An Investigation of the Relationship Between Estrogen, Estrogen Metabolites and Blood Cholesterol Levels in Ovariectomized Rats

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ABSTRACT

17β-Estradiol (E2) has long been known for protecting against coronary heart disease by lowering cholesterol levels in premenopausal women. A recent study in our laboratory suggested that two hydroxylated metabolites of E2, possess similar hypocholesterolemic effects in male rats. This effect has been further investigated with additional estrogen metabolites in ovariectomized rats with a view toward mimicking the true postmenopausal situation in humans. Their effects in reproductive tissues were also evaluated histologically. Fundamentally, the following issues were addressed: (1) Do oxidized metabolites of estradiol lower total cholesterol levels? (2) Can a hypocholesterolemic effect be achieved without eliciting estrogenic activities on reproductive tissues? The results of this investigation showed that a number of oxygenated metabolites of estradiol can lower cholesterol levels. Among them, 4-hydroxyestradiol (4-OHE2) produced a striking hypocholesterolemic effect and a substantial uterotopic effect. 2-Hydroxyestradiol (2-OHE2), 2-methoxyestradiol (2-meoE2) and 2-methoxyestrone (2-meoE1) produced a significant decrease in cholesterol levels at doses that did not produce significant uterotropic effects.

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ABBR EVIATIONS:  
E2, 17β-estradiol; ER, estrogen receptor; 2-OHE2, 2-hydroxyestradiol; 4-OHE2, 4-hydroxyestradiol; 2-meoE2, 2-methoxyestradiol; TAM, tamoxifen; 4-OHT, 4-hydroxytamoxifen; LDL, low density lipoprotein; TCL, total cholesterol level; CYP, cytochrome P450; CAD, coronary artery (or heart) disease; OVX, ovariectomized; AEBS, antiestrogen binding sites.
out eliciting estrogenic activities on reproductive tissues? We investigated these issues in ovariectomized rats with several oxygenated estradiol metabolites.

**Experimental Procedures**

**Materials.** E$_2$, 4-OHE$_2$, 2-OHE$_2$, 2-meoE$_2$, 2-meoE$_1$, 4-OHT and TAM were purchased from Sigma Chemical (St. Louis, MO). Their chemical structures are shown in figure 1. The reagent kits that were used to measure serum TCL levels were also purchased from Sigma Chemical.

**Animals.** Female, virgin Sprague-Dawley rats were purchased from Harlan Sprague-Dawley (Indianapolis, IN). They were 100 to 225 g, 10- to 11-week-old rats and were used in groups of four or five. All rats were kept in the vivarium at 24°C on a 12-hr light/dark cycle. The animals had free access to standard chow (#8604, obtained from Harlan Teklad) and water. Food was withheld for 8 hr before death.

**Surgical procedures.** Rats (except for intact controls) were anesthetized using ketamine hydrochloride (100–120 mg/kg) and xylazine hydrochloride (24 mg/kg) intramuscularly. A small midline dorsal skin incision (1–2 cm) was made just caudal to the 13th ribs. Bilateral ovariectomy was performed according to the procedures described by Waynforth and Flecknell (1992). Animals were kept warm during the procedure and recovery. On recovery from anesthesia, animals were randomly sorted into experimental groups of four or five rats each.

**Treatment.** There were two separate experiments. In each experiment, there were both intact control and ovariectomized control groups as shown in tables 1 and 2. In all cases, all compounds were administered by gastric gavage, and 1% methylcellulose was used as the vehicle. The total volume of solution gavaged for each dose was administered by gastric gavage, and 1% methylcellulose was used as the vehicle. The total volume of solution gavaged for each dose was administered to compare their biological activities. 4-OHE$_2$ was also given at a dose of 0.05 mg/kg to mimic physiological conditions. 4-OHT (1.5 mg/kg) was used to determine whether the effects of estradiol and oxidized estrogen metabolites on cholesterol and on reproductive tissues were mediated through ERs.

