To examine the expression of the estrogen phase I and phase II metabolism enzymes in ovarian endometriosis and normal endometrium at the mRNA level.

To study the protein expression of CYP1B1 and COMT in endometriotic normal endometrial tissues.

**INTRODUCTION**

Endometriosis is a complex estrogen-dependent disease defined as the presence of endometrial tissue outside the uterine cavity. Estrogens stimulate cell proliferation via the estrogen receptor and undergo extensive oxidative metabolism at different positions, catalyzed by various cytochrome P450 isoforms. Formation of 2- and 4-hydroxy-estrogens (catechol estrogens, CEs) is catalysed mainly by CYP1A1/1A2 and CYP1B1, respectively. CEs can be oxidized to the corresponding estrogen orthoquinones with concomitant formation of the reactive oxygen species (ROS). NADPH:quinone oxidoreductase (NQO1/2) regulate the reduction of toxic estrogen quinones back to catechols. Furthermore, estrogens can induce 16 a-hydroxylation, extracellularly catalysed mainly by CYP1A2 and CYP1A3. CE and 16 a-hydroxy-estrogens are further metabolised by the conjugative enzymes: catechol-O-methyltransferase (COMT), sulfotransferases (SULT), UDP-glucuronyltransferases (UGTs), and glutathione S-transferases (GSTs) and yields less harmful products (Figure 1).

Protein expression levels of CYP1B1 and COMT were examined in 19 samples of ovarian endometriosis and 29 normal endometrium samples (Figure 1). We found significantly increased in endometriosis (4 fold, \( p = 0.0005 \)) and unchanged mRNA levels of SULT1A1 and NQO2, while all the other gene levels were significantly decreased in endometriosis (2- and 4-, 16a-hydroxylation of estrogens. CYP1B1 and CYP3A5 expression was not altered, while CYP1A2 expression was not detected. The levels of CYP1A1, CYP1A3, and CYP1A7 were increased in endometriotic tissue (4 fold, \( p < 0.0001 \)).

**RESULTS AND DISCUSSION**

Expression levels of genes encoding phase 1 (CYP1A1, CYP1A2, CYP1B1, CYP1A3, CYP1A7) and phase II (SULT1A1, SULT1E1, SULT2B1, COMT, UGT2B7, NQO1, NQO2, GSTP1) estrogen-metabolizing enzymes were studied by real-time PCR in 31 samples of ovarian endometriosis and 30 samples of normal endometrium (Figure 2). The statistical calculations and tests were immediately performed using IBM SPSS Statistics 20 software. The results were assessed by Western blotting and showed unchanged protein levels of CYP1B1 in endometriosis (\( p = 0.0118 \)).

**CONCLUSION**

Our data show imbalance between the phase I and phase II enzymes in ovarian endometriosis, increased hydration and decreased conjugation of estrogens caused to excessive ROS formation, which may activate cytokines that control the implantation and the growth of endometrial cells outside the uterus and thus contribute to the development of ovarian endometriosis.

Additionally, we observed higher MB- and S-COMT ratio, suggesting that MB- and S-COMT play different roles in CE detoxification in endometriosis, where MB-COMT may be involved mainly in 2-CE metabolism. However, the exact role of each COMT isoform should be further studied.

**METHODS**

**Specimens.** A total of 60 specimens were collected by biopsies during laparoscopy: 31 of ovarian endometriomas (mean age, 33 ± 6 and 29 of control normal endometrium, from women undergoing sterilization (mean age, 41 ± 3) (Tables 2 and 3). The study was approved by the National Medical Ethics Committee of the Republic of Slovenia and tissue samples were obtained after hysterectomy or during sterilization procedure with the full and informed consent of the patients. The specimens were immediately placed into RNA Later (Qiagen) RNA stabilization solution, and kept at –20 °C until RNA extraction. Total RNA was extracted with Tri Reagent (Sigma-Aldrich). The purity and quality of RNA were assessed by spectrophotometry (Nanodrop, Thermo Scientific) and by gel electrophoresis.

Preeclampsia, Gestational hypertension, and the presence of proteinuria were noted in 14% of the blood samples obtained from pregnant women.

**Selection of normalization gene candidates.** A cohort of 15 reference genes (GAPDH, CDN1, TUBB, ACTB, ABPB, RPS26, RIK, TFO, TKR, P450, RPLP0, SULT1A1, and SULT2B1) were used as controls in the performance and interpretation of the qPCRs. Statistical calculations and tests were immediately performed using the SPSS software (SPSS Inc, USA). The Mann Whitney test for unequal sample size was used. All of the statistical tests were two-tailed, and differences in values of less than 0.05 were considered to be significant.

**Western blot analysis.** Proteins were isolated from the same tissues as RNA. Protein aliquots were separated by SDS-PAGE. Proteins were transferred from gels to membranes and blocked with 5% non-fat milk for 2 hours. Afterwards the membranes were incubated overnight with primary antibodies (anti-CYP1B1, anti-CYP3A5, 1:20000) and rabbit anti-COMT (Millipore, Febit, CA, USA). Quantification was accomplished with the LightCycler® 480 Real-Time PCR System (Roche) using TaqMan® Universal PCR Master Mix and universal thermocycling parameters recommended by Applied Biosystems. The relative expression levels of the genes were calculated using the 2^- delta delta CT method.

**Figure 1. Phase I and Phase II metabolism of estrogens.** Enzymes of our interest are shown in red. ↓ down regulated genes, → up regulated genes and = statistically insignificant change in expression levels. The scatter plot graph show relative expression levels of the CYP1A1, CYP1A2, CYP1A3, CYP1A7, COMT, SULT1A1, SULT1B1, SULT1E1, UGT2B7, NQO1, NQO2 and GSTP1 genes in normal endometrium and endometriosis. The levels of gene expression were multiplied by 10^14 and are presented on a logarithmic scale. Bars denote the median expression levels.

**Table 1. Patients details for endometriosis control group.**

<table>
<thead>
<tr>
<th>Total number</th>
<th>Mean age</th>
<th>Prognostic phase</th>
<th>Late postoperative early recovery</th>
<th>Secrecy phase</th>
<th>Phase not determined</th>
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<tbody>
<tr>
<td>33</td>
<td>33 ± 6</td>
<td>14</td>
<td>5</td>
<td>5</td>
<td>3</td>
</tr>
</tbody>
</table>

**Figure 2.** Western blot analysis of protein fractions of normal endometrium (N) and endometriotic tissue (E). Representative samples are shown, and cyclophilin A was used as normalisation control. Box and whiskers graph show S-COMT, MB-COMT, and CYP1B1 protein expression, as well as MB-/S-COMT ratio.