TAS-108, a Novel Oral Steroidal Antiestrogenic Agent, Is a Pure Antagonist on Estrogen Receptor α and a Partial Agonist on Estrogen Receptor β with Low Uterotrophic Effect

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ABSTRACT

Purpose: Investigators are currently conducting phase II trials on TAS-108, a novel oral steroidal antiestrogenic agent. The purpose of this study is to investigate the molecular and pharmacologic properties of TAS-108 compared with other antiestrogenic agents such as tamoxifen, raloxifene, and fulvestrant.

Experimental Design: The antagonistic or agonistic activities of these agents against both estrogen receptors (ER) α and β were compared in the reporter assay systems. Their effects on the uterus were evaluated in ovariectomized rat models. The antitumor activity of TAS-108 given p.o. was evaluated in both dimethylbenzanthracene-induced mammary tumor model and human breast cancer MCF-7 cell line xenografts.

Results: TAS-108 inhibited the transactivation of ERα under the presence of 17β-estradiol (E2) and did not induce the transactivation of ERβ in the absence of E2, unlike the agonistic activity of tamoxifen. On the other hand, it exhibited the most agonistic activity on ERβ among the antiestrogenic agents tested. When given p.o. in the ovariectomized rat, TAS-108 showed a much weaker estrogenic effect on uterine weight compared to tamoxifen, or with similar levels of raloxifene, a selective estrogen receptor modulator. Also, TAS-108 strongly inhibited tumor growth in dimethylbenzanthracene-induced mammary carcinoma in the rat, the endogenous E2 model, at a dosage of 1 to 3 mg/kg/day. It also inhibited high exogenous E2, inducing tumor growth against MCF-7 xenografts at a dosage of 1 mg/kg/day without any toxic manifestation.

Conclusions: Taken together, p.o. treatment with TAS-108 has a novel mode of action on ERs and inhibits E2-dependent tumor growth with little uterotrophic effect.
premenopausal women without complete estrogen blockade with luteinizing hormone–releasing hormone.

Thus, there has been no agent that is superior to tamoxifen with its clinical benefits and added beneficial effects in the treatment of osteoporosis and cardiac disease. In addition, although some clinical improvements have been made with these new types of hormonal agents, there will still be patients with disease refractory to these agents. Additionally, the treatment of metastatic and advanced breast cancers demands for new types of endocrine therapies. To address these issues, we screened for an endocrine drug that has little uterotrophic effect while retaining the beneficial effects of tamoxifen. As a result, the oral steroidal antiestrogenic compound TAS-108 (SR16234; Fig. 1) was found to be effective against breast cancer with low uterotrophic effect based on its novel molecular mechanisms.

MATERIALS AND METHODS

Chemicals, Cells, and Animals. TAS-108 (Fig. 1), raloxifene, and fulvestrant were synthesized in our institute. For the ER binding assay and cell proliferation assay, raloxifene, and fulvestrant were purchased from Sigma Chemicals (St. Louis, MO) and Toeris Cookson (Bristol, United Kingdom), respectively. Tamoxifen citrate salt (TAM) and 4-hydroxytamoxifen were purchased from Sigma Chemicals. 17β-Estradiol (E2) was purchased from Wako Pure Chemicals (Osaka, Japan). 7, 12-Dimethylbenzanthracene (DMBA) was purchased from Tokyo Chemicals (Osaka, Japan). 1H-Estradiol ([1H]-E2; 1.92 TBq/ mmol) was purchased from Amersham Biosciences (Piscataway, NJ). For xenograft study, estrogen pellets were made according to the method by Wieder and Shimkin (20). Estrogen and antiestrogens were first dissolved in DMSO to prepare stock solutions, then diluted with the buffer or culture media for the in vitro experiments. For the in vivo experiments, a suspension of the drug in a vehicle for oral administration (0.5% hydroxypropylmethylcellulose) was prepared.

