

Effect of toremifene and ospemifene, compared to acolbifene, on estrogen-sensitive parameters in rat and human uterine tissues

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Abstract

Background: Although the first generation selective estrogen receptor modulator (SERM) tamoxifen (TAM) is well known for its uterotrophic activity, this study compares the stimulatory effect of the TAM derivatives toremifene (TORE) and ospemifene (OSPE) on estrogen-sensitive parameters in rat and human uterine tissues.

Material and methods: Ovariectomized female rats were treated daily orally for 10 days with 0.75 mg/rat of TORE, OSPE or acolbifene (ACOL, a pure estrogen antagonist in the uterus and mammary gland), which was used for comparison. Human endometrial carcinoma Ishikawa cells were incubated for 5 days with increasing doses of compounds, in the absence or presence of 1 nM estradiol (E₂).

Results: TORE and OSPE revealed 52% and 56% increases, respectively, in uterine weight, whereas ACOL had no effect. Similar effects were observed on vaginal weight. Endometrial epithelial height increased from 15.82 ± 0.20 to 48.94 ± 2.12 and 42.14 ± 1.95 μm with TORE and OSPE, respectively, whereas ACOL had no effect. Alkaline phosphatase activity, an estrogen-sensitive parameter in Ishikawa cells, was increased by 144% and 135% with OH-TORE and OH-OSPE, respectively. Owing to their intrinsic estrogenic activity, at maximal concentrations, OH-TORE and OH-OSPE blocked the stimulatory effect of E₂ by only 89% compared to 100% with ACOL.

Conclusions: The present in vitro and in vivo data show similar stimulatory effects of 4-hydroxytoremifene (OH-TORE) and OH-OSPE on estrogen-sensitive parameters. ACOL, a third generation SERM, has no effect on any of these parameters. Such data add to the potential uterine safety limitations of triphenylethylene-derived SERMs for long-term use in humans.

Keywords: acolbifene; Ishikawa cells; ospemifene; rat uterus; toremifene.

Introduction

Selective estrogen receptor modulators (SERMs) could well become the best example of the success achievable by organic chemistry and pharmaceutical research and development. Each SERM induces ligand- and cell-specific three-dimensional structural changes of the estrogen receptors (ERs) which lead to a multitude of different activities of the SERM-ER complex. Such SERM-induced unique modifications of the three-dimensional structure of ER in combination with cell-specific coactivators and corepressors can lead, at one extreme, to a complete blockade of the normal action of estrogens in some tissues (pure estrogen antagonistic action), whereas in other tissues, the same SERM-ER complex mimics the natural action of estrogens (estrogen agonistic action) (1, 2).

The ideal SERM should have an excellent safety profile, particularly pure estrogen antagonistic activity in the mammary gland and uterus to ensure good tolerance during 20–40 years of a woman's life to prevent breast and uterine cancer (3). A compound that could efficiently prevent breast and uterine cancer, while at the same time preventing bone loss, would be a major breakthrough for the benefit of women's health.

Only two first generation SERMs, both triphenylethylene derivatives, namely tamoxifen (TAM) and toremifene (TORE) (Figure 1), are approved for the treatment of breast cancer while raloxifene, a second generation SERM, is approved only for breast cancer prevention. Although TAM has a positive effect on bone, the increased risk of endometrial cancer eliminates this compound as a possible therapy for osteoporosis or any other benign disease where long-term administration is required.

Because each SERM exerts different levels of tissue-specific antiestrogenic, estrogenic or mixed antiestrogenic/estrogenic activity, it is important to obtain information on the uterotrophic activity of compounds to support their long-term use and avoid the risk of endometrial stimulation (4, 5). It is thus of interest to study in well-established in vitro and in vivo models the estrogenic activity of two close analogs of TAM, namely ospemifene (OSPE) and TORE on in vitro alkaline phosphatase activity in human endometrial adenocarcinoma Ishikawa cells as well as in vivo on endometrial thickness and uterine weight in the rat and to compare with

