Effects of SP500263, a Novel Potent Antiestrogen, on Breast Cancer Cells and in Xenograft Models

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ABSTRACT

We have compared the antitumor activities of SP500263, a novel next-generation selective estrogen receptor modulator (SERM), tamoxifen, and raloxifene side-by-side in in vitro and in vivo MCF-7 breast cancer models. In vitro, SP500263 acted as an antiestrogen and potently inhibited estrogen-dependent MCF-7 proliferation with IC_{50} values in the nanomolar range. SP500263 also strongly inhibited MCF-7 proliferation in the absence of estrogen at all of the concentrations tested. To investigate the antitumor activity of SP500263 in animals, athymic nude mice were implanted with MCF-7 tumor in the presence of a tumor growth-supporting sustained release estrogen pellet. Treatment was initiated after tumors were established. SP500263, administered for 28 days through daily i.p. dosing, effectively reduced estrogen-stimulated tumor growth at 3 and 30 mg/kg. SP500263 was as efficacious as tamoxifen and superior to raloxifene at the corresponding doses. Maximum efficacy was reached with the 30 mg/kg dose. The observed effects were highly significant.

SP500263 represents a member of a novel series of SERMs that is structurally unrelated to SERMs currently on the market or in clinical development. The experiments described herein demonstrate that SP500263 is efficacious in the MCF-7 proliferation assay and in a murine model of breast cancer.

INTRODUCTION

The estrogen hormone has a broad spectrum of effects on tissues, including many positive biological effects such as maintenance of bone density, cardiovascular protection, and central nervous system functions (1–13). However, estrogen is also a potent growth factor in the breast, endometrium, and possibly the ovaries, which increases the risk of certain cancers (14–19). Approximately 70–80% of breast cancers express the ER and, therefore, are potentially responsive to treatments that inhibit the effects of estrogen. Also, a majority of ovarian and endometrial cancers express ER (17, 20–24).

SERMs are an expanding class of structurally diverse drugs that bind to ERs (25–34). SERMs can retain some of the positive effects of estrogen while preventing some of its adverse effects, namely the proliferative effects on reproductive tissues. Three well-known drugs that belong to the SERM drug class are tamoxifen (Nolvadex), toremifene (Fareston), and raloxifene (Evista). The most widely used hormonal therapy for breast cancer today is tamoxifen.

Breast cancer is the second most common cancer in women, with ~180,000 new cases occurring each year in the United States. The American Cancer Society estimates that 40,000 women will die from this disease in 2001. Whereas there are several therapies approved for the treatment and prevention of breast cancer, including hormonal therapies, there is still a need for therapeutic alternatives for women. SERMs have the potential to be useful as first, second, and third line therapy, including in hormonally refractory tumors.

In the present study, we had two principal aims. First, provide evidence that SP500263, a novel, potent SERM, acts as estrogen antagonist in a breast cancer proliferation assay. Second, evaluate the efficacy of SP500263 side-by-side with tamoxifen in a murine breast cancer xenograft model.

MATERIALS AND METHODS

In Vitro Proliferation Assay. MCF-7 cells were cultured in phenol red-free DMEM/Ham’s F12 (Invitro) containing 5% FCS (Hyclone), 3 mM β-mercaptoethanol (Life Technologies, Inc.), 43 ng/ml of sodium selenite (Life Technologies, Inc.), and 20 mM ethanolamine (Sigma Chemical Co.) at 37°C in 5% CO_{2}. Cells were plated in 96-well plates at 3000 cells/well in the above medium with charcoal/dextran-treated FCS (Hyclone). The next day, medium was removed by aspiration, and 200 μl of fresh medium was added. The compound was dissolved in DMSO at 30 mM. Appropriate dilutions of the compound in DMSO were added to cells in duplicate wells for a final DMSO concentration of 0.2%. This concentration was shown not to be toxic to the cells (data not shown). 17β-Estradiol was also dissolved in DMSO and added to cells where indicated at a final concentration of 10 nM in 0.2% DMSO.