The treatment and vehicle groups were given one daily dose for 7 consecutive days, and each dose followed the previous one by 24 hours. In experiment 1, the interval between surgery and the beginning of treatment was 14 days. Subsequently, in experiment 2, the interval was 7 days, since we learned that a 7-day interval was long enough for uteri to atrophy.

**Serum cholesterol measurement.** Blood samples were collected into sterile silicone-coated tubes (Terumo Venoject Evacuated Specimen Tubes) by cardiac puncture 24 hr after the last treatment. Animals were placed under carbon dioxide anesthesia for 1 to 2 min before we obtained 3 ml of whole blood. After collection, animals were killed using CO$_2$ anesthesia and cervical dislocation. Serum was then separated with a bench top centrifuge running at 3000 rpm for 15 min at room temperature. After separating the serum, the samples were immediately analyzed to determine concentrations of TCL. TCL levels were measured colorimetrically with reagents obtained from Sigma Chemical (Kit 352). Measurements were made using a Beckman DU640 spectrophotometer (Beckman, Fullerton, CA). The coefficient of variation for total cholesterol measurement is 1.4%. The assay is linear to 600 mg/dl, and the minimum detectable cholesterol concentration is 2 mg/dl.

**Histological study of uteri.** After death, uteri were removed, weighed and fixed in 10% neutral buffered formalin. Preserved uteri were stored for 1 month before histological study. Formalin-fixed uteri were processed for conventional paraffin embedding with hematoxylin and eosin staining. The sections were studied in our laboratory using a light microscope (Leica, Galen III) fitted with a micrometer. Epithelial cell height, myometrial thickness and endometrial stromal thickness were measured perpendicularly to the long side of the luminal oval at ×80 with the micrometer, and endometrial stromal eosinophil infiltrates were evaluated by counting the number of eosinophils within each field. The measured raw data were then converted to a scale of 1 to 7. For instance, each uterine wet weight value was divided by 100 mg, and each measured value for uterine stromal expansion, uterine epithelial height and uterine myometrial thickness was multiplied by 10/mm to give final numbers for each parameter ranging from 1 to 7, which were then summed to give the total estrogenicity score. The purpose for this conversion was to confer equal weights to each measured parameter for the overall estrogenicity score.

**Statistics.** One-way ANOVA, a Student-Newman-Keuls test and the Mann-Whitney test were used to analyze for differences among experimental groups for each parameter (TCL, uterine weight, uterus/body mass ratio and total estrogenicity score). Differences were considered significant at $P < .05$.

**Results**

**Body weight.** Ovariectomy was associated with a 5.4% weight gain compared with a 3.4% weight loss among intact controls during the 21-day period after surgery and before death in experiment 1, table 1). In experiment 2, OVX rats exhibited a 3.8% loss in weight compared with an 8.5% loss for intact controls during the 14-day period after surgery. The differences in weight losses (OVX vs. intact controls) were not significantly different in either experiment.

**Uterus.** The effects of each treatment in experiment 1 and 2 are presented in tables 1 and 2 respectively. As expected, uterine wet weights of the OVX rats are 40% to 48% lower than for the intact controls in both experiments (P < .01). E$_2$ at doses of 0.5 and 1 mg/kg completely restored uterine
weights to the intact control levels after a 7-day treatment, and produced significantly heavier uteri than those observed in the OVX control group rats (P < .01).

4-OHE2 at doses of 0.5 mg/kg and 1 mg/kg also restored the uterine wet weight to the intact control level and significantly increased weights compared with the OVX control rats (P < .01; tables 1 and 2). 4-OHE2 at 1.5 mg/kg did not alter uterine weight any more than the 1.0 mg/kg dosage. At a low dose of 0.05 mg/kg, 4-OHE2 increased the uterine weight relative to OVX controls, but the increase failed to achieve statistical significance. However, the ratio of uterus/body mass was significantly increased even at this low dose. On the other hand, 2-OHE2, 2-meoE1 and 2-meoE2 at 0.5 mg/kg showed no uterotrophic effect (tables 2 and 3).