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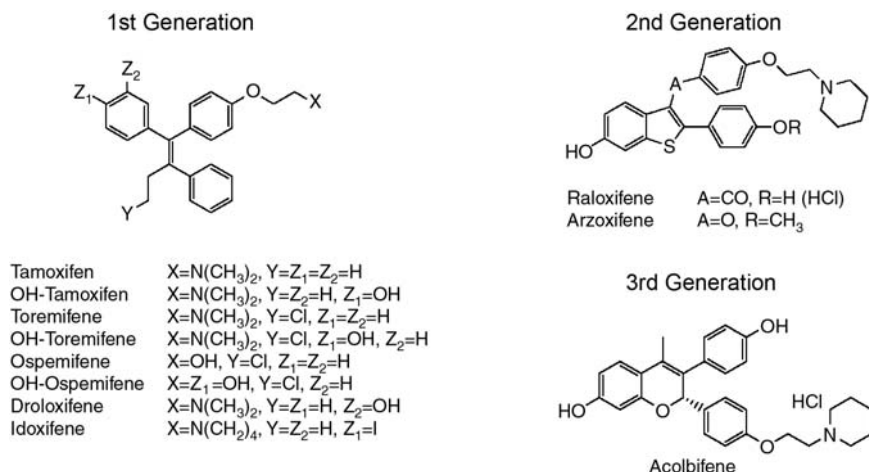


Figure 1 Chemical structure of some SERMs from first, second and third generation.

acolbifene (ACOL), a third generation SERM having pure estrogen antagonistic activity in the uterus.

Materials and methods

Synthesis of OSPE and 4-hydroxyospemifene (OH-OSPE)

Synthesis of OSPE was performed using the McMurry reaction strategy previously described for the synthesis of (Z)-TAM (6). The condensation of 4-(2-hydroxyethoxy)benzophenone (three steps with slight modifications (7)) and 3-chloropropiophenone was done with TiCl₄/Zn in refluxed THF yielded crude OSPE in a 3:1 *Z/E* ratio which was purified by twice recrystallization from methanol-water to yield a >100:1 *Z/E* ratio at a 21% yield of pure OSPE. The proton NMR spectrum (400 MHz) was in accordance with the literature (Sodervall M, Eloranta J, Kalapudas A. Hormos Medical Ltd, United States Patent Application Publication US2008/0207956, August 28, 2008): (CDCl₃) δ 1.92 (t, *J*=6.2 Hz, 1H), 2.93 (t, *J*=7.5 Hz, 2H), 3.42 (t, *J*=7.5 Hz, 2H), 3.88 (m, 2H), 3.95 (deformed t, *J*=4.4 Hz, 2H), 6.57 (d, *J*=8.8 Hz, 2H), 6.80 (d, *J*=8.8 Hz, 2H), 7.13–7.39 (m, 10H).

A new synthesis of OH-OSPE was also performed using the McMurry reaction strategy previously described by us for the synthesis of (Z)-4-hydroxyTAM and (Z)-4-hydroxytoremifene (8). (*E*)-1-(4-Hydroxyphenyl)-1-[4-(benzoyloxy)phenyl]-2-phenyl-4-chlorobut-1-ene cited by our group (8) was etherified with 2-hydroxyethyl benzoate in a Mitsunobu reaction (DIAD, PPh₃) to yield OH-OSPE dibenzoate at a 87% yield. Dibenzoate was cleaved with MeLi at –78°C to yield crude OH-OSPE at a >19:1 *Z/E* ratio which was purified by flash chromatography (ethyl acetate-toluene) and by twice recrystallization from methanol-water to yield a >100:1 *Z/E* ratio at a 20% yield of pure OH-OSPE in the *Z*-configuration. The proton NMR spectrum (400 MHz) was in accordance with the literature (DeGregorio M, Wiebe V, Kangas L, Härkönen P, Väänänen K, Laine A. Orion-Yhtymä Oy, United States Patent 5,750,576, May 12, 1998): (CD₃OD) δ 2.96 (t, *J*=7.5 Hz, 2H), 3.43 (t, *J*=7.5 Hz, 2H), 3.80 (t, *J*=4.7 Hz, 2H), 3.93 (t, *J*=4.7 Hz, 2H), 6.61 (d, *J*=8.8 Hz, 2H), 6.79 (d, *J*=8.8 Hz, 2H), 6.81 (d, *J*=8.6 Hz, 2H), 7.11–7.22 (m, 7H).

TORE, 4-hydroxytoremifene (OH-TORE) and ACOL

TORE citrate was obtained from Toronto Research Chemicals Inc and OH-TORE and ACOL were synthesized in our laboratory as previously described (8, 9, Labrie F. Endorecherche, Inc., United States Patent 6, 465445, October 15, 2002).

