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Role of steroid sulfatase in local formation of estrogen in post-menopausal breast cancer patients☆

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Abstract

More than two-thirds of breast cancers occur in post-menopausal women, and depend on the estrogens for their proliferation and survival. For the treatment of estrogen-dependent breast cancers, two major treatment options are now available. One is selective estrogen receptor modulator (SERM) such as Tamoxifen and another is aromatase inhibitor such as Anastrozole, Letrozole and Exemestane, which reduce local in situ formation of estrogens. Although these therapies are clinically active for advanced and early breast cancers, de novo and/or acquired resistance to SERM and/or aromatase inhibitors are also clinical problem. Recent studies suggest that local formation of estrogens in the breast tumors is more important than circulating estrogen in plasma for the growth and survival of estrogen-dependent breast cancer in post-menopausal women. The rationale for the importance of local formation of estrogens is based on the following evidences. Estradiol (E2) levels in breast tumors are equivalent to those of pre-menopausal patients, although plasma E2 levels are 50-fold lower after menopause. E2 concentrations in breast tumors of post-menopausal women are 10–40 times higher than serum level. Biosynthesis of estrogens in breast tumors tissues occurs via two major different routes, one is aromatase pathway and another is steroid-sulfatase (STS) pathway. Whereas many studies has been reported about aromatase inhibitor and its clinical trial results in breast cancer patients, limited information are available regarding to other estrogen regulating enzymes including STS, its role in breast tumors and STS inhibitors. STS is the enzyme that hydrolyses estrone 3-sulfate (E_1S) and dehydroepiandrosterone-sulfate (DHEA-S) to their active un-sulfoconjugated forms, thereby stimulating the growth and survival of estrogen-dependent breast tumors. It has been well known that E_1S level are much higher than E2 level both in plasma and tumor of post-menopausal patients. Recent reports show that more than 80% of breast tumors are stained with anti-STS antibody and the expression of STS is an independent prognostic factor in breast cancer. Taking these findings into consideration, local formation of estrogens could be partially synthesized from large amount of E_1S by STS, which exist in breast cancer. On the other hand, aromatase localizes in stroma and adipocyte surrounding breast cancer. Furthermore, since estrogen formation from E_1S and DHEA-S (STS pathway) cannot be blocked by aromatase inhibitors, STS is thought to be a new molecular target for the treatment of estrogen-dependent tumor post-SERM and/or aromatase inhibitors. In this symposium, these recent rationale for the importance of STS in post-menopausal breast cancer patients is reviewed as well as STS inhibitor.

Keywords: Breast tumor; Estrogen; Sulfatase; Inhibitor

1. Introduction

Estrogen-sensitive breast cancer cells are stimulated to proliferate by active estrogens synthesized in the ovary and/or in peripheral or breast tissues [1–3]. Enzymes involved in estrogen synthesis, therefore, should be excellent targets for therapeutic intervention for the treatment of breast cancer and other estrogen-dependent cancers. Estrogen levels in breast cancer tissues of post-menopausal women are 10–40 times higher than in plasma (serum) levels from the same individuals [4–7]. Furthermore, estrogen levels in breast tissue of post-menopausal women are nearly equivalent to those in pre-menopausal women

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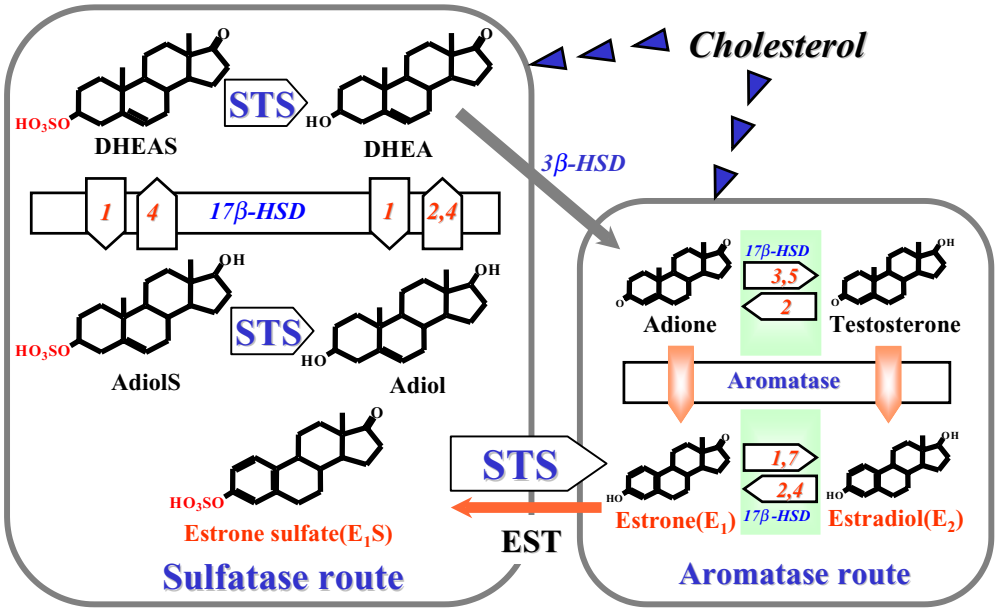

