Expression and cyclic variations of catechol-O-methyl transferase in human endometrial stroma

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Objective: To investigate the role of catechol-O-methyl transferase (COMT) in the regulation of estrogen metabolism in human endometrium.
Design: Laboratory study.
Setting: Academic research laboratory.
Intervention(s): Immunohistochemistry was used to localize COMT protein in human endometrial tissues. Catechol-O-methyl transferase promoter–luciferace reporter gene transactivation assay was used to assess COMT promoter activity in response to estrogen and progesterone treatment in primary human endometrial stroma (pHES) cells. Catechol-O-methyl transferase protein and mRNA expression were determined by Western blot and/or real-time polymerase chain reaction. The effect of 2-methoxy estrogen treatment on DNA proliferation, B-cell lymphoma 2, and vascular epithelial growth factor protein expression were assessed by Hoechst and Western blot analyses, respectively.
Main Outcome Measure(s): Catechol-O-methyl transferase protein and mRNA subcellular localization and expression in human endometrial tissues and pHES cells.
Result(s): Catechol-O-methyl transferase protein expression in human endometrial tissues was up-regulated in the proliferative phase and down-regulated in the midsecretory phase of the menstrual cycle. Estrogen induced a dose-dependent increase in COMT proximal promotor–luciferace transactivation in pHES cells whereas progesterone inhibited it. Estrogen up-regulated soluble COMT protein isoform expression whereas the addition of progesterone down-regulated it in pHES cells. High doses of 2-methoxy estrogen inhibited endometrial stroma cell proliferation, and down-regulated B-cell lymphoma 2 and vascular epithelial growth factor protein expression.
Conclusion(s): Catechol-O-methyl transferase expression is hormonally regulated in human endometrial stroma. Catechol-O-methyl transferase product, 2-methoxy estrogen, inhibited endometrial stroma cell proliferation and decreased vascular epithelial growth factor and B-cell lymphoma 2 protein expression. (Fertil Steril® 2008;90:789–97. ©2008 by American Society for Reproductive Medicine.)

Key Words: Catechol-O-methyl transferase, human endometrial stroma, catechol estrogen, 2-methoxy estrogen

The human endometrium is a metabolically active tissue that undergoes monthly cell proliferation, differentiation, and apoptosis during the female reproductive cycle. The molecular mechanisms that control synchronized endometrial development during each menstrual cycle are still poorly understood. Estrogen and progesterone are known to modulate the endometrium in a variety of ways. Estrogen stimulates endometrial cell proliferation whereas progesterone induces endometrial cell decidualization and prepares the endometrium for blastocyst implantation. Genes that are modified by estrogen and/or progesterone are likely to be important players in the monthly cyclic development of the endometrium and possible culprits in the pathogenesis of endometrial disorders. Catechol-O-methyl transferase (COMT) is an enzyme that catalyses O-methylation and inactivation of a variety of metabolically active catechol compounds such as epinephrine, norepinephrine, and dopamine. Catechol-O-methyl transferase is also involved in the final stages of estrogen metabolism.

Estrogen is oxidized by the cytochrome P450 group of enzymes (1A1 and 1B1) to catechol estrogens (2-hydoxy and 4-hydroxy estrogens, respectively). Catechol-O-methyl transferase converts 2-hydroxy estrogens (2-OHE) to their respective 2-methoxy estrogens (2-ME) by catalyzing the transfer of O-methyl group to one of the hydroxyl groups of the catechol substrate in the presence of MG^{+2} (1). 2-Hydroxy estrogens bind to estrogen receptors and works as an antiestrogen, while 2-ME has a 50% estrogen receptor-independent estrogenic effect at physiologic concentrations (1–4). 2-Methoxy estradiol (2-ME2) has a number of effects on its own, unrelated to estradiol, 2-hydroxy estradiol, or other methoxy estrogen derivatives (5). 2-Methoxy estradiol is one of the most potent endogenous inhibitors of angiogenesis known to man (6). It is a potent

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inhibitor of endothelial cell proliferation and migration as well as angiogenesis in vitro. It disturbs the function of the microtubules, leading to failure of microtubular polymerization (7, 8). It also plays a crucial role in cellular proliferation and differentiation through the induction of ERK mitogen-activated protein kinase (9). 2-Methoxy estradiol is exclusively produced by O-methylation of 2-hydroxy estradiol by the COMT enzyme in extrahepatic tissues (1).

The regulation of COMT expression and the role of perturbed COMT metabolites in human endometrium has not been well characterized. In this report we study the regulation and the potential role of COMT expression in human endometrium. We evaluated COMT mRNA and protein expression in human endometrial tissues and investigated the in vitro regulation of COMT using pHES cells. Furthermore, we assessed the effect of increased COMT product, 2-ME2, on the proliferation and expression of cell cycle regulatory proteins in primary endometrial stroma cells.