Animals and treatment

Female Sprague-Dawley rats (10- to 12-week-old) [CrI:CD® (SD)Br] (Charles River Laboratory, St-Constant, Canada) weighing approximately 225–250 g at the start of the experiment were used. The animals were acclimatized to the environmental conditions (temperature: 22 ± 3°C; humidity: 50 ± 20%; 12-h light/12-h dark cycles, lights on at 07:15 h) for 3 days before starting the experiment. The animals were housed up to 3 per cage in plastic cages and were allowed free access to water and a commercial rodent feed (Harlan # 2018, pellets). The experiment was conducted in accordance with the CCAC Guide for Care and Use of Experimental Animals in an animal facility approved by the Canadian Council on Animal Care (CCAC) and the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

A total of 30 female rats were randomly distributed into five groups as follows: 1) intact control; 2) OVX control; 3) OVX + acolbifene (ACOL, EM-652·HCl; 0.75 mg/rat); 4) OVX + toremifene citrate (TORE; 0.75 mg/rat); 5) OVX + ospemifene (OSPE; 0.75 mg/rat). On the first day of the study, all animals (except group 1) were bilaterally ovariectomized (OVX) under isoflurane anesthesia. ACOL, TORE and OSPE were administered as a suspension in 0.4% aqueous methylcellulose by oral gavage (0.5 mL/gavage/rat) once daily for 10 days (from Day 3 to Day 12 of the study).

On Day 13 of the study, approximately 24 h after last dosing, overnight fasted animals were euthanized by exsanguination at the abdominal aorta (under isoflurane anesthesia). Uteri and vagina were removed, weighed and kept in 10% buffered formalin for further histological procedures. Uteri were routinely processed in a tissue processor and embedded in paraffin blocks. For each animal, 4–5 μm-thick paraffin sections were cut and stained with hematoxylin-eosin for morphological examination and determination of endometrium epithelial height using a computerized-assisted program (Software Image-Pro Plus).

In a second experiment performed using experimental conditions as described above, 55 rats were randomly distributed between five groups of 11 animals per group as follows: 1) intact control; 2) OVX control; 3) OVX+acolibifene (ACOL, EM-652·HCl; 0.5 mg/rat); 4) OVX+raloxifene (RALOX; 0.5 mg/rat); 5) OVX+tamoxifen (TAM; 0.5 mg/rat). On Day 1 of the study, the animals of groups 2 to 5 were bilaterally OVX under isoflurane anesthesia. ACOL, RALOX and TAM were administered once daily by oral gavage as suspension in 0.4% methylcellulose (0.5 mL/rat) for 20 days (from Day 2 to Day 21 of the study). On Day 22 of the study, animals were euthanized and tissues were processed as described above. Immunohistochemistry of estrogen receptor alpha (ER α) was performed as described by Berger et al. 2005 (ref. 38).

Alkaline phosphatase activity in human endometrial adenocarcinoma Ishikawa cells

The effect of TORE, OH-TORE, OSPE, OH-OSPE and ACOL on basal and estradiol (E₂)-induced alkaline phosphatase activity in human Ishikawa endometrial carcinoma cells was determined as described previously (10). Alkaline phosphatase activity was measured after a 5-day exposure to increasing concentrations of compounds in the presence or absence of 1.0 nM E₂. Media were changed every 48 h and alkaline phosphatase activity was determined as described previously (11). Plates were monitored at 405 nm in an ELISA plate reader. Dose-response curves and EC₅₀ as well as IC₅₀ values were calculated using a weighted iterative nonlinear squares regression (12).

Statistical analysis

Data are presented as means \pm SEM. Statistical significance was determined according to the multiple-range test of Duncan-Kramer (13).

Results

As illustrated in Figure 2, a 65% loss in uterine weight from 526.5 \pm 44.0 mg to 186.3 \pm 8.7 mg ($p < 0.01$) was observed 10 days after OVX in the rat. In accordance with previous data, the third generation SERM ACOL (Figure 1) used as reference of pure antiestrogen in the uterus and mammary gland (9) had no effect on uterine weight (198.5 \pm 15.6 mg vs. 186.3 \pm 8.7 mg, 6.5%, NS) after 10 days of daily oral treatment of OVX animals with 0.75 mg of the compound (Figure 2). The analogs of TAM, namely TORE and OSPE, by contrast, increased uterine weight from 186.3 \pm 8.7 mg to 282.8 \pm 12.0 mg (52% increase over placebo, $p < 0.01$) and 289.8 \pm 13.0 mg (56% increase over placebo, $p < 0.01$), respectively. In fact, TORE and OSPE reversed by 28.4% and 30.4%, respectively, the OVX-induced inhibitory effect on uterine weight.