The MCF-7 cell line was obtained from American Type Culture Collection (Rockville, MD) and grown in RPMI 1640 medium, supplemented with 10% fetal bovine serum (HyClone, Logan, UT). After cell adhesion, the cells were treated with different concentrations of each antiestrogen, and reporter constructs (EREx3-Luc, 17m8-luc) have been described previously (22–25). The ligand binding domain (LBD) regions of ERα and ERβ expression vectors (HEG0, HEG19, pcDNA3ERα), VP16 fused TIF2 expression vector (VP-TIF2), and reporter constructs (EREx3-Luc, 17m8-luc) have been described previously (22–25). The ligand binding domain (LBD) regions of ERβ were inserted into the pM vector (Clontech, Franklin Lakes, NJ) to generate GAL-ERβ LBD.

For transfections, 293T cells were seeded in 12-well plates containing RPMI 1640 (phenol red–free) medium supplemented with 10% (v/v) charcoal dextran–treated fetal bovine serum (HyClone, Logan, UT). After cell adhesion, the cells were treated with different concentrations of each antiestrogen for 6 days. The cells were then fixed with glutaraldehyde and stained with crystal violet (21). The following formula was used to assess the number of cells (T/C%), based on dye concentration extracted from stained cells and measured by a spectrophotometer (λ = 540 nm):

\[ T/C(\%) = \left( \frac{absorption \ of \ treated \ group}{absorption \ of \ control \ group} \right) \times 100 \]

The IC50 value were determined based on the experimental data derived from experiments repeated six times.

Luciferase Assay and Mammalian Two-Hybrid Assay. ERα and ERβ expression vectors (HEG0, HEG19, pcDNA3ERβ), VP16 fused TIF2 expression vector (VP-TIF2), and reporter constructs (EREx3-Luc, 17m8-luc) have been described previously (22–25). The ligand binding domain (LBD) regions of ERβ were inserted into the pM vector (Clontech, Franklin Lakes, NJ) to generate GAL-ERβ LBD.

For transfections, 293T cells were seeded in 12-well plates in phenol red–free DMEM (Invitrogen) supplemented with 10% charcoal dextran–treated fetal bovine serum and l-glutamine. At 50% to 60% confluence, cells were transfected with the following plasmids: for luciferase assays, 100 ng EREx3-Luc plasmid was transfection.
cotransfected with 25 ng full-length ER expression vectors (HEG0, pcDNA3ERβ) or A/B region activation function (AF-1) deleted ERα expression vector (HEG19) by LipofectAMINE 2000 (Invitrogen); for mammalian two-hybrid assays, 1 μg 17m8-luc vector was cotransfected with 250 ng GAL-ERβEF constructs in combination with 250 ng VP-TIF2 plasmids or the mock plasmid VP by Lipofectin (Invitrogen).

As a reference plasmid to normalize for transfection efficiency, 5 ng pRL-cytomegalovirus vector (Promega, Madison, WI) for luciferase assay or 2.5 ng pRL-cytomegalovirus vector (Promega) for mammalian two-hybrid assay was cotransfected in all experiments. Six hours after transfection, media were replaced with fresh medium containing 10% fetal bovine serum. At this time, E2 and antiestrogens were added and cells incubated for an additional 24 hours. Preparation of cell extracts and dual luciferase assays were done following the manufacturer’s protocols (Promega). Individual transfections, each consisting of triplicate wells, were repeated at least three times. In mammalian two-hybrid assays, specific recruitment of TIF2 to ERα was evaluated by subtracting the mean measure of the wells transfected with VP-TIF2 by that for VP.

**Uterotrophic and Antiuterotrophic Assay in Ovariectomized Rats.** Four-week-old female Sprague-Dawley rats were ovariectomized, and randomly assigned to treatment groups (n = 7) 2 weeks after operation. TAS-108, tamoxifen, or raloxifene were prepared in 0.5% hydroxypropyl methylcellulose. Oral treatment by gavage was based on a total volume of 10 mL/kg body weight. E2 was prepared in 100% ethanol as a 100-fold stock and weighed. On day 4, animals were sacrificed and uteri were removed and weighed.

