Thiochroman Derivative CH4986399, A New Nonsteroidal Estrogen Receptor Down-regulator, Is Effective in Breast Cancer Models

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Abstract. Background: Tamoxifen, a selective estrogen receptor modulator, and fulvestrant, a selective estrogen receptor down-regulator (SERD), are now available for estrogen receptor-positive breast cancer patients. However, these patients acquire drug resistance during the treatments. We identified a new orally active nonsteroidal SERD, CH4986399, which is structurally unrelated to fulvestrant and tamoxifen. Materials and Methods: We examined the oral antitumor activity and down-regulation of ER by CH4986399 in human breast cancer Br-10 and ZR-75-1 xenografts. Results: In the Br-10 xenografts, CH4986399 (100 mg/kg p.o.) as well as fulvestrant (3 mg/body s.c.) strongly reduced tumor weight. In the ZR-75-1 xenografts, CH4986399 (100 mg/kg p.o.) strongly reduced tumor weight and ER content without agonistic activity. In contrast, tamoxifen (100 mg/kg p.o.) showed only moderate antitumor activity and no ER down-regulation. Conclusion: With a chemical structure different from both fulvestrant and tamoxifen, CH4986399, may help overcome drug resistance from the endocrine treatment sequence for breast cancer patients.

Currently, three classes of antiestrogenic agent, selective estrogen receptor modulators (SERMs), aromatase inhibitors (AIs) and selective estrogen receptor down-regulators (SERDs), are available for estrogen receptor (ER)-positive breast cancer patients. The SERM tamoxifen has been widely used for ER-positive breast cancer patients for many years (1). Recently, AIs, such as anastrozole, are replacing tamoxifen as first-line and adjuvant treatment (1). The SERD fulvestrant has proven to be as effective as anastrozole in patients who had relapsed during tamoxifen treatment (2, 3), and has shown efficacy similar to tamoxifen in postmenopausal women with ER-positive advanced breast cancer as the first-line treatment (4). Currently, a combination therapy of fulvestrant with anastrozole is being investigated as a first-line treatment for these patients (5, 6). However, fulvestrant has a steroidal structure and must be administered intramuscularly in a clinical setting due to its poor bioavailability. In addition, with fulvestrant, as with tamoxifen, patients acquire drug resistance during the treatment (1, 7, 8). Therefore, there is a need for new, non-crossresistant, orally active SERDs that are structurally unrelated to fulvestrant and tamoxifen.

In 2002 and 2007, Dardes et al. introduced and further studied an orally available nonsteroidal SERD (GW5638) with a chemical structure similar to that of tamoxifen and found it to be efficacious against ER-positive human breast cancer (9, 10). In 2006, we introduced another orally active nonsteroidal SERD, the thiochroman derivative CH4986399 (compound 19b in ref. 11) that is structurally different from both fulvestrant and tamoxifen (11). The present study shows that the effects of oral administration of CH4986399 on human breast cancer growth in Br-10 and ZR-75-1 xenografts in vivo.

Materials and Methods

Drugs. CH4986399, fulvestrant, arzoxifene, EM-652 and ERA-923 (Figure 1) were synthesized in Chemistry Research Department I, Chugai Pharmaceutical Co., Ltd., Japan. Tamoxifen, 4OH-tamoxifen, 17β-estradiol (E2) and 17β-estradiol 3-benzoate (17β-EB) were purchased from Sigma (St. Louis, MO, USA).

Animals. Female, 5-week-old athymic nude mice were purchased from Nihon Clea (Tokyo, Japan). Mouse chow and tap water were freely available to the mice. All experiments were approved by the
Animal Experimentation Ethics Committee in Chugai and carried out according to the Guidelines for the Care and Use of Laboratory Animals at Chugai Pharmaceutical Co., Ltd.

Cells. Human breast cancer cell line ZR-75-1 was purchased from the American Type Culture Collection (ATCC) (Manassas, VA, USA). The ZR-75-1 cells were cultured in RPMI-1640 supplemented with 10% fetal bovine serum (FBS).

Human breast cancer Br-10 tumors were purchased from Central Institute for Experimental Animals (Kanagawa, Japan). For the Br-10 xenograft experiments, Br-10 tumors were maintained by serial subcutaneous (s.c.) transplantation in the subaxillary region of female nude mice.