MATERIALS AND METHODS

Patient Selection and Human Tissues Processing

Endometrial tissue samples were collected from 16 women presenting with a benign gynecologic disorder such as uterine fibroids, endometriosis, and cervical dysphasia, and requesting definitive treatment in the form of hysterectomy. Patients' age ranged from 35 to 45 years. All patients have $COMT^{Met/Val}$ genotype, which has an intermediate COMT enzyme activity, compared with the high activity allele ($COMT^{Val/Val}$) and to the low activity COMT allele ($COMT^{Met/Met}$) as descibed below. Eight patients were in the secretory phase of their cycle and eight patients were in the proliferative phase of their cycle. Patients did not receive hormonal treatment for at least 6 weeks before sample collection. Endometrial samples were collected from the upper lateral anterior and posterior uterine walls after the completion of hysterectomy surgery. Tissue samples were submersed immediately in liquid nitrogen or formalin and stored until use. Part of the specimen was collected in sterile Hank's balanced salt solution (containing 25 mM of HEPES and antibiotics), and was used to establish primary endometrial stromal cell culture. Fixed formalin tissue blocks were used for immunohistochemistry, while protein was extracted from the frozen endometrial tissues for Western blot analysis. This study protocol was approved by the institutional review board of the University of Texas Medical Branch.

Genotyping of the COMT Gene

DNA was extracted from peripheral blood samples (10). The following primers 5′-CTC ATC ACC ATC GAG ATC AA-3′ (forward) and 5′-CCA GGT CTG ACA ACG GGT CA-3′ (reverse) were used in polymerase chain reactions (PCRs) using a DNA thermocycler Perkin-Elmer Cetus, GeneAmp PCR system 9600 (Perkin Elmer Cetus, Norwalk, CT) as described previously (11). Polymerase chain reaction product size was 109 bp. The PCR product was digested with 2 U of *Nla*III (New England BioLabs, Beverly, MA) at 37°C overnight, followed by 4.5% agarose gel electrophoresis. Catechol-O-methyl transferase genotypes (Val and Met alleles) were discriminated by the size of the restriction fragments. The Val/Val homozygotes (86 and 23 bp), Met/Met homozygotes (68 and 18 bp), and Val/Met heterozygotes (86, 68, 23, and 18 bp) were visualized by ethidium bromide staining.

Immunohistochemistry

Human endometrial tissue specimens were collected from representative areas. Tissues were fixed in phosphate-buffered saline (PBS) solution containing 10% formalin, embedded in paraffin, then sectioned. Immunohistochemistry was conducted using standard techniques (12). Paraffin-embedded tissue sections (5 μm) were deparaffinized and dehydrated by passage through xylene and graded ethanol solutions. Highly specific primary antihuman COMT polyclonal antibodies raised in the guinea pig (kind gift from Tenhunen, Orion Corporation, Orion-Farmos, Research Center, Helsinki, Finland) was applied to sections at a 1:250 dilution as described earlier (13). Diaminobenzidine served as the chromagen to detect COMT protein, and tissue sections were counterstained with hematoxylin. Negative controls included omission of the primary antibody or substitution of the COMT antiserum with normal serum from the guinea pig, the species used to generate the COMT antiserum. Catechol-O-methyl transferase immunoreactivity in endometrial tissues was visually assessed by assessing the intensity and counting the frequency of the brown staining (Fig. 1).

Cell Culture

Primary human endometrial stromal (pHES) cells were used to study the in vitro regulation of COMT expression in response to estrogen, progesterone, or 2-ME2 treatment. The cells were established from fresh endometrial tissues collected from hysterectomy samples, as have been described previously (14). Endometrial tissue was finely minced, and cells were dispersed by incubation in Hank's balanced salt solution containing HEPES (25 mM), penicillin (200 U/mL), streptomycin (200 μg/mL), collagenase (1 mg/mL, 15 U/mg), and DNase (0.1 mg/mL, 1,500 U/mg) for 20–30 minutes at 37°C with agitation. Stroma cells were separated from glandular cells by filtration through a wire sieve with 73-μ diameter pores. The stroma cells that were found in the filtrate portion were pelleted, washed, and suspended at 37°C in 5% CO_2/air in phenol red-free Dulbecco's modified Eagle's medium (DMEM), containing antibiotics, 2-mM L-glutamine, and 10% fetal bovine serum. The cells were passaged once and grown to confluence. Confluent monolayers were maintained in media containing 10% charcoal-stripped fetal bovine serum for 48 hours, and subsequently treated with different concentrations of 17-β-estradiol (10^{-10}–10^{-6} M), progesterone (10^{-8}–10^{-6} M) with or without 17-β-estradiol (10^{-8}), 2-ME2 (10^{-10}–10^{-6} M) in 0.01% (v/v) ethanol, or with only 0.01% ethanol as a negative