In experiment 1, E2 (1 mg/kg) with TAM was less effective than E2 alone in restoring uterine weight; however, uterine weight was still significantly greater than for OVX controls (P < .05). 4-OHT at a dose of 0.8 and TAM at a dose of 1 mg/kg blocked the uterotrophic effect of E2 (36% increase in uterine weight with E2 + TAM compared with 19% with E2 + 4-OHT), however, these differences in uterine weight gain were not statistically significant (table 1). Similar changes were observed in uterus/body mass ratio. In experiment 2, 4-OHT blocked the uterotrophic effect of both 4-OHE2 (0.5 mg/kg) and E2 (0.5 mg/kg) significantly (table 2).

**Serum cholesterol.** As expected, ovariectomy caused a substantial increase in TCL levels (30.9% and 35.1% in experiments 1 and 2, respectively) compared with those of intact control rats (figs. 2 and 3). In experiment 1, E2 produced a 65.2% decrease in total cholesterol levels at a dose of 1 mg/kg. At the same dose, 4-OHE2 produced an even greater decrease in total cholesterol levels, nearly depleting them (i.e., total cholesterol declined by 94.3%). 4-OHE2 at 1.5 mg/kg produced a 98.4% decrease in total cholesterol levels (fig. 2). TAM appeared to be ineffective in blocking the hypocholesterolemic effect of E2. 4-OHT may have partially blocked the hypocholesterolemic effects of E2 although the effect was not statistically significant (fig. 2). In experiment 2, E2 at a dose of 0.5 mg/kg produced a 60.6% decrease in total cholesterol levels. At the same dose, 4-OHE2 virtually depleted total cholesterol levels (fig. 3). At a dose of 0.05 mg/kg, 4-OHE2 reduced total cholesterol levels comparable to the reduction produced by E2 at a dose of .5 mg/kg. Although 2-OHE2, 2-meoE1 and 2-meoE2 did not affect cholesterol levels as dramatically as E2 or 4-OHE2, they nevertheless decreased total cholesterol levels significantly by 26.8%, 38.1%, and 33.6%, respectively. 4-OHT blocked the cholesterol lowering effect of 4-OHE2 to a significant extent (fig. 3). However, combined administration of E2 and 4-OHT produced a greater decrease in total cholesterol than E2 alone.

**Histological results.** Uterine sections obtained from the intact controls, E2-treated and 4-OHE2-treated rats show epithelial lining cells with typical elongated cell bodies and elongated, diffuse nuclei (fig. 4). Epithelial cells from OVX control rats, 2-OHE2-, 2-meoE1- and 2-meoE2-treated rats were more cuboidal in appearance with smaller, darker staining nuclei (fig. 4). Uterine epithelial cell height was 54.5% smaller in OVX control rats compared with intact controls. 4-OHE2 at a dose of 0.05 mg/kg had no statistically significant effects on uterine epithelial cell height compared with the intact control rats. At doses of 0.5, 1.0 and 1.5 mg/kg, 4-OHE2 produced a significant increase in epithelial cell height.
TABLE 3
Effect of different treatments on uterine wet weight and histological parameters in OVX rats
Uterine weight was divided by 100 mg before it was combined with other parameters to give the total estrogenticity score.

