Expression and regulation of estrogen-converting enzymes in ectopic human endometrial tissue

Sabine Fechner, Ph.D., a Bettina Husen, Ph.D., c Hubert Thole, M.D., c Markus Schmidt, M.D., b Isabella Gashaw, Ph.D., a Rainer Kimmig, M.D., b Elke Winterhager, Ph.D., a and Ruth Gru¨ mmer, Ph.D. a

a Institute of Anatomy and b Department of Gynecology, University Hospital Essen, Universitätsklinikum Essen, Essen; and c Solvay Pharmaceuticals Research Laboratories, Hannover, Germany

Objective: To investigate the regulation of estrogen-converting enzymes in human ectopic endometrial tissue.

Design: Animal study.

Setting: Academic medical center.

Animal(s): Sixty female nude mice with implanted human endometrial tissue.

Patient(s): Twenty-two premenopausal women undergoing endometrial biopsy or hysterectomy.

Intervention(s): Human endometrial tissue was implanted into the peritoneal cavity of nude mice, and the effect of therapeutic drugs on transcription of steroid receptors and estrogen-converting enzymes was analyzed.

Main Outcome Measure(s): Transcript levels of steroid hormone receptors, 17β-hydroxysteroid dehydrogenase type 1 and 2, aromatase, and steroid sulfatase as well as proliferation rate were analyzed in the human ectopic endometrial tissue.

Result(s): Steroid receptors and estrogen-converting enzymes were expressed in the ectopic human endometrial fragments. Application of medroxyprogesterone acetate, dydrogesterone, danazol, and the aromatase inhibitor finrozole significantly inhibited aromatase transcription. In addition, danazol caused a significant decrease in transcription of steroid sulfatase, and finrozole, of 17β-hydroxysteroid dehydrogenase type 1 in parallel to a decrease in proliferation rate in the ectopic human endometrial tissue.

Conclusion(s): Pharmacological regulation of transcription of estrogen-converting enzymes in human endometrial tissue cultured in nude mice may help to develop new therapeutic concepts based on local regulation of estrogen metabolism in endometriosis. (Fertil Steril 2007;88(Suppl 2):1029–38. ©2007 by American Society for Reproductive Medicine.)

Key Words: Aromatase, 17βHSD, aromatase, STS, endometriosis, estrogen metabolism, nude mouse

Endometriosis, defined by the presence of endometrium-like tissues outside the uterus, is one of the most frequent benign gynecological diseases that affects ≤60% of women of reproductive age with pelvic pain or infertility (1). Among the different theories discussed, one widely accepted mechanism for the development of endometriotic lesions is the implantation of menstrual endometrium on peritoneal surfaces via retrograde menstruation (2). However, because retrograde menstruation occurs in nearly all women of reproductive age, additional factors are assumed to contribute to the establishment of this disease.

There is a large body of evidence that suggests an important role for estrogen in the establishment and maintenance of endometriosis (3, 4). This disease develops mostly in women of reproductive age and regresses after menopause or ovariectomy (5). Because estrogen withdrawal causes the involution of endometriotic lesions in patients, the current therapy is focused on the lowering of endogenous estrogen to pharmacological castration levels, and surgical therapy to remove endometriotic lesions is widely combined with medical therapy to induce a hypoestrogenic state in patients (1). Suppression of the estrogen level, for example, by danazol or GnRH agonists, provides the regression of lesions; however, these drugs that induce a hypoestrogenic state cannot be used for prolonged duration because of severe side effects. In addition, recovery of the estrogen level after discontinuation of the therapies results in a high recurrence rate after these medical therapies (6–8).

Hence, there is a definite need for developing new drugs to provide long-term and more efficacious therapeutic alternatives with an improved side-effect profile. It has recently been reported that in addition to ovarian estrogen synthesis, estrogen is produced locally within endometriotic lesions (9), whereas estrogen biosynthesis does not take place in healthy uterine tissues (10). This local estrogen biosynthesis may be caused by an increased expression of the estrogen-synthesizing enzymes aromatase and 17β-hydroxysteroid dehydrogenase type 1 (17βHSD-1) (9, 11), accompanied by deficient 17βHSD-2 expression in the endometriotic epithelial cells (11, 12). Particularly, significant levels of aromatase activity and messenger RNA (mRNA) could be demonstrated in pelvic endometriotic implants (13, 14), and by using an aromatase inhibitor, a successful treatment of an unusually aggressive type of recurrent postmenopausal endometriosis has been reported (15).
One focus for new therapeutic approaches could be this local estrogen production, which leads to a sustained environment of elevated estrogen exposure in the endometriotic tissue. Because occurrence of spontaneous endometriosis is restricted to human beings and subhuman primates, we used an animal model to investigate therapeutic concepts for the treatment of endometriosis by xenotransplantation of human endometrial fragments into the peritoneal cavity of immunodeficient nude mice (16). These fragments implant and form endometriotic-like lesions, which resemble lesions found in patients in terms of macroscopic and histological appearance (16–19), steroid responsiveness (17, 19), and vascularization (16, 20).

