In vivo Efficacy of STX213, A Second-Generation Steroid Sulfatase Inhibitor, for Hormone-Dependent Breast Cancer Therapy

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Abstract

Purpose: Steroid sulfatase (STS) inhibitors that can decrease or prevent the biosynthesis of estrogenic steroids via the sulfatase route may play an important role in the treatment of breast cancer. We compare the in vivo efficacy of two potent STS inhibitors, STX64 and STX213, in a xenograft breast cancer model.

Experimental Design: MCF-7 cells stably expressing STS cDNA (MCF-7STS) were generated. Ovariectomized MF-1 female nude mice receiving s.c. injections of estradiol sulfate (E2S) and bearing both MCF-7STS and wild-type MCF-7 (MCF-7WT) tumors were orally treated with STX64 and STX213. Treatment was given for 49 days followed by a recovery period of 35 days in which animals received only E2S. Mice were weighed, and tumor measurements were taken weekly.

Results: STX64 and STX213 exhibited potent STS inhibition in vivo. However, STX213 showed a greater duration of activity. In vehicle-treated nude mice receiving E2S, tumor volumes increased 5.5-fold for MCF-7WT and 3.8-fold for MCF-7STS after 49 days compared with day 0. MCF-7WT tumor growth was reduced by 56% by STX213 over the dosing period, and subsequent growth was retarded during the recovery period. All treatments fully inhibited growth of MCF-7STS tumors, and recovery of these tumors was significantly retarded ($P < 0.01$). All compounds completely inhibited liver and tumor STS activity. Additionally, STS mRNA expression in the MCF-7STS tumors directly correlated with the corresponding STS enzyme activity.

Conclusions: This study indicates that STS inhibitors attenuate hormone-dependent human breast cancer growth and therefore offer a potentially novel treatment for this condition.

The importance of estrogens in the development and maturation of human breast cancer in both premenopausal and postmenopausal women is now well established. In postmenopausal women, current therapies aim to block the action of estrogens with antiestrogens or inhibit the synthesis of estrogens by use of an aromatase inhibitor (1, 2). However, the sulfatase pathway is another important route by which estrogens can be synthesized in postmenopausal women. Estrone (E1) and dehydroepiandrosterone can be formed from E1 sulfate (E1S) and dehydroepiandrosterone sulfate, respectively, and can be reduced to steroids with potent estrogenic properties, estradiol (E2) and androstenediol, by 17beta-hydroxysteroid dehydrogenase type 1, which is present in breast tissues (3). Although the development of antiestrogens and aromatase inhibitors has led to major advances in the treatment of breast cancer, many women will progress on these therapies, although their tumors remain estrogen receptor (ER) positive on relapse (4).

Therefore, it seems reasonable to conclude that some ER’ human breast cancers are reliant on estrogens, or steroids with estrogenic properties, formed via the steroid sulfatase (STS) pathway. In postmenopausal women, the production rates of E1 and E2 are approximately 40 and 6 μg/d, respectively (5). Significant quantities of estrogen, formed by peripheral conversion of androstenedione to E1, can be converted to E1S by the actions of E1 sulfotransferase (3, 6, 7). Estrogen sulfates are biologically inactive because they are unable to bind to ER. However, their high concentrations and prolonged half-life in blood indicates that they may act as a reservoir for the formation of physiologically active estrogens on reaction with STS (8). It is believed that STS is virtually ubiquitous throughout the body and therefore able to generate significant amounts of active estrogens (9).

There is increasing evidence to support the importance of STS in human breast cancers. Breast tumor tissue of postmenopausal women can have as much as 10 times the estrogen levels than is seen in the plasma of the same patients (10). Furthermore, STS activity is at least 50 times greater in both premenopausal and postmenopausal breast tumors compared to normal breast tissue (8). This implies that STS could be a potential target for breast cancer therapy.
with normal breast tissue (11). STS expression is detected in 90% of breast tumors (12, 13), whereas aromatase expression is only found in 60% to 70% (14, 15) and that activity of STS in breast tumors is much higher than that of the aromatase complex (16). The result of this increased STS activity could be as much as a 10-fold greater amount of E1 originated via the sulfatase route than via the aromatase pathway (17). Additionally, real-time reverse transcription-PCR experiments have shown that STS mRNA expression in malignant breast tissue is significantly higher than in normal tissue (18). Clinical studies have now shown that STS mRNA expression may be a predictor of recurrence in breast cancer patients (19) and that this association and prognosis is applied only to ER+ tumors (20).