Antiestrogenic and estrogenic activity of CH4986399 in vivo. The estrogenic and antiestrogenic activities of CH4986399 were evaluated by measuring the uterotrophic and antiuseterminic effects in Institute of Cancer Research (ICR) mice. Mice were ovariectomized 2 weeks before the administration of the drug. To evaluate the antiuseterminic activities of the drug, ovariectomized (OVX) mice were subcutaneously administered 0.1 μg of 17β-EB and then orally administered 1, 3 or 10 mg/kg of CH4986399 once a day for 3 days. To evaluate the estrogenic activity of the drug, the OVX mice were orally administered 100 mg/kg of CH4986399 once a day for 3 days. Uterine weights were determined on day following the last administration.

Effect of CH4986399 on ER expression in ZR-75-1 cells in vitro. To determine the effects of the tested drugs on the cellular level of ER, 6×10^5 ZR-75-1 cells were seeded onto a 6-cm dish and cultured for 3 days. CH4986399, fulvestrant, 4OH-Tamoxifen, Arzoxifene, EM-652, or ERA-923 was then added to the culture at concentrations ranging from 0.1 to 10000 nM, and the cells were further cultured for 2 days. At the end of the culture, the cells were collected and

Figure 1. Chemical structures of fulvestrant, CH4986399, tamoxifen, arzoxifene, EM-652 and ERA-923.
Effects of CH4986399 on OVX ZR-75-1 xenografts. OVX female nude mice were implanted s.c. with E2 implants (E2:C=1:99) 4 days before ZR-75-1 cell transplantation to maintain the ZR-75-1 tumors. The E2 implants were removed from the ZR-75-1 xenografts when the tumor volume reached about 50 mm³; CH4986399 (100 mg/kg) or tamoxifen (100 mg/kg) were then orally administered 5 times per week for 5 weeks. Tumor volumes were measured once a week. Tumor volume (V) was estimated using the equation V=ab²/2, where a and b are tumor length and width, respectively.

**Statistical analysis.** To compare differences in tumor weights, Student’s unpaired t-test was carried out.

**Results**

Antiestrogenic and estrogenic activity of CH4986399 in vivo. In an attempt to identify an orally active nonsteroidal SERD, we synthesized a number of nonsteroidal compounds and tested their inhibitory effects against the binding of estrogen (E2) to ER in vitro and their antiestrogenic and estrogenic activities in OVX mice in vivo. One of the compounds, CH4986399, inhibited the binding of E2 to ER with an IC₅₀ value of 6.4 μM, and reduced E2 (17β-EB)-stimulated uterine growth in OVX mice in vivo. As shown in Table I, oral administration of CH4986399 (1, 3, 10 mg/kg) to the OVX mice that had received 17β-EB reduced uterine weight in a dose-dependent manner and, at 10 mg/kg, CH4986399 lowered the uterine weight to that of the OVX control (E2(–) cont.).

**Table I.** Antiestrogenic and estrogenic effects of CH4986399 in OVX mice. E2(–) control: Control OVX mice that did not receive 17β-EB. E2(+) control: Control OVX mice that received 17β-EB.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Uterine weight (mg/100g body weight)</th>
<th>Antiestrogenic activity (% inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2(–) cont.</td>
<td>63.2±3.0</td>
<td>100.0%</td>
</tr>
<tr>
<td>E2(+)</td>
<td>382.0±39.1</td>
<td>0.0%</td>
</tr>
<tr>
<td>E2(+) + CH4986399 (1 mg/kg p.o.)</td>
<td>261.9±12.1</td>
<td>37.7%</td>
</tr>
<tr>
<td>E2(+) + CH4986399 (3 mg/kg p.o.)</td>
<td>171.6±11.5</td>
<td>66.0%</td>
</tr>
<tr>
<td>E2(+) + CH4986399 (10 mg/kg p.o.)</td>
<td>93.4±2.6</td>
<td>90.5%</td>
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</tbody>
</table>

**B. Estrogenic activity of CH4986399.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Uterine weight (mg/100g body weight)</th>
<th>Estrogenic activity (% change)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2(–) cont.</td>
<td>65.7±4.1</td>
<td>0.0%</td>
</tr>
<tr>
<td>E2(+) + CH4986399 (100 mg/kg p.o.)</td>
<td>63.4±2.7</td>
<td>–0.8%</td>
</tr>
<tr>
<td>E2(+) cont.</td>
<td>339.7±23.6</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

At the end of the experimental period, the tumors were collected and frozen at –140°C in a deep freezer. The level of ER protein was determined as above.