As shown in Figure 3, endometrial epithelial height was decreased by 48% from 30.67 \pm 1.85 μ m to 15.82 \pm 0.20 μ m ($p < 0.01$) 10 days following OVX. Although ACOL has no significant effect on endometrial epithelial height (15.97 \pm 0.38 μ m vs. 15.82 \pm 0.20 μ m, 1%, NS), TORE and OSPE reveal respective increases to 48.94 \pm 2.12 μ m (206% over OVX control) and 42.14 \pm 1.95 μ m (164% over control, $p < 0.01$) (Figure 3). The important stimulatory effect of

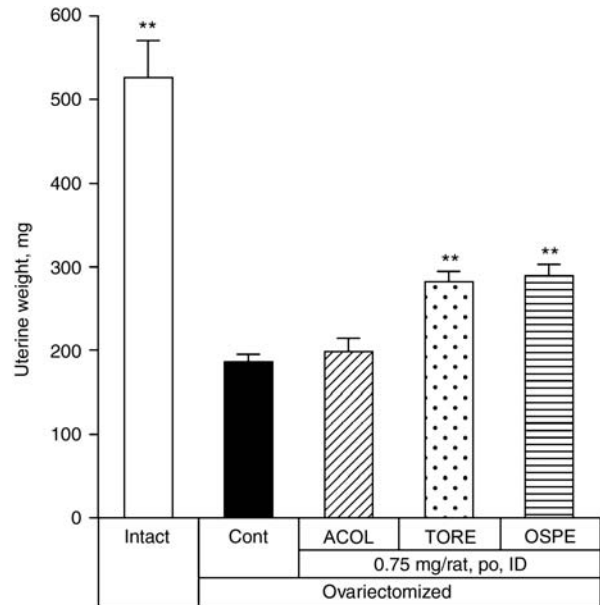


Figure 2 Effect on uterine weight of 10 days of ovariectomy (OVX) and treatment with 0.75 mg/rat of acolibifene (ACOL), toremifene (TORE) or ospemifene (OSPE) administered orally to OVX female rats. Data are expressed as the means \pm SEM of 6 animals per group. ** $p < 0.01$, experimental vs. OVX-control rats.

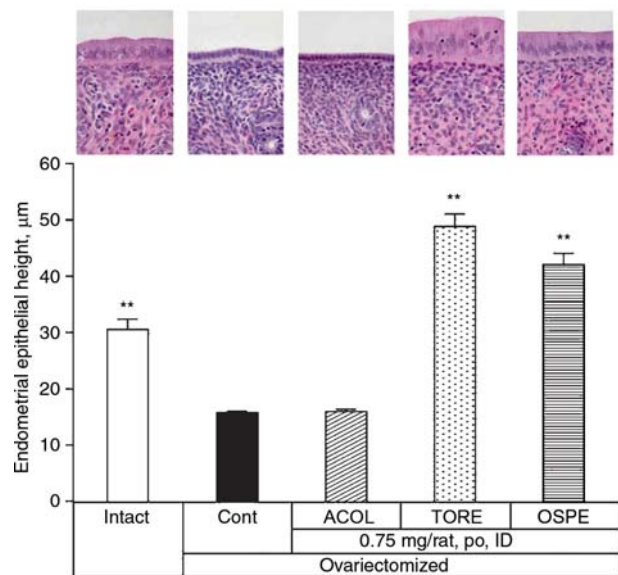


Figure 3 Effect on endometrial epithelial height of 10 days of ovariectomy (OVX) and treatment with 0.75 mg/rat of acolibifene (ACOL), toremifene (TORE) or ospemifene (OSPE) administered orally to OVX female rats. Data are expressed as the means \pm SEM of 6 animals per group. ** $p < 0.01$, experimental vs. OVX-control rats. A representative hematoxylin and eosin-stained section of rat uteri illustrating the epithelial lining cells for each group is added. Note the absence of stimulatory effect of ACOL on the endometrial epithelial cells compared with the OVX-control group, whereas a hypertrophic effect of TORE and OSPE on uterine epithelial cells is observed.

TORE and OSPE on endometrial epithelial cells is clearly illustrated on representative hematoxylin and eosin-stained sections of rat uteri shown in the upper part of Figure 3. In fact, as an indication of the high sensitivity of the epithelium of the endometrium to these two compounds, TORE and OSPE increased endometrial epithelial thickness by 60% ($p < 0.01$) and 37% ($p < 0.01$), respectively, above the value found in intact animals after only 10 days of daily oral administration.