There is a further compelling reason to develop STS inhibitors. Androstenediol, although an androgen, can bind to the ER and stimulate the growth of ER+ breast cancer cells in vitro and carcinogen-induced mammary tumors in ovariec-tomized rats (21, 22). It is derived from dehydroepiandroster-one sulfate in a pathway that is independent of aromatase, relying mainly on STS for conversion (23).

These observations strongly suggest a crucial role for STS in human breast cancer growth and development. However, the models used to study the effects of STS inhibitors on breast tumors in vivo have, to date, been limited. James et al. (24) have developed a STS-overexpressing MCF-7 cell line that was able to establish tumors in ovariectomized nude mice. Despite being unable to obtain growth rates, they did show that overexpression of STS led to an increase in tumorigenesis. Interestingly, they further showed that animals supplemented with E2 sulfate (E2S) reached a greater final tumor volume than those supplemented with E2.

We have previously shown the development and effects of STS inhibitors on STS activity in rodent liver and nitrosmethylurea-induced mammary tumors (25). Estriol-3-O-sulfamate was identified as the first irreversible STS inhibitor (26) but unexpectedly proved to be estrogenic in rodents (27). In the pursuit of alternative nonestrogenic mimics of estriol-3-O-sulfamate, a large number of steroidal and nonsteroidal sulfamates were then synthesized and tested as STS inhibitors. STX64, also known as 667 Coumate, (27, 28) a tricyclic coumarin sulfamate, resulted from such a study, and this compound has been selected for further clinical development. In the nitrosmethylurea-induced mammary tumor model, STX64 caused significant regression of E1S-stimulated tumor growth at 10 mg/kg (85 ± 5%) and 2 mg/kg (56 ± 13%). Recent phase I clinical studies testing STX64 in female breast cancer patients have shown very encouraging results (29). However, there is a further need for the development of more potent STS inhibitors. Building on initial earlier work (30, 31) that showed the promise of D-ring-modified steroid sulfamates as sulfatase inhibitors, we describe the activity of a second-generation STS inhibitor and the establishment of an in vivo tumor model using ovariectomized nude mice supplemented with E2S and bearing tumors derived from both STS-overexpressing (MCF-7STS) and wild-type (MCF-7WT) human breast cancer cells. Furthermore, we also show the effect of the nonestrogenic STS inhibitors, STX64 and STX213, on tumor growth and intratumor STS activity. Finally, we show for the first time that tumor STS activity and STS expression are directly correlated in the nude mouse model.

### Materials and Methods

#### Compounds

All compounds used were synthesized as described previously. STX64 was synthesized according to Woo et al. (27). The synthesis of STX213 has been published elsewhere (30, 32). Both compounds exhibited spectroscopic and analytic data in accordance with their structures (Fig. 1) and were pure as shown by high-performance liquid chromatography.

#### Cell culture.

MCF-7 cells, a human breast cancer ER+ cell line, and JEG-3 cells, an ER+ choriocarcinoma cell line, were obtained from the American Type Culture Collection (LGC Promochem, Teddington, United Kingdom) and grown in 10% serum-supplemented RPMI 1640 (Sigma, Poole, United Kingdom). All cells were cultured at 37°C under 5% CO2 in a humidified incubator.

**In vitro inhibition of STS activity.** The extent of in vitro inhibition of STS activity by STX64 and STX213 was compared by using intact JEG-3 cells. Cells were seeded into 24-well culture plates and maintained in MEM (Flow Laboratories, Irvine, United Kingdom) containing supplements and used when 80% confluent. For the assay of STS activity, cells were incubated with [6,7-3H]E1S (5 pmol, 7 × 105 dpm, 60 Ci/mmol; Perkin-Elmer LS, Wellesley, MA) in the presence or absence of 0.001-10,000 nmol/L STS inhibitor. The product E1 was separated from E1S by toluene partition using [4-14C]E1 to monitor procedural losses, and radioactivity was measured by liquid scintillation spectrometry (31).

**Inhibition and recovery of rodent liver STS activity.** Female adult Wistar rats (180-200 g) were purchased from Charles River UK Ltd. (Margate, Kent, United Kingdom). Experiments were carried out under conditions that complied with institutional requirements. Groups of three rats each received a single oral dose of either vehicle (10% tetrahydrofuran: 90% propylene glycol) or STS inhibitor at 0.1, 1.0, or 10 mg/kg. Twenty-four hours later, rats were terminally anesthetized and liver samples were taken and stored at −20°C. The recovery of liver STS activity after inhibition was also measured. In the recovery studies, rats were given a single dose of STS inhibitor at 10 mg/kg or vehicle control and then killed at 1, 3, 5, 7, 10, and 15 days after dosing and their livers were removed for STS analysis.