Fig. 1. Synthesis route of estrogens and androgens. These steroidal compounds originated from cholesterol. The working points of aromatase and STS are shown. 17β HSD: 17β hydroxysteroiddehydrogenase, DHEA(S): dehydroepiandrosterone (sulfate), Adiol(S): androstenediol (sulfate) EST: estrone sulfotransferase.

[8,9]. The higher levels of estrogens in breast cancer tissues are thought to be due to local formation of estrogens via two different routes, namely, the aromatase pathway (from androstenedione (ADIONE)) or sulfatase pathway (from estrone 3-sulfate (E_1S)) [10–12] (Fig. 1).

Recently, inhibitors of aromatase have been shown to be more effective against recurrent and progressive breast cancer patients than the anti-estrogen drug, Tamoxifen [13], suggesting that the inhibition of estrogen synthesis is clinically very important for the treatment of estrogen-dependent breast cancer. Recent evidences suggested that steroid sulfatase (steryl sulfatase, EC 3.6.1.2 (STS)) could also be an important target for hormonal therapy of breast cancer in addition to aromatase inhibitors. Firstly, the expression level of STS has been observed in tumor tissue but not in surrounding normal tissue [14]. Secondly, the activity of STS in breast cancer tissue was shown to be at least 100 times higher than that of aromatase [15–17]. Thirdly, andro-5-ene-3β,17β-diol (Adiol), which is also the active estrogen, is derived from dehydroepiandrosterone-sulfate (DHEA-S) in a pathway involving sulfatase pathway that is independent of the aromatase pathway [18–20]. Fourthly, E_1S should be a reservoir for formation of active estrogens in breast cancer tissue, because E_1S has a higher tissue concentration and longer half-life [6,21]. Finally, recent clinical studies revealed that STS mRNA expression could be an independent predictor of recurrence in breast cancer patients [22]. These results suggested that inhibitors of STS might be effective against recurrent and progressive estrogen-dependent breast cancer or may provide an addition to aromatase inhibitors for complete estrogen blockade therapy.

Over the past several years, we and other researchers have reported on the efficacy of steroidal [23–26] and non-steroidal inhibitors [27–29] of STS. We have focused upon cell-free enzyme inhibitory activity as well as anti-proliferative activity or estrogenic activity in cultured breast cancer cells. Only few reports have previously addressed in vivo anti-cancer activity or estrogenic activity of these STS inhibitors [30].

In this report, we describe the anti-cancer activity of the non-steroidal STS inhibitor compound 9 ((*p*-*O*-sulfamoyl)-*N*-tetradecanoyl tyramine), which lacks estrogenic activity in vitro. This STS inhibitor suppressed E_1S-dependent growth in nude mice of MCF-7 cells, which over-expresses STS. Our results suggest that our STS inhibitors may have a potential as a therapeutic agent for estrogen-dependent breast cancer.