For comparison, as shown in Figure 4, 20 days of daily oral treatment of OVX rats with 0.5 mg of RALOX or TAM significantly increased the endometrial epithelial height by 24% ($p < 0.01$) and 164% ($p < 0.01$), respectively. The important stimulatory effect of TAM on this parameter is thus similar to the above-described stimulatory effect of TORE and OSPE. By contrast, although the uterine luminal and glandular cell nuclei as well as most of the stromal cell nuclei were strongly labeled for ER α in the OVX group, treatment with ACOL completely eliminated ER α labeling (Figure 4A). Meanwhile, treatment with TAM and RALOX led to moderate stained nuclei for ER α .

As illustrated in Figure 5, vaginal weight was decreased from 158.3 ± 11.8 mg to 113.5 ± 5.2 mg (28% decrease,

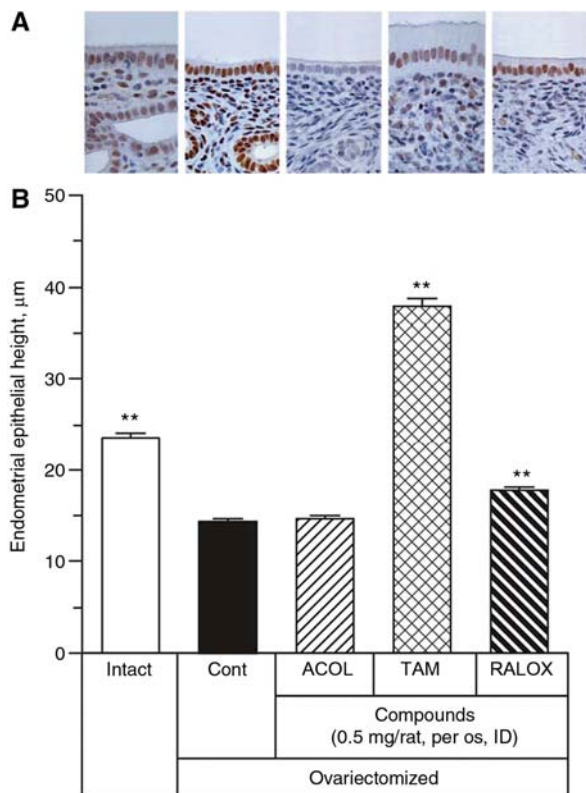


Figure 4 Effect on uterine estrogen receptor (ER α) immunostaining (A) and on endometrial epithelial height (A,B) following 20 days of ovariectomy (OVX) and treatment with 0.5 mg/rat of acolbifene (ACOL), tamoxifen (TAM) and raloxifene (RALOX) administered orally to OVX female rats. Data are expressed as the means \pm SEM of 11 animals per group. ** $p < 0.01$, experimental vs. OVX-control rats.

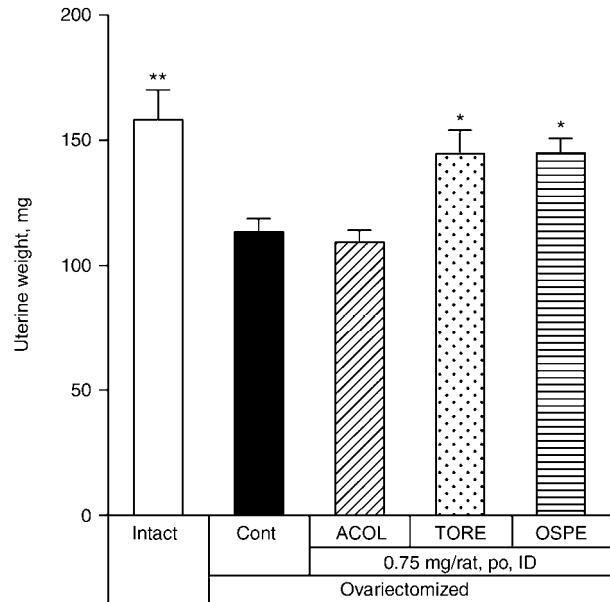


Figure 5 Effect on vaginal weight of 10 days of ovariectomy (OVX) and treatment with 0.75 mg/rat of acolbifene (ACOL), toremifene (TORE) or ospemifene (OSPE) administered orally to OVX female rats. Data are expressed as the means \pm SEM of 6 animals per group. * $p < 0.05$, ** $p < 0.01$, experimental vs. OVX-control rats.