**STS biochemical assay.** STS activities of MCF-7 cell and tissue samples obtained from animals were measured as described previously (31). For cell STS activity, the method done was as described above. Tissues were homogenized in ice-cold PBS (pH 7.4) containing 250 mmol/L sucrose, and supernatants were prepared by centrifugation (2,000 × g, 4°C for 10 minutes). Aliquots of tissue supernatants were incubated...
with [6,7-3H]E1S (4 × 10^6 dpm; Perkin-Elmer LS, Boston, MA) and adjusted to a final concentration of 20 μmol/L with unlabeled E1S (Sigma). [4,14C]E1 (1 × 10^6 dpm; Perkin-Elmer) was included in the reaction mixture to monitor procedural losses. Samples were incubated for 60 minutes at 37°C after which the product E1 was separated from E1S by partition with toluene. An aliquot of toluene was removed and the 3H and 14C radioactivity was measured by liquid scintillation spectrometry. The mass of E1S hydrolyzed was calculated from the 3H counts detected corrected for procedural losses. A protein measurement was also obtained for the tissue using the Bradford assay method. Results are determined as nmol product formed per hour per mg protein and expressed as the percentage inhibition or activity compared with the control.

**Generation of MCF-7STS cell line.** MCF-7 cells were seeded into six-well plates (4 × 10^4 well) and, 24 hours later, transfected with a pc1-neo (Promega, Southampton, United Kingdom) construct containing the complete coding sequence for the human STS gene using FuGene 6 (Roche, Welwyn Garden City, United Kingdom) transfection reagent in accordance with the manufacturer’s instructions. Twenty-four hours after transfection, cells were exposed to 800 μg/mL G418 (Promega) for 5 days, after which cells were split 1:500 and infected reagent in accordance with the manufacturer’s instructions. This resulted in a total study time of 84 days. Throughout the study animals were terminated and the tumors and liver were removed for STS mRNA expression and activity measurements. At the end of the study, tumors and livers were collected for the measurement of STS activity. Blood samples were taken from tumor-bearing mice treated with STX213 or MCF-7WT for measurement of E2 concentrations.

**Plasma E2 measurement.** Blood taken from mice was collected into heparinized tubes and centrifuged at 13,000 rpm for 1 minute to remove erythrocytes. The plasma was collected and stored at −20°C until assayed. Plasma E2 levels were measured using a Coat-A-Count Estradiol RIA kit (Diagnostic Products Corp., Los Angeles, CA) according to the manufacturer’s instructions. The intra-assay coefficient of variation for these measurements of plasma E2 concentrations by this method was <10%.

**STS mRNA expression.** For tumor samples, 20 to 40 mg of tissue were excised from the tumor xenograft and transferred to 2 mL of RNalater solution (Ambion, Inc., Austin, TX). The tissue sample was transferred to 600 μL of RNeasy lysis buffer (Qiagen, Ulm, Germany) plus 1% β-mercaptoethanol (Promega, Madison, WI) and homogenized. The homogenate was centrifuged (3 minutes at 10,000 × g), and the RNA was isolated from the supernatant using the RNeasy kit (Qiagen) and stored at −80°C. A 5 μg aliquot of each RNA sample was reverse transcribed in a final volume of 33 μL to generate cDNA using the First-Strand cDNA Synthesis kit (Amersham Biosciences Ltd., Little Chalfont, United Kingdom) with 1 μL cDNA in a final volume of 25 μL using Excite 2X Master Mix (BioGene, Kimbolton, United Kingdom). Primers and hydrolysis probes for STS (35) and the internal control HPRT-1 (36) were synthesized by Thermo Hybaid (Dreieich, Germany). The conditions were as follows: 95°C for 10 minutes followed by 40 cycles of 95°C for 30 seconds, 60°C for 45 seconds, and 72°C for 45 seconds. The relative C_t values were calculated, and the values presented are representative of one of three such independent experiments.

**Statistical analysis.** An ANOVA was done to determine tumor growth differences. Where only two groups are compared, a Student’s t test was applied. A linear regression curve was calculated to compare STS mRNA expression against STS activity. All values are represented as the mean ± SE.

