ± 58.7 ^a
17β-E2 + ICI 182 780 + SSE 50	198.1 ± 93.0 ^a
17β-E2 + ICI 182 780 + SSE 100	201.7 ± 73.0 ^a

Groups with different letters are significantly different (Tukey-Kramer test).

Figure 2 Effect of treatments on relative uterus weight (mg/g body weight) of ovariectomized athymic mice (mean $\pm$ s.d. $n=8$ mice/group). One day before tumor inoculation, mice were implanted with 17 β -estradiol pellet (17 β -E2, 0.18 mg/pellet). Starting from the day after tumor cell implantation, mice were treated with 0 (positive control) or ICI 182 780 (5 mg/mouse, once weekly, 4 consecutive weeks); the SSE 50 and SSE 100 groups also received either 50 or 100 mg/kg per day SSE, by oral gavage, five consecutive days per week, until the end of the study. A negative control group (NC), without 17 β -estradiol supplementation, was also included in the study. Groups with different letters are significantly different ($P<0.001$, by Tukey-Kramer test).

tumor regression (Wakeling *et al.* 1991). Concomitant administration of 50 mg/kg per day SSE slightly potentiated the inhibitory activity of the drug, while at 100 mg/kg per day, SSE partially negated its activity. It is worth to note that dosages of SSE used in the present study were previously seen to be devoid of proliferative activity in ovariectomized MCF-7-bearing mice, the extract also not affecting the estradiol-sustained growth of MCF-7 (Gallo *et al.* 2006a). Thus, although in analogous experimental conditions SSE was devoid of intrinsic stimulatory activity, it was able – at a certain concentration range – to modulate the inhibitory effect of ICI 182 780 on tumor growth. In keeping with these *in vivo* outcomes, we observed that the level of cyclin D1 (and PR) in MCF-7 xenografts was considerably reduced by ICI 182 780, an effect enhanced by concomitant treatment with 50 SSE, but reduced by the higher dosage (i.e. 100 mg/kg per day). TSP-1 and KLK6 levels were also reduced following ICI 182 780, although to a lesser degree; it has to be mentioned, however, that tumor samples were harvested $\sim$ 4 weeks after the last administration of the drug, and thus a significant regulation could have been no longer detectable. Again, combined ICI 182 780 and SSE produced a dose-dependent regulation in TSP-1 and KLK6 tumor level, with a further reduction in the mRNA genes expression at 50 SSE (compared with ICI 182 780) and a partially reversion of the anti-estrogen-induced down-regulation at 100 mg/kg per day. All together, these findings suggest that SSE induced, in our

experimental model, a biphasic effect that potentially occurs as a result of an interference in both estrogen-dependent and -independent pathways. Indeed, phytoestrogens, 17 β -E2 and ICI 182 780 seem to interact reciprocally via rather complex mechanisms; this is particularly true when considering that i) our previous study showed that 100 mg/kg per day SSE reduced the expression of cyclin D1, KLK6, and TSP-1, both in the absence and in the presence of estrogenic supplementation (Gallo *et al.* 2006a); and ii) in the current study no interactions were noticed at the uterus level, where the concomitant administration of ICI 182 780 and SSE did not modify the rate and extent of uterine involution elicited by the anti-estrogen. One possibility to explain findings observed in the present study could be that at a dosage of 100 mg/kg per day, SSE may, in some way, boost tumor cells to circumvent the imposed growth inhibition, by activating compensatory survival pathways that ultimately allow the development of drug resistance.

Data from a recent study showed that hKLK6 was specifically up-regulated upon estradiol treatment in MCF-7, as well as in other breast cancer cell lines (Paliouras & Diamandis 2007). Notably, findings from the present study confirm that the regulation of KLK6 expression is under the control of estradiol, since the ER antagonist ICI 182 780 down-regulated its expression in 17 β -E2-supplemented tumors. The discovery that kallikreins are able to hydrolyze a number of different substrates, taken together with the dysregulated expression of these proteins in breast, prostate, and ovarian cancer, raises the possibility that kallikreins could contribute to the invasiveness and/or progression of these cancers (Borgono & Diamandis 2004). Also TSP-1 production is under the control of estradiol: Ghosh *et al.* (2000) found an approximately fourfold up-regulation of expression in MCF-7 cells in response to 17 β -E2 treatment and, more recently, Buterin *et al.* (2006) reported that TSP-1 is up-regulated in an estrogen-dependent manner in T47D cells, an effect also elicited by genistein, although to a lesser degree. TSP-1 is a multi-functional protein that has received great attention due to observations which suggest that it reduces angiogenesis, leading to the inhibition of tumor growth (Ren *et al.* 2006). However, the role of TSP-1 in breast cancer is controversial, with studies showing that paradoxically, TSP-1 production by breast cancer cells is associated with tumor cell proliferation rather than regression (Wang *et al.* 1996). It has also been demonstrated that patients with breast, lung, colon, and gynecological malignancies have significantly elevated serum levels of TSP-1 compared with non-tumor bearing human controls (Esemuede *et al.* 2004).

IGF-1 and estrogens have been shown to be intimately linked to the progression of a number of human cancers, notably breast cancer (Yee & Lee 2000). Evidence for a cross-talk between the IGF-I and estrogen signaling pathways in the regulation of breast cancer cell proliferation have been recently reported, with studies showing an up- and a down-regulation of IGF-1 receptor expression following exposure of MCF-7 cells to 17 β -E2 (Stewart *et al.* 1990) and ICI 182 780 (Huynh *et al.* 1996) respectively. Recent studies also suggest a role for soybean phytochemicals in the modulation of IGF-1 signaling pathway: specifically, a reduction in circulating levels of this growth factor was reported in mice bearing human prostate or bladder tumors following consumption of dietary soy products (Zhou *et al.* 1999, Singh *et al.* 2006). Results obtained in the current study did not show any modulation of IGF-1 serum levels either upon treatment with ICI 182 780 or upon concomitant administration of the anti-estrogen and SSE. The reason why effects of soy isoflavones on IGF-I signaling are seen in some experimental systems but not others are not understood, but may involve differences in both cell lines and/or experimental conditions.

In conclusion, results from this study raise concerns about the consumption of isoflavone supplements in conjunction with ICI 182 780 (fulvestrant) therapy in postmenopausal women with estrogen-dependent breast cancer. Additional studies are warranted to develop a more complete understanding of the mechanisms involved in the interaction between the complex soy products that people actually consume and anti-cancer therapies. Indeed, the consideration of whether a particular exposure could have effects and whether these would be positive or negative will likely depend on the product, level of exposure and dosing regimen, and the particular end point being examined. Such fundamental studies are long overdue to allow individuals, including physicians, to make informed decisions on the value or otherwise of the current unregulated, and potentially harmful, consumption of phytoestrogen products by breast cancer patients.

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