$p < 0.01$) 10 days after OVX. Although ACOL had no significant effect on vaginal weight (109.4 ± 4.7 mg vs. 113.5 ± 5.2 mg, 3.6%, NS), TORE and OSPE prevented the OVX-induced decrease in vaginal weight by 69% ($p < 0.05$) and 70% ($p < 0.05$), respectively.

It is of interest to visualize the relative efficacy of the two first-generation TAM-derived SERMs on the above-described parameters of estrogenic activity in the rat. It can be seen in Figure 6 that although TORE and OSPE reverse the effect of OVX on uterine weight by approximately 30%, the estrogenic effect of the two SERMs is much more potent on endometrial epithelial height where the effect of OVX is not only completely reversed at 10 days of treatment with the two estrogenic compounds but where the values observed are 60% and 37% above those found in intact animals. The effect of the two SERMs on vaginal weight, by contrast, is intermediate with a 70% reversal by treatment with the two compounds.

Following the above-described data showing the comparable stimulatory effects of the two TAM analogs OSPE and TORE on uterine and vaginal weight, particularly the effect on endometrial epithelial thickness, in the OVX rat, it is of special interest to study the effect of the same two compounds on a well-recognized parameter of estrogenic activity in the human endometrium, namely alkaline phosphatase activity in human endometrial Ishikawa carcinoma cells (10). As illustrated in Figure 7, in the absence of E $_2$, ACOL has no stimulatory effect on basal alkaline phosphatase activity, whereas the same compound completely reverses the stimulatory effect of E $_2$ at an IC $_{50}$ value of 2.06 ± 0.34 nM.

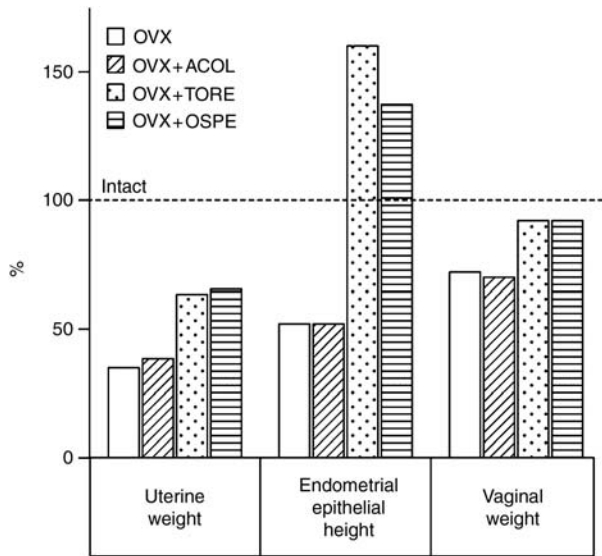


Figure 6 Relative efficacy of ACOL, TORE and OSPE on estrogen-sensitive parameters in the rat. Data are expressed as %, taking into account the values obtained in intact animals correspond to 100%.

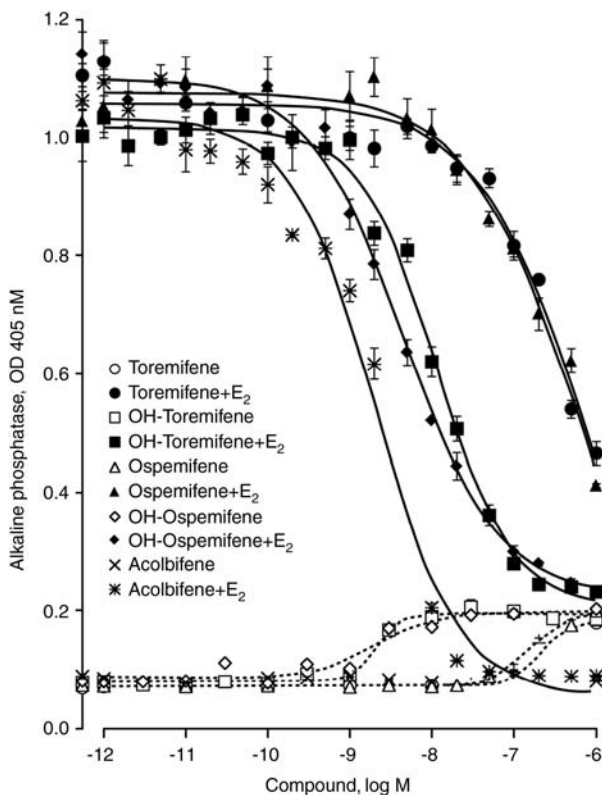


Figure 7 Effect of increasing concentrations of TORE, OH-TORE, OSPE, OH-OSPE and ACOL on alkaline phosphatase (AP) activity in human Ishikawa endometrial carcinoma cells. AP activity was measured after a 5-day exposure to increasing concentrations of the indicated compounds in the presence or absence of 1.0 nM E_2 . Data are expressed as the means \pm SEM of four wells. When the SEM overlaps with the symbol used, only the symbol is shown.