**DMBA Chemical-Induced Tumor Model.** Mammary tumors were induced in 50 ± 1-day-old female Sprague-Dawley rats by a single p.o. dose of 20 mg DMBA in 1.0 mL of sesame oil (26, 27). Drug treatment was started when the tumors reached a diameter of about 10 mm. TAS-108 was given p.o. daily for 4 weeks.

The size of the tumors was recorded once a week, using the two perpendicular dimensions, and the tumor volume (TV) was calculated from the following formula:

\[
TV = \frac{1}{2} \times \text{length} \times \text{width} \times \text{height}
\]

The relative tumor volume (RTV) was then calculated as the ratio of the TV on day n to that on day 1, according to following formula:

\[
RTV = \frac{TV \text{ on day } n}{TV \text{ on day } 1}
\]

**Xenograft Study in Nude Mice.** A 2-mm³ tumor fragment was implanted into the right flank of a female nude mouse. Estrogen supplementation was provided by the s.c. implantation of an E2 pellet into the left flank of the mouse. When a tumor grew to the size of a diameter of about 6 mm, mice were allocated to the experimental groups and given TAS-108 p.o. daily for 3 weeks.

The size of the tumors was recorded on the day following the last administration (day 22), using the two perpendicular dimensions, the TV was calculated from the following formula:

\[
TV = \frac{1}{2} \times \text{length} \times \text{width} \times \text{height}
\]

The RTV was then calculated as the ratio of the TV on day 22 to that of day 1, according to following formula:

\[
RTV = \frac{TV \text{ on day } 22}{TV \text{ on day } 1}
\]

**RESULTS**

**The Binding Affinity of TAS-108 to ERα and ERβ.** To estimate the binding affinity of TAS-108 to ERs in comparison with other known antiestrogenic agents, competition assay using radiolabeled E2 was carried out. RBA was calculated from the ratio between the concentrations of test compounds and nonradioactive E2 necessary for 50% reduction of ER-specific binding. TAS-108 exhibited high binding affinity to both ERα and ERβ, and the RBAs were 80% and 98%, respectively, which were much higher than tamoxifen and were similar to that of 4-hydroxytamoxifen, an active metabolite of tamoxifen, and fulvestrant (Table 1). On the other hand, the binding affinity of raloxifene against ERα showed similar levels to TAS-108, but its binding affinity against ERβ was almost 10 times weaker than ERα.

**In vitro Anti-Proliferative Activity of TAS-108 Against ER-Positive MCF-7 Cell Line.** The antiproliferative activity of TAS-108 on ER-positive/estrogen-responsive MCF-7 cells, growing in the presence of E2, was determined and compared with antiestrogens. TAS-108 inhibited the estrogen-dependent cell growth of MCF-7 with an IC₅₀ value of 34 nmol/L (Fig. 2). Tamoxifen weakly inhibited the proliferation of MCF-7 cells at an IC₅₀ value of 1.5 μM. Fulvestrant, raloxifene, and 4-hydroxytamoxifen exhibited equally strong growth inhibitory activities with IC₅₀ values of 3.6, 4.2, and 5.5 nmol/L, respectively (Fig. 2).

**Agonistic/Antagonistic Activity of TAS-108 on ERα and ERβ in the Reporter Assay System.** The agonistic and antagonistic activities of TAS-108 on ERα and ERβ were compared with other known antiestrogenic agents. First, to compare the agonistic activities, ligand-induced transactivation of ERα and ERβ was examined using a transient expression assay. An ERα reporter plasmid (EREx3-Luc) and an ERα or ERβ expression vector were cotransfected into 293T cells, and then cells were incubated in the presence of E2 with or without agents. As shown in Fig. 3A, TAS-108 inhibited the transactivation of ERα stronger than 4-hydroxytamoxifen, such as fulvestrant, a pure antiestrogen, and raloxifene, a selective estrogen receptor modulator. Interestingly, the antagonistic potencies of these agents against ERβ were more varied (Fig. 3B). Fulvestrant effectively