2. Methods

2.1. Chemicals

Compound 9 (Fig. 2) was synthesized by published methods [17]. Tris, E_1S, Tamoxifen, dimethyl sulfoxide (DMSO), and NP-40 were obtained from Sigma (St. Louis, MA). Ovahormon Depot™ (containing estradiol (E2) dipropionate 5 mg/ml) was obtained from Teikoku Zoki Seiyaku (Tokyo, Japan). Calf serum (CS) was obtained from Hy-clone (Logan, UT, USA). Dextran coated charcoal (DCC) treated CS was prepared as previously described [31]. Phenol red (PR) free modified Eagle medium (MEM)

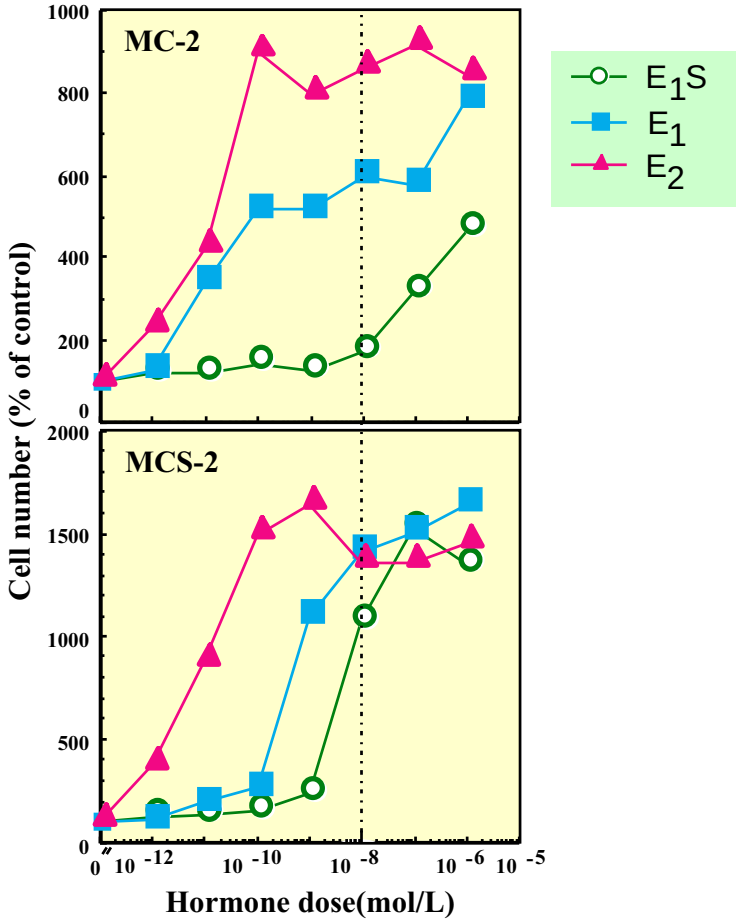

Fig. 2. Hormone dependent growth of human breast cancer MCF-7 which over-express human steroid sulfatase (STS) induced cell line MCS-2 and vector control MC-2 cells. Transfected cells were pre-cultured with estrogen free medium for 5 days and re-seeded with the same medium. The next day of re-seeding, estrogens were added and the cell number was counted at 6 days later.

was obtained from Nissui Seiyaku (Tokyo, Japan). Basement Matri-Gel™ was obtained from Becton Dickinson Labware (Bedford, MA, USA).

2.2. Animals

Six-week-old female nude mice were obtained from Clea, Japan (Tokyo, Japan). All animals were kept in the condition of 23 °C and 55% humidity, lighted every 12 h, and filtered water and feed chow were freely given. All animal studies were carried out under the authorized conditions guided by Animal Moral Committee of the Pharmaceutical Research Institute of Kyowa Hakko Kogyo Co. Ltd.

2.3. Cell culture

The MCS-2 cell line was established by introduction of the human STS gene into estrogen-dependent human breast cancer MCF-7 cells (unpublished data). MCS-2 cells were cultured with 5% DCC-CS/PR-free MEM for 5 days as an estrogen depletion condition. After starvation of estrogen, the cells were re-seeded with the same medium in 24-well plate at 5000 cells per well/0.5 ml. STS inhibitors and E_1S (10 nmol/l) were added 24 h later and the cell numbers were counted 144 h later.

2.4. Effects of STS inhibitor on E_1S-stimulatd MCF-7 cells which over-express STS (MCS-2 cells) in nude mice

MCS-2 cells were cultured with 5% DCC-CS/phenol red (PR)-free MEM/10 nmol/l E_1S and were transplanted into nude mice at 1×10^{-7} cells per mouse with an equal volume of Matri-Gel™ (0.1 ml per mouse), and on the same day, Ovahormon Depot™ were injected into the thigh muscle. Two weeks after transplantation, tumor sizes were measured, and E_1S (0.1 mg/kg per day) were administrated subcutaneously at another side of tumor. After daily E_1S injection for 7 days, growth stimulated tumors were chosen and these tumor bearing mice were divided into four groups. One group received E_1S (0.1 mg/kg per day, s.c.) daily and the other group was treated with E_1S (0.1 mg/kg per day, s.c.) and the STS inhibitor for 18 days. Tumor volume was calculated from measured diameters as previously described [32].