Effects of CH4986399 on ER down-regulation in ZR-75-1 cells in vitro. The ER down-regulation rate (%) indicates the mean value of four independent experiments.

<table>
<thead>
<tr>
<th>ER down-regulation (% inhibition)</th>
<th>IC₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>SERDs</td>
<td></td>
</tr>
<tr>
<td>CH4986399</td>
<td>n.t.</td>
</tr>
<tr>
<td>Fulvestrant</td>
<td>95%</td>
</tr>
<tr>
<td>SERMs</td>
<td></td>
</tr>
<tr>
<td>4OH-Tamoxifen</td>
<td>127%</td>
</tr>
<tr>
<td>Arzoxifene</td>
<td>258%</td>
</tr>
<tr>
<td>EM-652</td>
<td>70%</td>
</tr>
<tr>
<td>ERA-923</td>
<td>89%</td>
</tr>
</tbody>
</table>

n.t., Not tested.

Effect of tamoxifen, arzoxifene, EM-652, ERA-923, fulvestrant and CH4986399 on ER down-regulation in ZR-75-1 cells in vitro. The ER down-regulation rate (%) indicates the mean value of four independent experiments.
Effect of CH4986399 on ER expression in ZR-75-1 cells in vitro. As CH4986399 caused an antiestrogenic effect without estrogogenic effect in vivo, we expected that, like fulvestrant, CH4986399 has the ability to down-regulate ER. To address this question, we examined its effect on ER expression in ZR-75-1 cells by EIA. As shown in Table II, both CH4986399 and fulvestrant reduced the level of ER in a concentration-dependent manner with IC50 values of 295 and 67 nM, respectively, whereas SERMs tamoxifen and arzoxifen increased the level of ER. The other SERMs, EM-652 and ERA-923, showed moderate effects.

Antitumor activity of CH4986399 in Br-10 xenografts. In the ZR-75-1 cell experiments, the IC50 of CH4986399 for ER down-regulation was greater than that of fulvestrant. Nevertheless, CH4986399 should exhibit strong antitumor activity in vivo if efficiently absorbed and a sufficient concentration in blood attained for tumor growth inhibition. In fact, the bioavailability of CH4986399 was 34.3% in rats (data not shown, compound 19b in ref.11); therefore, we examined its antitumor activity using human breast cancer Br-10 xenografts, in which both fulvestrant and tamoxifen have exhibited antitumor activity (14). As shown in Figure 2, CH4986399 at 100 mg/kg p.o. inhibited tumor growth to almost the same extent as fulvestrant (3 mg/body s.c.). On the other hand, the antitumor activity of tamoxifen at 10 mg/kg p.o. was only slight.

Discussion

Tamoxifen, an oral SERM, has been the standard endocrine therapy for ER-positive breast cancer patients for many years (1); however, most patients ultimately become resistant to this drug (1, 8). Some antiestrogenic agents such as the AI anastrozole and the SERD fulvestrant, with modes of action different from tamoxifen, have been developed to overcome tamoxifen resistance (15-17). Even so, most patients experience relapse or disease progression following endocrine treatment sequences (7, 16). Therefore, new, non-crossresistant antiestrogenic agents are needed for endocrine treatment in breast cancer patients. The present study shows that the thiochroman derivative CH4986399, a new orally active nonsteroidal SERD with a chemical structure different from both fulvestrant and tamoxifen, is efficacious against human breast cancer tumors both in vitro and in vivo.

In the OVX mice experiments in vivo, oral administration of CH4986399 reduced E2-stimulated uterine growth in a dose-dependent manner without estrogenic activity (Table I). In the ZR-75-1 cells in vitro, CH4986399 as well as fulvestrant reduced the level of ER protein in a concentration-dependent manner (Table II). Thus, CH4986399 has the characteristics of an orally available SERD. On the other hand, the SERMs tested (tamoxifen,
arzoxifene, EM-652, and ERA-923) increased the ER levels or showed only partial down-regulation of ER.