Because the active metabolites of TAM and its derivatives TORE and OSPE are the 4-hydroxylated compounds, we studied the estrogenic and antiestrogenic activities of 4-OH-TORE and 4-OH-OSPE. As can be seen in Figure 7, incubation of Ishikawa cells with OH-TORE or OH-OSPE alone stimulates phosphatase activity to similar maximal levels from 0.0789 ± 0.0028 to 0.1931 ± 0.0033 OD₄₀₅ units (144% over control) at an ED₅₀ value of 2.003 ± 0.240 nM for OH-TORE and from 0.0843 ± 0.0061 to 0.1974 ± 0.0095 OD₄₀₅ units (134% over control) at an ED₅₀ value of 2.015 ± 0.785 nM ($p < 0.01$) for OH-OSPE. When incubated in the presence of 1.0 nM E_2 , OH-TORE and OH-OSPE only partially reversed the stimulatory effect of E_2 at IC₅₀ values of 16.95 ± 1.21 nM and 7.15 ± 0.56 nM, respectively. The precursors TORE and OSPE, by contrast, had a maximal stimulatory activity similar to their active 4-OH metabolites but at lower ED₅₀ values, namely 200.7 ± 8.94 nM and 153 ± 13.5 nM, respectively. Estimated IC₅₀ values for inhibition of E_2 -stimulated alkaline phosphatase activity of 536 ± 164 nM and 465 ± 113 nM were observed for TORE and OSPE, respectively.

Discussion

The present data show that TORE and OSPE, two derivatives of TAM (Figure 1), have similar and relatively potent stimulatory effects on two well-recognized parameters of estrogenic activity in the rat uterus, namely uterine weight and endometrial epithelial thickness. In agreement with the recognized predictive value of the estrogenic effects observed in the rat uterus, comparable stimulatory effects of the two SERMs are also observed in a well-established estrogen-sensitive human in vitro model of endometrial estrogenic activity, namely alkaline phosphatase activity in human Ishikawa endometrial carcinoma cells.

The present in vitro data are comparable to the effects previously observed with 4-hydroxytamoxifen (OH-TAM) on alkaline phosphatase activity in human Ishikawa cells (10). Consequently, the present data combined with previous observations permit to conclude that OH-TAM, OH-TORE and OH-OSPE have similar potency on alkaline phosphatase activity. Two other analogs of TAM, namely droloxifene and idoxifene, have also shown comparable stimulatory effects on alkaline phosphatase activity (10, 14). By contrast, the second generation SERMs raloxifene and arzoxifene (Figure 1) also stimulate alkaline phosphatase activity in human Ishikawa endometrial carcinoma cells (10, 14).

Contrary to the effects of the first and second generation SERMs, the third generation SERM ACOL used as reference in the present study has no stimulatory effect on any estrogen-sensitive parameter, in agreement with previous data (9, 10, 14, 15). As mentioned above, for long-term use for any medical indication, it is important to have access to compounds exerting no estrogenic activity in the mammary gland and uterus, an objective which is very difficult to achieve because it requires a ligand which induces changes in the three-dimensional conformation of ER which prevents bind-

ing of the cell-specific coactivators and/or favors binding of the corepressors, thus rendering ER inert and unable to induce transcription of the estrogen-sensitive genes in the presence of estrogens (3).

OSPE, first designated FC1271a, was originally described as a compound having a "tissue-selective profile of estrogen agonistic and weak antagonistic effects" (16). In fact, OSPE has a structure similar to TAM and TORE (Figure 1). OSPE is even a known metabolite of TORE (17).

In a previous study performed in immature rats treated for only 3 days, a clear stimulatory effect of OSPE was observed at 150%–200% over control at the 10 mg/kg dose (16). This effect was similar to that of droloxifene and TORE, two analogs of TAM whereas raloxifene was approximately 10 times less potent to stimulate uterine weight. In that study, the thickness of the endometrial epithelium was significantly increased at the 10 mg/kg dose of OSPE. As inhibitor of body weight increase induced by OVX in the rat, a typical estrogenic effect, OSPE and TORE were three times more potent than raloxifene (16). In addition, serum LH was decreased in OVX animals receiving OSPE at a dose of 3 mg/kg, whereas raloxifene had no significant effect at the same dose (16). As measured by the effect on serum FSH, OSPE was also found to be more estrogenic than raloxifene (18). Similar differences between TAM, TORE and raloxifene have been observed on the thickness of the endometrial epithelium in the mouse (19).