Cells were incubated 37°C in 5% CO_{2} for 48 h. [H]thymidine (Amersham) was added to each well (final concentration 0.15 μCi/μl). Cells were incubated at 37°C in 5% CO_{2} for 6 h. Medium was removed, 200 μl of H_{2}O added to each well, and the plate incubated for 15 min. Cells were harvested on a Millipore filter plate using a Packard Cell Harvester (Packard Instruments, Meriden, CT). Scintillation fluid (50 μl) was added to each well, and counts/well were determined using a Packard TopCount.

Proliferation was normalized to 100% compared with the vehicle control (DMSO) or 17β-estradiol, respectively. All of the values are derived from triplicate determinations for each concentration of compound. All of the experiments were repeated at least four times.

Mouse Xenograft Model. Female, 7-week-old, NC-nu athymic nude mice were purchased from Frederic Cancer Research Development Center (Frederick, MD) and acclimated in the laboratory 1 week before experimentation. The animals were housed in microisolator cages, five per cage, in a 12-h light/dark cycle. The animals received filtered sterilized water and sterile rodent food ad libitum. The animals were observed daily, and clinical signs were noted. Tumor fragments (30–40 mg) of MCF-7 human mammary tumor were s.c. implanted in mice near the right axillary area using a 12-gauge trocar needle and allowed to grow. To support the growth of the estrogen-dependent MCF-7 tumor, a 0.72-mg 17β-estradiol 60-day release pellet (Innovative Research of America, Sarasota, FL) was implanted s.c. on the opposite side to the tumor implant side of the mouse on the day of tumor implantation. The tumors were allowed to reach 88–196 mg in weight before the start of the treatment. Those animals selected with tumors in the proper size range were randomly divided into the various treatment groups. Each treatment group consisted of 10 animals except for 94 animals in the vehicle control group.

Animals were treated with compound once daily by i.p. injection for a total of 28 days. All of the compounds were administered in the vehicle of 30% PEG-400, 20% propylene glycol, 15% cremophor EL, and 35% distilled water. Compounds were administered by exact body weight, with the injection volume being 0.05 ml/10 g body weight.

The s.c. tumors were measured and the animals weighed twice weekly.
Effect of SP500263 in the Estrogen Agonist MCF-7 Assay. In the first experiment, SP500263 (Fig. 1), a novel SERM structurally unrelated to tamoxifen, was tested in an MCF-7 breast cancer proliferation assay. MCF-7 has been used for many years as model cell line to investigate hormonally responsive breast cancer (35). To evaluate the estrogenic properties of SP500263, MCF-7 cells were treated with increasing concentrations of SP500263 in the absence of estrogen. For comparison, tamoxifen and raloxifene were included. As can be seen in Fig. 2A, SP500263 at none of the concentrations tested showed any growth stimulatory activity. In fact, all of the compound concentrations tested down-regulated basal proliferation. In contrast, 17β-estradiol at 10 nM potently stimulated MCF-7 proliferation ∼7-fold.

Effect of SP500263 in the Estrogen Antagonist MCF-7 Assay. We next evaluated the antiproliferative activity of SP500263 in the presence of 10 nM of 17β-estradiol. Under these conditions, estrogen strongly stimulates MCF-7 proliferation ∼7-fold, and an antiestrogen, therefore, is expected to inhibit proliferation. Tamoxifen and raloxifene decreased proliferation of estrogen-stimulated MCF-7 cells by 50% at concentrations of 336 ± 168 nM and 1205 ± 935 nM, respectively (Fig. 2B). SP500263 also effectively blocked the stimulatory effect of estrogen on MCF-7 proliferation with an IC50 value of 421 ± 185 nM.