<table>
<thead>
<tr>
<th>Group</th>
<th>Uterine weight (mg)</th>
<th>Uterine epithelial height (×10^1 mm)</th>
<th>Uterine myometrial thickness (×10^1 mm)</th>
<th>Uterine stromal expansion (×10^1 mm)</th>
<th>Uterine stromal eosinophiliaa</th>
<th>Total estrogenticity score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact control</td>
<td>382 ± 108a</td>
<td>2.2 ± 0.4a</td>
<td>3.7 ± 0.8a</td>
<td>6.4 ± 1.1a</td>
<td>3.2 ± 0.8a</td>
<td>19.4 ± 2.8a</td>
</tr>
<tr>
<td>OVX control</td>
<td>155 ± 14</td>
<td>1 ± 0a</td>
<td>2.8 ± 1.1a</td>
<td>5.6 ± 0.5a</td>
<td>0.5 ± 0.6</td>
<td>10.1 ± 2.0</td>
</tr>
<tr>
<td>OVX +4-OHE2 (0.05 mg/kg)</td>
<td>216 ± 98</td>
<td>1.6 ± 1.3a</td>
<td>3.0 ± 0.7a</td>
<td>3.8 ± 0.5a</td>
<td>0.6 ± 1.1a</td>
<td>20.2 ± 1.2</td>
</tr>
<tr>
<td>OVX +4-OHE2 (0.5 mg/kg)</td>
<td>345 ± 37a</td>
<td>2.6 ± 0.5a</td>
<td>3.6 ± 0.4a</td>
<td>4.4 ± 0.1a</td>
<td>0.5 ± 0.6</td>
<td>13.8 ± 5.2</td>
</tr>
<tr>
<td>OVX +4-OHE2 (1 mg/kg)</td>
<td>345 ± 20a</td>
<td>2.8 ± 0.4a</td>
<td>3.6 ± 0.6a</td>
<td>5.3 ± 1.3a</td>
<td>0.5 ± 0.6</td>
<td>23.4 ± 2.5</td>
</tr>
<tr>
<td>OVX +4-OHE2 (1.5 mg/kg)</td>
<td>339 ± 56a</td>
<td>3.3 ± 0.5a</td>
<td>4.6 ± 0.9a</td>
<td>7.2 ± 1.3a</td>
<td>0.5 ± 0.6</td>
<td>24.9 ± 2.4</td>
</tr>
<tr>
<td>OVX +4-OHE2 (0.5 mg/kg) +4-OHT (1.5 mg/kg)</td>
<td>239 ± 48a</td>
<td>3.6 ± 0.5a</td>
<td>6.8 ± 0.8a</td>
<td>1.3 ± 1.5a</td>
<td>0.5 ± 0.6</td>
<td>17.8 ± 2.1</td>
</tr>
<tr>
<td>OVX +4-OHE2 (0.5 mg/kg) +E2 (0.5 mg/kg)</td>
<td>155 ± 10</td>
<td>1 ± 0a</td>
<td>3.2 ± 0.8a</td>
<td>6.0 ± 3.8a</td>
<td>0.5 ± 0.6</td>
<td>23.6 ± 6.7</td>
</tr>
<tr>
<td>OVX +4-OHE2 (0.5 mg/kg) +E2 (1 mg/kg)</td>
<td>340 ± 145a</td>
<td>3.4 ± 0.9a</td>
<td>6.8 ± 0.8a</td>
<td>6.0 ± 3.8a</td>
<td>0.5 ± 0.6</td>
<td>23.6 ± 6.7</td>
</tr>
<tr>
<td>OVX +4-OHE2 (0.5 mg/kg) +E2 (1 mg/kg)</td>
<td>367 ± 99a</td>
<td>4.0 ± 0.8a</td>
<td>6.0 ± 1.4a</td>
<td>5.3 ± 1.5a</td>
<td>0.5 ± 0.6</td>
<td>23.4 ± 1.5</td>
</tr>
<tr>
<td>OVX +2-meoE1 (0.5 mg/kg)</td>
<td>256 ± 44a</td>
<td>3.4 ± 0.5a</td>
<td>5.4 ± 0.5a</td>
<td>3.4 ± 1.5a</td>
<td>0.5 ± 0.6</td>
<td>18.2 ± 2.2</td>
</tr>
<tr>
<td>OVX +2-meoE1 (0.5 mg/kg)</td>
<td>150 ± 10</td>
<td>1.2 ± 0.4a</td>
<td>5.4 ± 0.5a</td>
<td>0.2 ± 0.5a</td>
<td>0.5 ± 0.6</td>
<td>10.9 ± 1.3</td>
</tr>
<tr>
<td>OVX +2-meoE1 (0.5 mg/kg)</td>
<td>141 ± 10</td>
<td>1.2 ± 0.4a</td>
<td>5.6 ± 0.9a</td>
<td>0 ± 0a</td>
<td>0.5 ± 0.6</td>
<td>10.2 ± 1.1</td>
</tr>
</tbody>
</table>

* P < .05 compared with OVX controls.
✿ Number of eosinophils per high microscope field (×80).