**Results**

**Inhibition of STS activity in vitro.** The potencies of STX64 and STX213 as STS inhibitors were initially compared by testing their ability to inhibit STS activity in intact JEG-3 cells. Using this assay, the IC_{50} value for STX213 was 0.5 nmol/L, whereas that for STX64 was 1.5 nmol/L, indicating that in this system STX213 is 3-fold more potent than STX64.

**Inhibition and recovery of rat STS activity.** Rat liver STS activity was measured to determine the inhibitory activity of STX64 and STX213. Significant decreases in STS activity were seen in both STX64 and STX213 treatments even at the lowest dose tested (0.1 mg/kg; P < 0.05). More than 95% inhibition was observed for both of the compounds at doses of 1 and 10 mg/kg (Fig. 2A). STS recovery was determined after a single oral dose of 10 mg/kg in livers taken at 1, 3, 5, 7, 10, and 15 days after dosing (Fig. 2B). There was a rapid recovery of STS activity after STX64 dosing, with 50% recovery occurring by day 4. Full recovery was observed by day 8. In contrast, STX213 resulted in a longer duration of STS inhibition, with only 50% recovery taking place by day 12 but with almost complete recovery by 15 days.

**STS activity in cells stably expressing STS cDNA.** To explore the possible role of STS in human breast cancer, several MCF-7 cell lines stably expressing STS cDNA were generated. The MCF-7 clone exhibiting the highest STS expression and activity was chosen for this study. Before cellular inoculation into mice, it was important to determine the STS activity of both MCF-7WT and MCF-7STS cell lines. This was to confirm that MCF-7STS cells exhibited a greater STS activity compared with MCF-7WT cells. STS activity was significantly elevated in the MCF-7STS cell line.
(2,775 ± 443 nmol/h/10⁶ cells) compared with the MCF-7 WT cells (122 ± 16 nmol/h/10⁶ cells). Therefore, MCF-7 STS cells had an ~20-fold increase in activity compared with MCF-7 WT cells.

**Inhibition of tumor growth by STS inhibitors.** After inoculation, both MCF-7 WT and MCF-7 STS cells developed into stable growing tumors in mice treated with E2S, with a take rate of 83% and 80% for each cell line, respectively. However, in mice not given E2S supplements, neither cell type was able to develop into a tumor. It was expected that the maturation would be accelerated in the MCF-7 STS tumors due to the greater levels of E2 created by those tumors. However, this did not prove to be the case. Growth rates for the MCF-7 WT tumors were similar when compared with the MCF-7 STS tumors.

Once tumors had reached approximately 100 to 150 mm³ over a 28-day period, compound dosing commenced for a period of 49 days. This was followed by a 35-day recovery period during which animals received E2S s.c. injections only. At the end of dosing (day 49), growth of the MCF-7 WT tumors (Fig. 3A) was significantly inhibited by STX64 at 20 mg/kg by 39% (325 ± 102%; P < 0.05) and by STX213 at 10 mg/kg by 56% (230 ± 48%; P < 0.05) when compared with controls receiving just vehicle (527 ± 87%). The lower dose of STX64 (10 mg/kg) did not significantly attenuate MCF-7 WT tumor development. At the end of study (i.e., 35 days after cessation of dosing), only those animals that had received STX213 continued to have the growth of their tumors significantly attenuated (765 ± 169%; P < 0.05) when compared with controls (1,412 ± 301%).
The MCF-7WT tumors did not respond to dosing with STX64 or STX213 as well as the MCF-7STS tumors (Fig. 3B). Dosing with STX64 at 10 and 20 mg/kg resulted in no significant MCF-7STS tumor growth at day 49 compared with day 0, whereas STX213 caused a 39% regression of growth. This inhibition of tumor growth was maintained throughout the recovery period, although some growth was seen toward the completion of the study (day 84). For animals dosed with STX64 at 10 mg/kg, growth was inhibited by 76% (297 ± 86%; P < 0.05), with STX64 at 20 mg/kg inhibited by 79% (268 ± 71%; P < 0.01) and with STX213 at 10 mg/kg inhibited by 72% (352 ± 202%; P < 0.05) compared with vehicle-treated growth from day 0.

Figure 3C shows the changes in the weights of mice throughout the study. No weight loss occurred, indicating that the animals tolerated the compounds throughout the study without any apparent toxicity.