Effect of SP500263 in the Nude Mouse MCF-7 Xenograft Model. The purpose of this study was to evaluate the in vivo efficacy of SP500263 on established MCF-7 tumors grown in the presence of estrogen. Hormone-responsive breast tumors can be formed in the athymic mouse using human breast carcinoma MCF-7 cells and estradiol supplementation (36, 37). A schematic outline of the experimental protocol is shown in Fig. 3. Animals were treated in the presence of the estrogen pellet with 0.3, 3, and 30 mg/kg of SP500263, tamoxifen, or raloxifene through once daily i.p. dosing. We chose i.p. dosing for this proof-of-concept study to allow all of the compounds to reach maximum exposure regardless of their oral bioavailability. After 4 weeks of dosing, the drug treatment was discontinued, and the animals were monitored until the expiration of the estrogen pellet at day 60.

Median tumor weights were graphed over time (Fig. 4). A different presentation of the mean tumor data are shown in Fig. 5 for statistical purposes. The % T/C value on the last day of dosing (day 36) is graphed for all of the treatment groups. Continuous treatment with estrogen resulted in the expected growth of the tumor over the 56-day period (vehicle group in Fig. 4). Each test compound exhibited a dose-dependent decrease in tumor volume compared with the vehicle control (Figs. 4 and 5). A marginal but not statistically significant reduction in tumor growth was observed with the low dose (0.3 mg/kg) of SP500263 and raloxifene (Fig. 5). In contrast, tamoxifen was effective at this dose (P < 0.01 versus vehicle). Significant reduction in tumor growth was observed with all three of the compounds at the medium dose (3 mg/kg) and high dose (30 mg/kg; P < 0.01 versus vehicle). At 3 and 30 mg/kg, SP500263 and tamoxifen showed better efficacy than raloxifene at the corresponding dose (P < 0.05 versus corresponding dose of raloxifene). SP500263 and
tamoxifen reduced tumor growth by ~70% at the medium dose and close to 90% at the high dose (Fig. 5). Therefore, this study demonstrates that SP500263, administered once daily i.p. at 3 or 30 mg/kg, is effective in reducing the growth of MCF-7 tumors in a breast cancer xenograft model in mice.

SP500263 administered at doses as high as 30 mg/kg i.p. for 4 weeks was well tolerated and did not result in any drug-related deaths. Furthermore, no significant changes in body weight gain compared with the vehicle control group were observed with SP500263 (Fig. 6).

Results of the calculated tumor growth delay are presented in Table 1. SP500263 showed measurable antitumor activity at the highest dose tested, producing a median tumor growth delay of >183%. Tamoxifen exhibited comparable activity to SP500263 with a growth delay of >183%. Raloxifene treatment at the highest dose resulted in a 92% growth delay, which was significantly lower than that obtained with tamoxifen or SP500263.

All of the animals displayed accelerated tumor growth after being taken off drug on day 36 after 4 weeks of dosing (Fig. 4). Raloxifene-treated animals showed the biggest gain in tumor weight.

![Fig. 5. Dose response to SP500263, tamoxifen, and raloxifene in the MCF-7 xenograft.](image)

**Fig. 5. Dose response to SP500263, tamoxifen, and raloxifene in the MCF-7 xenograft.** Athymic nude mice implanted with the MCF-7 tumor were treated with the indicated concentrations of compounds through daily i.p. administration for 28 days (day 9 to day 36). Values are the mean body weight for each group.

![Fig. 6. Dose response to SP500263, tamoxifen, and raloxifene in the MCF-7 xenograft.](image)

**Fig. 6. Dose response to SP500263, tamoxifen, and raloxifene in the MCF-7 xenograft.** Athymic nude mice implanted with the MCF-7 tumor were treated with the indicated concentrations of compounds through daily i.p. administration for 28 days (gray bar; day 9 to day 36). Values are the mean body weight for each group.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg)</th>
<th>Median tumor growth delay (T—C) (days)</th>
<th>(T—C)*100%&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP500263</td>
<td>30</td>
<td>&gt;37.0</td>
<td>183%</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3.8</td>
<td>19%</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>0.3</td>
<td>1.8</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4.1</td>
<td>20%</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>1.4</td>
<td>0%</td>
</tr>
<tr>
<td>Raloxifene</td>
<td>30</td>
<td>18.4</td>
<td>91%</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.2</td>
<td>6%</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>1.6</td>
<td>0%</td>
</tr>
</tbody>
</table>

<sup>a</sup> Tumor growth delay in days of treated (T) versus control (C) animals based on time to reach 350 mg.