*Discussion*

Estrogen, both natural and synthetic (e.g., ethinyl estradiol), can induce profound hyperplasia at pharmacological doses (Davis and Roheim, 1978; Chao et al., 1979; Frolik et al., 1987; Fewster, 1989). After administration of E2 at a dose of 2 mg/kg compared to controls at the same dose (unpublished observations), Tselog et al. (1983) and Lui et al. (1997) observed that 4-OHE2, 4-OHE2, 2-meoE1, and 2-meoE2 each at a dose of 0.5 mg/kg elicited by E2 at the same dose (unpublished observations). These studies on both cholesterol and reproductive tissues have reported on the biological activities of oxygenated estradiol metabolites (Martucci and Fishman, 1979). Efforts have been made to lower cholesterol levels in male Sprague-Dawley rats treated with a maximum of 70% decline at a dose of 2 mg/kg compared to controls. The objectives of this research project were to investigate the biological properties of a series of oxygenated estradiol metabolites by using antihypertensive substances and by creating an OVX rat model. The advantages for the use of OVX rats are that no statistically significant effects on uterine epithelial cell height, Uterine myometrial thickness, stromal expansion, and estradiol binding were noted between controls and treated rats compared with the untreated OVX controls (P > 0.05, see Table 3).

*Fig. 2.* Treatment effects on total cholesterol levels in experiment 1. Rats were dosed p.o. once a day for 7 days. At 24 hr after the last dose, total cholesterol was determined. Results are presented as mean ± SD. Ctrl (control), 1 mg/kg 4-OHE2 ( ), 2 mg/kg 4-OHE2 ( ), 4 mg/kg 4-OHE2 ( ), 1 mg/kg mesoE2 ( ), 2 mg/kg 2-meoE2 ( ), 4 mg/kg 2-meoE2 ( ) and treated with tamoxifen ( ).
are that (1) ovariectomy can minimize the interference of endogenous estrogens, (2) ovariectomy mimics the true postmenopausal condition and (3) the model permits comparison of the simultaneous effects of oxygenated estradiol metabolites and E2 on reproductive tissue (uterus) and TCI permitting inferences to be drawn about the role of ERs in both effects.

The results of the study reported herein are quantitatively different from the previous study in male rats. The maximal hypocholesterolemic effect (E\text{max}) for 4-OHE\textsubscript{2} occurred at a dose of 0.05 mg/kg compared with 2.0 mg/kg in male rats. ERs are conceivably different between male and female rats both quantitatively and qualitatively, and this may contribute to the differential potencies between genders, as could differences in pharmacokinetic parameters.

In the present study, 4-OHE\textsubscript{2} at a dose of 0.05 mg/kg produced a significant decrease in cholesterol levels that was comparable to that elicited by E\textsubscript{2} at a 10 times higher dose. 4-OHE\textsubscript{2} also appeared to be more potent than the other three metabolites (2-OHE\textsubscript{2}, 2-meoE\textsubscript{1} and 2-meoE\textsubscript{2}). At doses of 0.5 mg/kg and higher, 4-OHE\textsubscript{2} virtually depleted the total cholesterol levels. It was interesting to observe that 2-OHE\textsubscript{2}, 2-meoE\textsubscript{1} and 2-meoE\textsubscript{2} were able to significantly decrease total cholesterol levels without stimulating the uterus, since it signals that these two effects may be mediated by different mechanisms. This finding may open the door for developing new hypolipidemic drugs devoid of the side effects observed in estrogen replacement therapy. However, further study is warranted to better understand the underlying mechanism(s).