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Binding affinity of TAS-108 and various antiestrogens to ERs</th>
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<tbody>
<tr>
<td></td>
<td>ERα</td>
</tr>
<tr>
<td>IC₅₀ (nmol/L)</td>
<td>RBA (%)</td>
</tr>
<tr>
<td>E2</td>
<td>8.8</td>
</tr>
<tr>
<td>TAS-108</td>
<td>11</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>76</td>
</tr>
<tr>
<td>4-Hydroxytamoxifen</td>
<td>6.3</td>
</tr>
<tr>
<td>Raloxifene</td>
<td>22</td>
</tr>
<tr>
<td>Fulvestrant</td>
<td>34</td>
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Note: Each value was the mean of three independent experiments.
agonized ERβ similar to ERα. Although TAS-108 and raloxifene inhibited transactivation of ERβ, when compared with the case of ERα, its antagonistic activities were much weaker. On the other hand, OHT inhibited the transactivation of ERβ more prominent than that of ERα.

Next, we compared the agonistic activity of these agents against ERs. Only 4-hydroxytamoxifen activated transcription of ERα (Fig. 4A); however, other agents such as TAS-108, did not exhibit transactivation of ERα. In the ERα mutant deleted AF-1 domain, 4-hydroxytamoxifen showed no agonistic activity. We previously reported that the agonistic activity of tamoxifen came from AF-1 activation (25). Strikingly, in contrast with ERα, TAS-108 exhibited an agonistic activity against the transactivation of ERβ similar to 4-hydroxytamoxifen (Fig. 4B). Fulvestrant and raloxifene did not exhibit agonistic activity against ERβ similar to ERα.

It has been well established that ERs exhibit two types of transactivation, AF-1 and AF-2, and they are characterized by several coregulator binding ERs (28, 29). To clarify which type of transactivation or which co-activator affects the agonistic activity of TAS-108 against ERβ, we examined the transactivation activity of ERβ by TAS-108 using mammalian two-hybrid assay between GAL4-fused ERβ-LBD and VP16-fused TIF2, a major co-activator in AF-2. Figure 4C clearly showed that TAS-108 induced the strongest ERβ transactivation among the antiestrogenic agents, which means that TAS-108 induces the AF-2 transactivation of ERβ by recruiting coactivator TIF2 to ERβ. On the other hand, 4-hydroxytamoxifen induced only weak transactivation in this system in spite of exhibiting agonistic activity against full-length ERβ. Its agonistic activity may depend on AF-1 as we have previously shown in the case of ERα (25). The partial agonistic property of TAS-108 to ERβ, with the addition of a pure antagonistic feature against ERα, is the unique mode of action of ERs that have never been observed in other antiestrogenic agents.

**Agonistic/Antagonistic Activity Of TAS-108 as Determined in the Model of Ovariectomized Rat Uterus.** To test the uterotrophic and antiuterotrophic activity of TAS-108, the uterine response in weight of ovariectomized rats was tested in the presence and absence of E2, respectively. Tamoxifen alone exhibited estrogenic activity on uterine weights in ovariectomized rats under the non-E2 supplement condition (Fig. 5A), and a little antiestrogenic activity under the E2 supplement condition (Fig. 5B). Raloxifene exhibited little estrogenic activity in the absence of E2 (Fig. 5A), and exhibited antiestrogenic activity in the presence of E2 (Fig. 5B). Like raloxifene, TAS-108 significantly antagonized the E2 action on...
uterus with little agonistic activity (Fig. 5A and B). TAS-108 seemed more potent as an antagonist in the uterine response than raloxifene in the absence of E2. Thus, unlike tamoxifen, TAS-108 acted as a potent antagonist in the uterus and had low risk for uterotrophic activity similar to raloxifene.

In vivo Antitumor Activity in the DMBA-Induced Rat Mammary Tumor Model and in the MCF-7 Human Mammary Tumor Xenograft. To determine the antitumor activity, TAS-108 was given p.o. in the DMBA-induced rat mammary tumor model. This model does not require exogenous
E2 and tumor growth depends on endogenous estrogen. This was confirmed in ovariectomized rats. In ovariectomized rats, the growth of DMBA-induced mammary tumor was completely suppressed by depletion of endogenously produced estrogen (Fig. 6).