<sup>b</sup> Percentage tumor growth delay of treated (T) versus control (C) animals.

### DISCUSSION

These studies demonstrate the efficacy of SP500263 in the MCF-7 in vitro proliferation assay and in the nude mouse xenograft model. We have shown that SP500263 acts as an antiestrogen on MCF-7 proliferation. Whereas 17β-estradiol at 10 nM resulted in an ~7-fold increase of MCF-7 proliferation, SP500263 demonstrated no estrogenic activity on its own in this assay. SP500263 also effectively antagonized the effect of estradiol on MCF-7 proliferation with IC<sub>50</sub> values similar to tamoxifen.

SP500263 was discovered in a screen using a small molecule compound library to identify estrogen agonists in bone (38). SP500263 represents a novel SERM with a structure unrelated to other SERMs currently in the clinic or in clinical development. SP500263 has high affinity for both ERs (ER-α and ER-β) but functionally acts through ER-α only (38). Furthermore, SP500263 acts as an estrogen agonist in a bone cell line but behaves as an estrogen antagonist in a breast cancer cell line (Fig. 2). Therefore, SP500263 represents a novel class of SERMs that act as selective ER-α agonists in bone cells, potentially reducing bone loss, and as antiestrogen in breast cancer cells, potentially preventing breast cancer progression.

A primary aim for developing novel SERMs for treatment of breast cancer is to find a SERM with less estrogenic activity on uterine tissue than tamoxifen but to retain its potent antiproliferative activity in the breast. SP500263 is not estrogenic in a reporter gene assay using an estrogen response element (38). In vivo studies with the MCF-7 nude mouse xenograft model revealed that SP500263 acted as an antiestrogen with a potency and efficacy similar to that of tamoxifen (Figs. 4 and 5). In contrast, raloxifene, a SERM approved for treatment of osteoporosis, demonstrated the lowest efficacy in these studies. Tamoxifen at all three of the doses was statistically significantly more efficacious than raloxifene tested at the same dose. Likewise, SP500263 at 3 and 30 mg/kg demonstrated superior efficacy compared with the corresponding doses of raloxifene. These studies were conducted using i.p. administration to give each compound the chance to reach highest bioavailability. SP500263 also did not stimulate uterine wet weight in immature rats and adult OVX rats after 28 days of treatment. This is in contrast to the known uterine stimulatory effects of tamoxifen (39, 40). Thus, SP500263 or a similar entity from the same compound series may be a safer therapeutic in the clinic.

Clinically, ~60% of all ER-positive breast cancer patients initially benefit from antiestrogen therapy such as tamoxifen. However, 40%
of the presumably ER-positive breast cancer patients do not respond to tamoxifen, and the majority of patients on tamoxifen therapy develop resistance within 5 years of treatment. The mechanism of acquired tamoxifen resistance is not well understood. More recent scientific findings in tamoxifen-resistant breast cancer support a change in the cofactor expression profile, which transforms tamoxifen into a growth stimulatory agent (41). Because SP500263 is structurally different from tamoxifen, it may induce a different conformational change to the ER (29, 42, 43). This may result in different cofactor recruitment. Therefore, it is possible that tumors resistant to tamoxifen still respond to SP500263. Along those lines, researchers have shown that tamoxifen-resistant tumors still respond to the structurally unrelated ICI-182,780 compound but are cross-resistant to the related toremefine (44–46).

In summary, these studies suggest that SP500263, a novel SERM structurally unrelated to other known SERMs, has demonstrated efficacy in in vitro and in vivo models of breast cancer. Additionally, in vivo efficacy and safety studies should show how suitable this class of SERMs is for development as anticancer agents.

REFERENCES

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