In a study performed in adult OVX rats treated for 4 weeks, a 48%–71% increase in uterine weight was observed with 0.3–10 mg/kg doses of OSPE (16). At 3 mg/kg, raloxifene revealed a 42% increase in uterine weight, a stimulatory effect illustrated in Figure 4 and seen in other *in vivo* studies (20–23). This stimulatory effect of raloxifene on the endometrium probably explains why, in the STAR study, the effect of raloxifene on endometrial hyperplasia was not significantly different from the well-known stimulatory effect of TAM (24).

It is of particular interest to observe the relative sensitivity of uterine weight, endometrial epithelial height and vaginal weight to the estrogenic activity of the SERMs studied. Although ACOL shows no estrogenic stimulatory effect on uterine weight, endometrial epithelial height or vaginal weight, a relatively potent estrogenic stimulatory effect is observed with both TORE and OSPE on all three parameters. The data show that both TORE and OSPE have a much more potent stimulatory effect on the endometrial epithelium than on the vagina. Taking OSPE as an example, this compound administered to OVX animals at a dose of 0.75 mg orally for 10 days reveals an increase in endometrial epithelial height 37% above the intact control value, whereas the effect on vaginal weight is limited to a 70% reversal of the effect of OVX. Such data indicate the relatively high sensitivity of the endometrial epithelium to the two SERMs.

A similar inhibitory potency of OSPE on both serum LH and FSH levels has been observed in postmenopausal women (25), thus indicating an estrogenic activity of the compound

in women. The relatively strong stimulatory estrogenic effect of OSPE in women was also seen on the serum levels of SHBG, a well-characterized estrogen-sensitive parameter. The stimulatory effect of OSPE on SHBG was statistically significant at the relatively low daily dose of 3.0 mg (25).

TAM, although shown to have benefits on survival at all stages of breast cancer, has shown negative effects on breast cancer under long-term use (26). In fact, the data have shown that continuation of TAM for 4 additional years after a treatment period of 5 years yields results inferior to those obtained after only 5 years of treatment. Such an observation seriously limits the applicability of compounds of the class of TAM for the prevention of breast cancer and other medical uses where long-term treatment is needed. The reasons for this time-limited beneficial effect of TAM are likely to include the well-recognized stimulatory estrogenic effects exerted by TAM on breast cancer cell proliferation at the preclinical and clinical levels (27–31).

In addition to its limited duration of beneficial effects in breast cancer, a serious problem associated with TAM is its stimulatory effect on the endometrium leading to endometrial hyperplasia and even cancer (26, 32, 33). Both TAM and TORE are well known to exert stimulatory effects on the human endometrium (5, 34). Following a publication by Fornander et al. in 1989 (35), endometrial cancer in women taking TAM has been the subject of many reports (4, 35–37). In a study by Fisher et al. (4), which included 13,175 women, the relative risk of endometrial cancer was increased by 2.53-fold (risk ratio, 2.53; 95% confidence interval, 1.35–4.97), an observation which confirms previous data on the stimulatory effect of TAM on the endometrium.

Analogues of TAM have been synthesized and developed to various stages, including clinical trials and commercialization. These compounds include TORE, droloxifene, idoxifene, TAT-59, GW5638 and OSPE. Based on the present data, both TORE and OSPE, however, exert a stimulatory effect on the uterus comparable with that of TAM (3).

In a short 3-month clinical study performed in postmenopausal women treated with OSPE, uterine volume increased slightly (8.4–14.7%) (25). Although a 3-month treatment period with estrogenic compounds is usually considered as being too short to induce significant changes at histopathological examination of the human endometrium, the frequency of proliferative endometrium increased by 6, 3 and 5 at the 30, 60 and 90 mg daily doses of OSPE compared to 2 in the placebo group (25). Endometrial thickness, however, was significantly increased at 3 months in all groups treated with OSPE compared to placebo.

The present data, combined with previous observations, permit to conclude that TAM, TORE and OSPE have significant and comparable estrogenic effects on estrogen-sensitive parameters, whereas ACOL, a third generation SERM, has no stimulatory effect on any of these parameters. The present data add to the potential uterine safety limitations of triphenylethylene-derived SERMs for long-term use in humans.

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