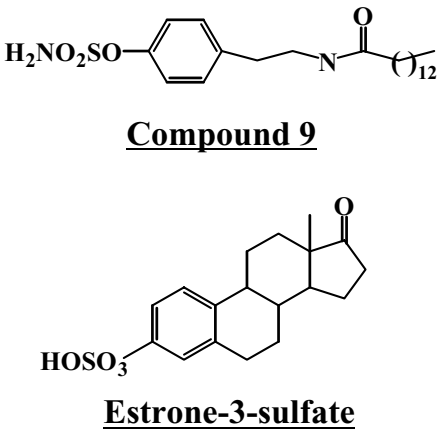

Fig. 3. Structures of compound 9 and estrone-3-sulfate.

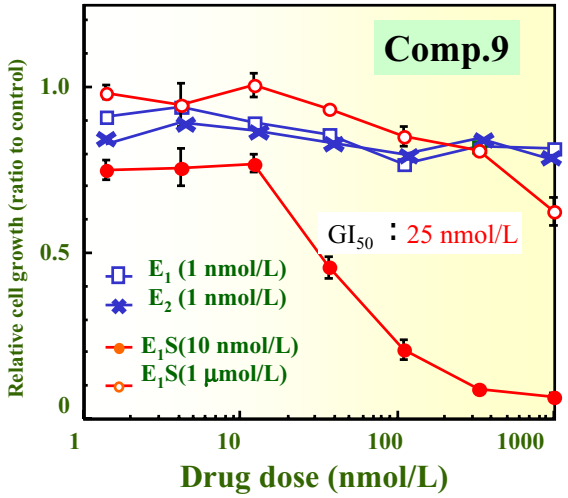

Fig. 4. Growth inhibitory effects of compound 9 against human breast cancer MCF-7 which over-express human STS, MCS-2 cell. MCS-2 cells were pre-cultured with estrogen free medium for 5 days and after that, re-seeded with the same medium. The next day of re-seeding, compound 9 and estrogens were added and 6 days later, cell numbers were counted. Cell numbers of added estrogen alone were as the controls.

3. Results

As shown in Fig. 2, MCF-7 cells which over-express the STS enzyme (MCS-2 cells) proliferated in the presence of low physiological concentrations (10 nmol/l) of E_1S. Hundred times higher concentrations of E_1S (1000 nmol/l) are needed to support the growth of vector control cells (MC-2 cells). With these data in mind, we utilized these MCS-2 cells for further validation of our STS inhibitors in vitro and in vivo.

Compound 9, a non-steroidal STS (Fig. 3) inhibitor which has an IC_{50} value of 56 nmol/l against the human STS enzyme in a cell-free system (data not shown), inhibited E_1S-stimulated growth of MCS-2 cells in a concentration-dependent manner, with a GI_{50} value of 25 nmol/l. Importantly, compound 9 did not affect E_1 or E_2-stimulated growth of MCS-2 cells (Fig. 4), suggesting that the compound acts on the pathway involved in the formation E_1 or E_2 from E_1S rather than blocking downstream events such as the inhibition of 17β-HSD or estrogen receptor. Compound 9 (at 1000 nmol/l) did not stimulate the growth of MCS-2 cells in the absence of estrogen, suggesting that this compound lacks the estrogenic activity (data not shown).

To examine if compound 9 would show growth inhibitory activity on E_1S-stimulated growth of MCS-2 cells in vivo, 1×10^7 of MCS-2 cells were transplanted into the flank of female nude mice with Matri-Gel™. After transplantation,