It is currently proposed that ERs can exist in the cell in multiple conformations that represent the inactive state, the active state and several intermediate states and that ligands exert their biological activities by stabilizing a specific conformation. In the absence of ligand the inactive conformation is preferred. Interaction of ER with E\textsubscript{2} stabilizes the complex in a conformation that facilitates transactivation (McDonnell et al., 1995). The relative agonist/antagonist balance of other ER modulators is determined by the intermediate conformation promoted by the particular compound (McDonnell et al., 1995). The findings of this study were consistent with the notion that 4-OHT is a partial ER agonist. A number of published studies showed 4-OHT has a similar affinity for ER as that of E\textsubscript{2} (McDonnell et al., 1995; Osborne et al., 1992). 4-OHT and its parent drug, TAM, are classified as type IV antiestrogens, which stabilize ER in a conformation that allows it to exhibit transcriptional activity on a limited subset of ER-responsive genes (McDonnell et al., 1995). It was shown by Klinge et al. (1996) that 4-OHT-ligated ER binds the ERE DNA with high affinity, but at its saturation ERE binding capacity is consistently half that of E\textsubscript{2}-ER, which means that one molecule of 4-OHT ligand dissociates from the ER dimer as a consequence of ERE binding. In a later separate experiment conducted in our lab, 4-OHT alone was shown to be able to produce both hypcholesterolemic and uterotrophic effects in OVX rats, which is consistent with the findings of other studies with regard to its partial ER agonism.

The results of these experiments suggest that the hypcholesterolemic effects of 4-OHE\textsubscript{2} are largely mediated through ER. Additional evidence is as follows: (1) partial ER antagonists partly but effectively blocked the hypcholesterolemic and uterotrophic effects of 4-OHE\textsubscript{2}; (2) the hypcholesterolemic effect of 4-OHE\textsubscript{2} in OVX rats is more potent than that observed in male rats, which is likely due to more ER present in female tissues; and (3) in vitro studies showed 4-OHE\textsubscript{2} has an affinity for ER close to that for E\textsubscript{2} (Tanaka et al., 1986; Martucci and Fishman, 1976; Merrian et al., 1980; Davies et al., 1975; Schutze et al., 1993). (4) In a similar study in OVX rats, E\textsubscript{2} and 4-OHE\textsubscript{2} produced comparable levels of ER occupation in limbic brain, pituitary and uterus, and similar behavioral and gonadotropic responses were observed (Jellinck et al., 1981).

That the hypcholesterolemic effect of estrogen metabolites can be at least partially dissociated from ERs is suggested by the following: First, among these four E\textsubscript{2} metabolites, 4-OHE\textsubscript{2} has the strongest affinity for ER, followed by 2-OHE\textsubscript{2}, whereas the methylated products of 2-OHE\textsubscript{2} and 2-OHE\textsubscript{1}, 2-meoE\textsubscript{2} and 2-meoE\textsubscript{1}, respectively, virtually have no affinity for ER (Martucci and Fishman, 1976; Merrian et al., 1980) yet they retain significant, although smaller, hypcholesterolemic effects. Thus, it may be inappropriate to attribute the hypcholesterolemic effects of 4-OHE\textsubscript{2}, 2-OHE\textsubscript{2}, 2-meoE\textsubscript{2} and 2-meoE\textsubscript{1} solely to the ligand-ER interactions. Second, 4-OHE\textsubscript{2} is a much more potent hypcholesterolemic agent than E\textsubscript{2}, even though they have similar affinity to ER. Third, both 2-OHE\textsubscript{2} and 4-OHE\textsubscript{2} are eliminated from the body at a much faster rate than E\textsubscript{2} with metabolic clearance rates in an apparent ratio of 1:4:11 (E\textsubscript{2}/4-OHE\textsubscript{2}/2-OHE\textsubscript{2}) (Ball et al., 1983; Emons et al., 1982). This pharmacokinetic
property would be expected to diminish the apparent potency of 4-OHE$_2$ relative to E$_2$ because one would expect lower tissue concentrations of 4-OHE$_2$ than for E$_2$ when the same dose is administered. Finally, nonsteroidal compounds, such as TAM and benzofurans, can bind to another class of intracellular binding sites, often termed “antiestrogen binding sites” or “AEBS,” which do not bind E$_2$ (Lazier and Bapat, 1988; Teo et al., 1992). It has been shown that selective ligands of AEBS are very likely to be involved in the inhibition of de novo cholesterol biosynthesis in cell culture (Teo et al., 1992; Cypriani et al., 1988). One interpretation of our findings is that non-ER-based mechanisms may play a role in the cholesterol-lowering effect of the oxygenated estrogen metabolites, and this would be consistent with a possible role at non-ER binding sites. It is well known that estrogen can lower cholesterol levels through up-regulating LDL receptors on the hepatic cell surface by acting at ER and subsequently accelerating the plasma clearance of cholesterol. The cholesterol synthetic pathway is another potential site of action for estrogen metabolites. There are several steps in the synthesis of cholesterol, each catalyzed by enzymes, which could be inhibited by estrogen metabolites in a manner akin to the inhibition of HMG COA reductase by lovastatin. Similarly, cholesterol catabolism in the liver could also be expedited by the stimulation of enzymes that convert cholesterol into bile acids. There is no proof for these hypotheses, and further investigations are warranted.