Tissue STS activity and plasma E2 levels. It was important to ascertain that the compounds were inhibiting STS activity in both tumors and livers of the mice. Consequently, STS activity was evaluated on tissues removed at days 49 and 84 of the study (Fig. 4). All drug treatments were able to inhibit both tumor and liver STS activity by >90% at day 49. Not all MCF-7STS tumor STS activities were measured (Fig. 4C and D) at this time point, as tumor growth had been inhibited to such an extent to result in insufficient tumor tissue in which to measure STS activity. However, once treatment had been ceased over the 35-day recovery period, STS activity was restored (Fig. 4B). Encouragingly, MCF-7STS tumors exhibited a higher level of STS activity compared with MCF-7WT in all treatment groups, indicating that these tumors had maintained elevated STS activity over the study period. Measurement of mouse plasma E2 indicated that E2S supplementation increased plasma E2 concentrations and that this was significantly reduced by STX213 (P < 0.05; Fig. 4E).

Figure 4. STS activity measured in mouse tumor and liver tissue. A to D, STS activity in tissue removed from animals at day 49 (white columns) was significantly inhibited in all treatment groups. Black columns, recovery of STS activity in tissues at day 84. MCF-7STS tumor STS activity is elevated compared with that of MCF-7WT tumor tissue in all groups at day 84. E, E2S-induced increase in mouse plasma E2 levels was significantly inhibited by STX213 at 10 mg/kg. Columns, mean (n = 4); bars, SE. *, P < 0.05.

Tumor STS mRNA expression. To assess the relationship between tumor STS activity and mRNA expression, RNA was isolated from tumors at the conclusion of the study. Real-time reverse transcription-PCR was used to assess the STS mRNA expression in both the MCF-7STS and the MCF-7WT tumors from each animal. The endogenous housekeeping gene HPRT (35) was used to normalize the STS expression between samples. The results are presented (Table 1) as the fold increase in MCF-7STS tumors relative to MCF-7WT tumor on the opposing flank of the animal. It can be seen that tumor STS mRNA expression had a direct significant correlation with tumor STS activity (r = 0.92; P < 0.01; Fig. 5). Furthermore, all MCF-7STS tumors maintained an elevated level of mRNA and activity compared with the MCF-7WT controls.
Table 1. The fold increase in MCF-7<sub>STS</sub> tumors of STS mRNA levels and STS activity compared with animal-matched MCF-7<sub>WT</sub> tumors

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<th>Animal no.</th>
<th>MCF-7&lt;sub&gt;STS&lt;/sub&gt; tumor RNA vs MCF-7&lt;sub&gt;WT&lt;/sub&gt;</th>
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**Discussion**

In contrast to aromatase inhibitors that are now used clinically, the development of STS inhibitors is still at an early stage. The nonsteroid-based inhibitor STX64 was the first inhibitor to be tested in a phase I clinical trial in postmenopausal women with breast cancer (29). At 20 mg/d, it was found to almost completely block STS activity in peripheral and tumor tissues. Although STX64 was therefore effective in the clinical setting, there is a need to develop second-generation inhibitors and in vivo models of human breast cancer in which the efficacy of these inhibitors can be tested.

STX213 was developed as a D-ring-modified steroid-based inhibitor and is 18 times more active than the original steroidal inhibitor estrone-3-O-sulfamate, with an IC<sub>50</sub> value of 1 nmol/L in placental microsomes (32). In the present study, the potency of STX213 was initially compared with that of STX64 in JEG-3 cells and found to be three times more active. Administration of STX213 at 10 mg/kg/d orally to ovariectomized rats had previously shown that it had no effect on uterine growth, indicating that it is not estrogenic (32). Livers from these rats revealed that, at this dose, STX213 inhibited STS activity by 99%. In the present study, STX213 was tested at 0.1, 1.0, and 10 mg/kg for its ability to inhibit rat liver STS activity 24 hours after a single oral dose. This resulted in almost complete liver STS inhibition at the 1.0 and 10 mg/kg doses, confirming results from previous work. At 0.1 mg/kg, the effect of STX64 and STX213 was similar. In contrast, however, when the duration of rat liver STS inhibition was investigated, STX213 proved to have a considerably longer duration of action than STX64. The times taken to recover 50% of liver STS activity were approximately 4 days for STX64 and 12 days for STX213. As the turnover time for STS is ~3.5 days, this prolonged activity of STX213 suggests that it is sequestered into, and released from, body tissues. Thus, STX213, with its greater potency in vitro activity and longer duration of action compared with STX64, is suitable for preclinical and clinical testing.