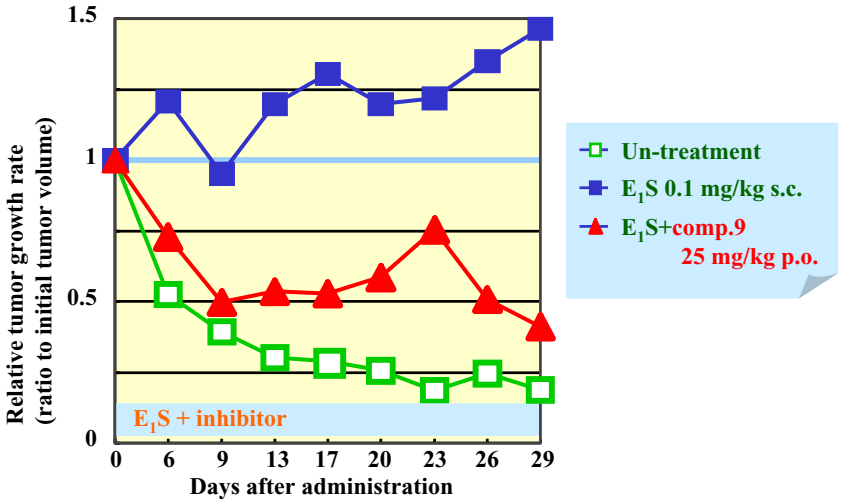

Fig. 5. Anti-tumor activity of STS inhibitors against STS over-expressed human breast cancer MCS-2 cells transplanted in female nude mice. MCS-2 cells were injected into nude mice with Matri-Gel™ and were selected for the E_1S dependency (see Section 2). Mice with E_1S dependent tumors were divided into sub-groups. STS inhibitors were administrated for 18 days.

mice were injected with Ovahormon Depot™ to establish tumor growth, and then were treated with E_1S at 0.1 mg/kg per day subcutaneously. The mice with tumors that grew in the presence of E_1S were selected for further therapeutic experiments (Fig. 5). In the absence of E_1S, tumor size decreased by 75% after 18 days, however, 18 consecutive daily administrations of E_1S at 0.1 mg/kg per day stimulated the growth of tumor 1.2-fold on that day. In addition, 18 consecutive daily oral administrations of compound 9 (10 mg/kg) blocked the E_1S-stimulated tumor growth of MCS-2 cells (Fig. 5). These results suggested that the STS inhibitor; compound 9 could inhibit the conversion of E_1S to E_1 in tumor or other tissue(s) in animal body.

4. Discussion

Estrogen-dependent breast cancers are prevalent in post-menopausal women, despite low circulating estrogen levels, suggesting that the breast tumors are themselves producing estrogens from circulating precursors [33,34]. Estrogens may be formed in breast tumors by two pathways, namely the aromatase pathway and sulfatase pathway. To date a lot of steroidal or non-steroidal STS inhibitors have been reported [23–29], however, at least to our knowledge, few report actually revealed in vitro and in vivo growth inhibitory activity of the inhibitors using the same cell line.

To simulate the post-menopausal breast cancer patient, we transfected the estrogen-dependent MCF-7 cells with the human STS gene to provide a source of non-ovarian estrogen in cell culture or nude mice. Using this STS over-producing cells, named MCS-2, which can grow in the presence of physiological concentration range of E_1S as low as 10 nmol/l, we revealed that our non-steroidal (compound 9) STS inhibitors could inhibit E_1S-stimulated growth of the cells giving GI_{50} value of 27 nmol/l (compound 9, data not shown). The inhibitory activity of this STS inhibitor is proved to be selective to STS pathway because GI_{50} value was significantly increased when higher concentrations of E_1S was used (Fig. 4), and this inhibitor did not inhibit E_1 or E_2-stimulated growth of the cells in vivo. Additionally, we also revealed that our non-steroidal STS inhibitor could inhibit E_1S-stimulated growth of MCS-2 cells in female nude mice model in vivo for the first time (Fig. 5).

Adiol, originated from DHEA, can be metabolized into testosterone by several steps and then into estradiol by aromatase, respectively (Fig. 1). In our data, Adiol can stimulate the cell proliferation of MCS-2 cell at concentrations of nanomoler range (i.e. 1–10 nmol/l). In contrast, testosterone cannot stimulate at the same concentrations and higher concentrations (i.e. 1–10 μmol/l) are required for the stimulation, presumably due to lower activity in aromatase. These data suggest that estradiol, which is originated from a physiological concentration of Adiol, hardly stimulate the proliferation of MCS-2 cells.

In summary, our present results strongly suggested that these STS inhibitors should be potent therapeutic agents for treatment of estrogen-dependent breast cancers and other hormone-dependent tumors.

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