Uterine wet weight has long been used as a reliable parameter in evaluating the uterotrophic effects of certain estrogenic compounds (Levin et al., 1968; Levin et al., 1967). The recent application of histological markers makes the evaluation a more convincing one (Black et al., 1994). In this study, 2-OHE$_2$, 2-meoE$_1$ and 2-meoE$_2$ showed no uterotrophic activity, but they significantly decreased cholesterol levels, albeit not as much as E$_2$ or 4-OHE$_2$. It is conceivable that pharmacokinetic differences between these three compounds could contribute to these observations. In addition, 2-meoE$_1$ and 2-meoE$_2$ can undergo demethylation and produce corresponding 2-hydroxyestrogens (Martucci and Fishman, 1993), which may actually mediate the hypocholesterolemic effect of 2-meoE$_1$ and 2-meoE$_2$. However, it is equally likely that these three oxygenated estrogen metabolites may exert their

Fig. 4. Hemotoxylin and eosin-stained sections of rat uteri, illustrating epithelial lining cells, obtained from intact controls (A), ovariectomized controls (B) and ovariectomized rats treated for 7 days with E$_2$ (0.5 mg/kg; C), 4-OHE$_2$ (0.5 mg/kg; D), 2-meoE$_1$ (0.5 mg/kg; E) and 2-OHE$_2$ (0.5 mg/kg; F). The marked hypertrophic effect of E$_2$ on uterine epithelial cells is not observed in the uteri obtained from 2-OHE$_2$ and 2-meoE$_2$ (×100).
hypocholesterolemic effect via an ER-independent pathway, in a manner analogous to the inhibition of de novo cholesterol synthesis by benzofurans in cells which lack ER (Teo et al., 1992). This hypothesis is supported by the finding that 2-meoE2 inhibits tubulin polymerization by acting at an ER-independent colchicine site, thereby inhibiting angiogenesis and breast cancer in mice (Klauber et al., 1997). Additionally, Joselsson and Tarkowski (1997) reported that 2-meoE2 can suppress angiogenesis without showing feminizing effects on sex organs. In light of the serious human health consequences of coronary heart disease, the implication that substances related to 2-OHE2, 2-meoE2, and 2-meoE2 might offer a useful therapy for postmenopausal women to maintain lower serum cholesterol without affecting reproductive tissue merits further investigation.

Finally, the different roles of the two ER subtypes, ERα and ERβ, warrant brief comment. ERα refers to the classic ER. ERβ was cloned in 1995 (Kuiper et al., 1996) and has since been found to exist in a number of tissues in both humans and animals (Mosselman et al., 1996; Arts et al., 1997). A recent study showed that ERα and ERβ signal in opposite ways when complexed with the E2 from an activator protein-1 site. E2-ERα activated transcription, whereas E2-ERβ inhibited transcription. Moreover, TAM, raloxifene, and ICI 164384 were shown to be potent transcriptional activators with ERβ at an activator protein-1 site. Thus, the two ERs signal in different ways depending on the ligand and response element (Paech et al., 1997). The difference in the distribution densities of these two ER subtypes may also partly explain the different pharmacological responses we observed in this study. Characterization of the distribution of each ER subtype in uterus and liver is warranted.

In conclusion, the present study on estradiol metabolites has provided evidence that oxygenated estradiol metabolites possess hypocholesterolemic activities that can be separated, in part, from their uterotropic effects in ovariectomized rats.

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