Brodie et al. (37) pioneered the use of MCF-7 breast cancer cells stably expressing the human aromatase cDNA to investigate the efficacy of breast cancer therapies in in vivo models. They showed that these MCF-7 cells proliferated in response to supplementation with androstenedione (38). Using a similar approach, MCF-7 cells stably expressing human STS cDNA were generated and used in a nude mouse model to test the efficacy of STX64 and STX213. In the absence of E2S supplementation, neither MCF-7<sub>WT</sub> nor MCF-7<sub>STS</sub> cells grew, indicating their absolute dependence on estrogen for growth in ovariectomized animals. E2S supplementation stimulated the growth of both MCF-7<sub>STS</sub> and MCF-7<sub>WT</sub> tumors. This suggests that sufficient E2 is generated in peripheral tissues to support the growth of MCF-7<sub>WT</sub> tumors. In contrast, previous work by James et al. (24) in which MCF-7 cells transfected with STS cDNA or MCF-7<sub>WT</sub> were inoculated into nude mice, supplementation with E2S from a silastic implant did not increase the incidence of tumors derived from MCF-7<sub>WT</sub> cells.

Given the high level of peripheral STS activity, it is not surprising that sufficient estrogen is generated from E2S in peripheral tissues to support the growth of MCF-7<sub>WT</sub> tumors in nude mice. However, it is not possible to know from this study whether the E2 is generated in peripheral tissues, such as the liver, or results from E2 generated by the STS in the MCF-7<sub>STS</sub>-derived tumors on the opposite flanks of the animals. Using an in vivo model in which tissues were derived from MCF-7 aromatase-overexpressing cells or MCF-7<sub>WT</sub> cells, insufficient estrogen was found in tumors overexpressing aromatase to support the growth of MCF-7<sub>WT</sub> tumors (38). In mice, there is little peripheral aromatase in contrast to the high levels of STS activity present in tissues. In the present study, STS activity in liver tissue was of a similar level to that detected in MCF-7<sub>STS</sub> tumors. There was no significant difference in the volumes of tumors, which grew from MCF-7<sub>WT</sub> or MCF-7<sub>STS</sub> cells, indicating that both peripheral and tumor STS activities are likely to be important in making estrogen available from estrogen sulfates. This is in contrast to aromatase activity where in situ activity is of greater importance than peripheral activity (38).

The E2S-stimulated growth of MCF-7<sub>WT</sub> and MCF-7<sub>STS</sub> tumors was significantly reduced by STX64 and STX213. However, in MCF-7<sub>WT</sub> cells, STX213 induced a greater degree of inhibition of tumor growth than STX64, and the growth of these tumors continued to be inhibited throughout the 35-day recovery period. This may be because of the greater potency and longer duration of action of STX213 compared with STX64. In contrast, these compounds were both very effective at inhibiting the growth of tumors derived from MCF-7<sub>STS</sub> cells.
growth was almost completely inhibited at the end of the treatment period by these compounds, and this was maintained for 20 to 25 days after the cessation of dosing. Thus, the results from this study confirm the importance of estrogen sulfates as substrates for the formation of active estrogen in peripheral and tumor tissues and indicate that this model can be used to evaluate STS inhibitors.

To confirm the effectiveness of both inhibitors in blocking STS activity, tissue and plasma samples were obtained from tumor-bearing animals at the end of the dosing period. Tumor and liver samples revealed that tissue STS activity was inhibited by >90% by STX64 and STX213, in keeping with previous results obtained with these inhibitors (31, 32). Furthermore, circulating E2 levels were significantly reduced by STX213 treatment, indicating the ability of this compound to inhibit peripheral conversion of E2S. Tumor samples taken from animals treated with vehicle confirm that STS activity was elevated in MCF-7STS tumors compared with MCF-7WT tumors. STS mRNA expression was also determined in these tumor samples and, although variable, was consistently higher in tumors derived from MCF-7STS cells than in those derived from MCF-7WT cells. Furthermore, and for the first time as far as we are aware, it was possible to show a significant correlation between STS mRNA expression and STS activity in these tissues.

In summary, an effective second-generation STS inhibitor, STX213, has been identified, which is more potent in vitro and has a longer duration of action in vivo than STX64. Having identified this inhibitor, a nude mouse model was established using MCF-7WT and MCF-7STS cells. Both cell types grew on supplementation with E2S, indicating that peripheral, in addition to in situ, STS activity is important to support the growth of tumors in this model. STX213 was active against tumors derived from MCF-7STS cells expressing elevated levels of STS mRNA and having increased STS activity. As many breast tumors have high levels of STS expression and activity, STX213 may have considerable therapeutic potential for the treatment of hormone-dependent breast tumors.

References


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