TAS-108 given p.o. also markedly inhibited tumor growth in a dose-dependent manner at the dose range from 0.3 to 30 mg/kg/day (Fig. 6). There was no obvious toxic manifestation (data not shown). It is noteworthy that this dose range was similar to that used in the uterotrophic assay in Fig. 5. This means that TAS-108 inhibits E2-dependent tumor growth within the dose range exhibiting little uterotrophic activity. Next, the antitumor effect of TAS-108 was studied in the MCF-7 human breast cancer xenograft models, high exogenous E2 supplement model. TAS-108 also exhibited significant tumor growth inhibition from the dose of 1 mg/kg/day in a dose-dependent manner (Fig. 7A).

The antitumor activity of TAS-108 was similar or potentially higher than tamoxifen, both compounds did not affect the body weight of nude mice (Fig. 7B). Thus, TAS-108 efficiently inhibited E2-dependent tumor growth under both low endogenous and high exogenous E2 levels without risk of uterotrophy.

DISCUSSION

Tamoxifen has played a major role in the hormonal therapies for both early and advanced ER-positive breast cancers (1, 2). However, the drawback of tamoxifen therapy is that it is associated with an increased risk of developing endometrial cancer, cataracts, and thromboembolic events based on its agonistic activity on ERs (8, 9). Some estrogen depletion strategies such as aromatase inhibitors or pure antiestrogens have overcome these drawbacks and exhibited some improvement for metastatic and advanced breast cancer therapy (16–18). However, these drugs have no agonistic activity on ERs and lack the clinical benefits of tamoxifen’s agonistic properties, such as the prevention of osteoporosis and cardiac disease. On the other hand, raloxifene has gained approval for the prevention and treatment of osteoporosis, but it has not exhibited superiority over tamoxifen in advanced cancer therapy (13). Thus, there has been no anticancer agent which is superior to the clinical benefits of tamoxifen while continuing to provide favorable effects in the treatment of osteoporosis and cardiac disease treatment. Here we have shown that TAS-108 given p.o., with its novel mode of action on ERs, exhibited antitumor activities against E2-dependent tumor growth of MCF-7 xenografts and DMBA-induced rat mammary carcinoma models with little uterotrophic effect.

TAS-108 had high binding affinity to ERα (Table 1) and inhibited its transactivation without any agonistic properties (Figs. 3A and 4A), which is in striking contrast to the agonistic property of tamoxifen (Fig. 4A). This is because tamoxifen can inhibit AF-2 situated in COOH-terminal E domain of ERα but induces AF-1 activation, situated in NH2-terminal A/B domain, whereas TAS-108 inhibits both AF-1 and AF-2 transactivation by promoting the recruitment of the SMRT co-repressor as we have previously reported (25). The transactivation of ERα AF-1 by tamoxifen is thought to be the mechanism for the agonistic property of tamoxifen leading to its resistance (28, 30, 31), and
the risk of endometrial cancers in patients with breast cancer (8, 28, 30). TAS-108 is expected to overcome at least some type of tamoxifen resistance without the risk of endometrial cancer (25).

In fact, TAS-108 given p.o. showed little uterotrophic effect on ovariectomized rats under the non-E2 supplement condition at the dose level exhibiting significant tumor growth inhibition (Figs. 5A and 6), and it exhibited more potent antagonistic activity on rat uterus than that of tamoxifen under the E2 supplement condition (Fig. 5B). Recently, this beneficial property in the uterus of TAS-108 has been examined in phase I clinical trials (32).