In the Br-10 xenografts in vivo, in which both fulvestrant and tamoxifen showed antitumor activities (14), CH4986399 at 100 mg/kg reduced tumor weight to the same level as in the OVX control (Figure 2). In the ZR-75-1 xenografts in vivo showing partial tamoxifen resistance (13), CH4986399 at 100 mg/kg decreased both tumor weight and the ER content to levels observed in the OVX control. On the other hand, tamoxifen at 30 and 100 mg/kg showed only modest antitumor activity and no down-regulation of ER (Figure 3). In addition, in the OVX ZR-75-1 xenografts, tamoxifen at 100 mg/kg led to an increase in tumor volume, whereas CH4986399 at 100 mg/kg did not increase tumor volume (Figure 4). Thus, the results of our human breast cancer Br-10 and ZR-75-1 xenograft in vivo studies show that CH4893237, unlike tamoxifen, demonstrated potent efficacies without agonistic activity, suggesting that CH4893237 would have clinical efficacy in tamoxifen-resistant ER-positive breast cancer patients.

Although the mechanisms of tamoxifen- and fulvestrant-resistance are not clearly understood, antiestrogenic agents chemically different from fulvestrant or tamoxifen are surmised to be efficacious against fulvestrant- or tamoxifen-resistant tumors. Wittmann et al. suggested the possibility that GW5638, a nonsteroidal tamoxifen derivative with a chemical structure different from that of fulvestrant, is active against fulvestrant-resistant tumors because the mechanisms of ER down-regulation are not the same as with fulvestrant (10). In addition, Brady et al. suggested the possibility that SP500263, a nonsteroidal SERM with a chemical structure different from that of tamoxifen, shows efficacy against tamoxifen-resistant tumors because of a difference in the induction of the conformational changes in the ER (18). Thus, an antiestrogenic agent whose structure is unrelated to both fulvestrant and tamoxifen has the possibility of having efficacy against both fulvestrant- and tamoxifen-resistant tumors. In fact, our data

Figure 3. Tumor weight in ZR-75-1 xenografts and degradation of ER in the ZR-75-1 tumors. OVX nude mice carrying the E2 implant (E2:C=1:99) were inoculated with ZR-75-1 cells. Drug administration was initiated when the tumor volume reached between 50 and 150 mm$^3$. A: The mice were orally administered 30 or 100 mg/kg of CH48986399 or 30 or 100 mg/kg of tamoxifen 5 times per week for 6 weeks. Control mice received only vehicle. Tumor weights were determined on the next day after the final administration. B: At the end of the study, the tumors were collected, frozen (–140°C), and the ER content quantified by ER-EIA. Bars represent the mean value and SEM of seven tumors. P<0.01 (unpaired t-test). E2(+) control: Control OVX mice that received estrogen; E2(−) control: Control OVX mice that did not receive estrogen.

Figure 4. Effects of tamoxifen and CH4893237 in OVX ZR-75-1 xenografts. OVX female nude mice carrying the E2 implants (E2:C=1:99) were inoculated with the ZR-75-1 cells. The E2 implants were removed from the ZR-75-1 xenografts when the tumor volume reached about 50 mm$^3$, and then 100 mg/kg of CH4893237 or 100 mg/kg of tamoxifen were orally administered 5 times per week for 5 weeks. Data points represent the mean value and SEM of six tumors. OVX-control: Control OVX mice that did not receive estrogen.
indicate that CH4986399, a thiochroman derivative chemically different from tamoxifen, was efficacious against the partial tamoxifen-resistant ZR-75-1 xenograft (Figure 3). As the chemical structure is also unrelated to fulvestrant, in addition to tamoxifen-resistant tumors, CH4986399 may have efficacy against fulvestrant-resistant tumors.

The bioavailability of CH4986399 needs to be clarified in a clinical setting; however, liver microsome experiments in vitro indicate that it is stable in human microsomes compared with in rats (data not shown). CH4986399, therefore, may be useful in endocrine treatment sequences for breast cancer patients with fulvestrant- or tamoxifen-resistant tumors.

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References


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