The action on ERβ of TAS-108 was another unique characteristic among antiestrogen agents. As shown in Fig. 4B and C, it induced transactivation of ERβ such as tamoxifen and dramatically induced the AF-2 transactivation of ERβ much more than any other antiestrogenic agent. This clearly indicates that TAS-108 acts as a partial agonist of ERβ through the recruitment of TIF2 coactivator in contrast to its pure antagonistic action on ERα. The biological function of ERβ is still not well understood and controversial (33, 34), and we have not had enough data to link the agonistic action of TAS-108 and ERβ to its beneficial effect. However, reports have indicated that ERβ is expressed in many tissue organs and has an important biological function in several tissues such as bones, lungs, or others (35–37). Also, there have been reports that ERβ acts as an inhibitor of ERα function (38), and the amount of ERβ expression is reported to be negatively correlated with the malignancy of breast cancer (33, 39). For example, genistein, an ERβ-selective agonist, is reported to prevent osteoporosis (40). Therefore, we expect that the prominent agonistic property TAS-108 on ERβ may beneficially affect some organs such as bone or cardiovascular systems similar to the agonistic property of tamoxifen on ERα. We are now examining the effects of TAS-108 on bone metabolisms. Preliminary data shows that TAS-108 prevents osteoporosis in ovariectomized rats or mice (data not shown).

The characteristic summaries of the unique mode of action of TAS-108, when compared with other known antiestrogenic agents are follows: in comparison to tamoxifen, TAS-108 is a pure antagonist on ERα unlike tamoxifen (25). On ERβ, it acts as a partial agonist similar to tamoxifen but both modes of action are different. Tamoxifen induces no AF-2 transactivation of ERβ, whereas TAS-108 markedly induces AF-2 transactivation by promoting the recruitment of TIF-2, a co-activator (Fig. 4). In the comparison to raloxifene, a selective estrogen receptor modulator, although raloxifene is a pure antagonist of ERα, it acts as an agonist on D351Y mutant ERα derived from a tamoxifen-resistant breast cancer cell line. TAS-108 acts as an ERα antagonist even in this mutant (25). The antagonistic activity of raloxifene on ERβ is much weaker than that on ERα (Fig. 3A and B). This is because its binding affinity to ERβ is 10 times weaker than that of ERα (Table 1; ref. 41). On the other hand, TAS-108 binds both ERs with the same affinity (Table 1). In comparison, fulvestrant acts as a pure antiestrogen on both ERα and ERβ via down-regulation of ERs and binding inhibition of ERs to DNA (42). This is completely different from the mode of action of TAS-108 which modulates the recruitment of coregulators to ERs and exhibits agonistic properties on ERβ in the absence of E2 (25).

Finally, TAS-108 given p.o. significantly inhibited tumor growth against DMBA-induced rat mammary carcinoma, physiologic endogenous E2 models, at a dosage of 1 to 3 mg/kg/day (Fig. 6). This dosing level is similar to that used in the uterotrophic test of Fig. 5. TAS-108 also inhibited E2-dependent tumor growth against MCF-7 xenograft, exogenous high E2 supplement models, at a dosage of 1 mg/kg/day. This indicates that TAS-108 can inhibit tumor growth even under high E2 concentrations in the blood at approximately the same dosage level range inhibiting tumor growth in low E2 concentrations (43–46). These will also be important results when its clinical usage is considered because tamoxifen is currently one of the few choices for hormonal therapy in premenopausal women with advanced breast cancer. Aromatase inhibitors cannot be used in premenopausal women without complete estrogen blockade with luteinizing hormone–releasing hormone. If TAS-108 can inhibit ERα transactivation in patients with premenopausal breast cancer in high E2 concentration in the blood, it would provide patients another choice for treatment with low risk for endometrial cancer.

In summary, TAS-108 has a novel characteristic for ER modulation, a pure antagonist of ERα and a partial agonist of ERβ exhibiting strong antigluttumor effects with little uterotrophic effects. It will be expected that TAS-108 becomes an ideal endocrine therapy which has efficacy for advanced breast cancer, safety for uteri, and agonistic benefits for osteoporosis and cardiac disease via its novel mode of action on ERs. We are now developing phase II clinical trials for advanced postmenopausal and premenopausal breast cancer.

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REFERENCES


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