By using the nude mouse as an experimental model, we obtained evidence that these endometriotic-like lesions exhibit expression of estrogen-converting enzymes, and our studies have shown that transcription of these genes within the human ectopic tissue is affected by exogenously applied drugs, meaning that drug effects can be assessed via expression analysis of specific genes.

MATERIALS AND METHODS
Human Endometrial Tissue
Endometrium of the proliferative phase of the menstrual cycle was obtained from 22 premenopausal women who were undergoing endometrial biopsy or hysterectomy at the Department of Gynecology, University Hospital Essen (Essen, Germany). Institutional ethical approval was obtained, and all women provided written informed consent. The stage of the menstrual cycle was confirmed by serum hormone determination by using competitive immunoassays for estrogen (Bayer Diagnostics, Leverkusen, Germany) and P (Bayer Diagnostics; P concentrations of >1.0 ng/mL were allocated to the secretory phase, and samples below that level were allocated to the proliferative phase) and by histological staging according to the method of Noyes and coworkers (21).

Explanted endometrial tissue was cut into fragments of 1–2 mm in diameter under sterile conditions and was left for 1 hour in culture medium (Dulbecco’s minimum essential medium–Ham’s F-12, 1:1; Biochrom KG, Berlin, Germany) supplemented with Pen/Strep (Invitrogen, Karlsruhe, Germany) and transplanted for 5 days from transplantation of human tissue onward. Animals in the control group were treated with vehicle only. Each drug was applied to at least four mice that were transplanted with endometria from different patients.

Animals
Athymic female nude mice (Han:nMRI nu/nu) were maintained in a barrier unit in a controlled pathogen-free environment and regulated 12:12-hour light–dark cycle. All equipment and food entering the barrier was autoclaved. Mice had free access to food and water. All experiments were performed in accordance with German laws for animal protection and with permission of the state.

Human endometrial tissue was transplanted into cyclic or into hormone-substituted ovariectomized mice. In the latter group, bilaterally ovariectomized nude mice were left untreated for ≥14 days. 17β-Estradiol and P (both, Sigma, Munich, Germany) were dissolved in benzylbenzoate; then, 0.05 μg of E2 + 30 μg of P per mouse per day was administered SC in 100 μL of arachis oil, beginning from the day of transplantation and continuing throughout the experiment.

Transplantation of Fragments and Tissue Processing
Of each patient, four human fragments per mouse were fixed intraperitoneally to the lateral abdominal wall of nude mice by laparotomy with surgical sutures, as described elsewhere (16). Implanted endometrial lesions were dissected 3–28 days after transplantation and were either frozen directly in liquid nitrogen or fixed with formalin and embedded in paraffin. For each experimental approach, endometrial tissue of at least four different patients was transplanted to separate mice.

Application of Drugs
Danazol (Sigma Aldrich, Munich, Germany), dydrogesterone (Solvay Pharmaceuticals Hannover, Germany), medroxyprogesterone acetate (MPA; Sigma Aldrich), and finrozole (MPV-2213ad; Hormos Medical Ltd, Turku, Finland) were diluted in benzylbenzoate (Synopharm GmbH, Barsbüttel, Germany)–castor oil (Fisher Scientific GmbH, Niddereau, Germany) 1:4 and were injected SC in a volume of 100 μL per animal. Mice were injected daily with 50 μg (MPA, finrozole, or dydrogesterone) or with 500 μg (danazol), for 5 days from transplantation of human tissue onward. Animals in the control group were treated with vehicle only. Each drug was applied to at least four mice that were transplanted with endometria from different patients.

Extraction of RNA and Reverse-Transcription Polymerase Chain Reaction
Isolation of total RNA from endometrial tissue was performed by using the RNAsin Minikit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. The concentration of RNA was determined spectrophotometrically, and the RNA was stored at −80°C until use.

Two micrograms of total RNA were digested with DNase I (Invitrogen, Germany) and transcribed into complementary DNA (cDNA) by reverse transcription with M-MLV Reverse Transcriptase (Invitrogen, Germany) by using an oligo (dT)16 primer in a total volume of 50 μL. Polymerase chain reaction (PCR) amplifications were obtained by using gene-specific primers (Table 1). For aromatase, the target and the endogenous control (β-actin) were run in separate tubes. For 17βHSD-2 and aromatase, 50 pmol of oligonucleotides per reaction was used; for β-actin, 2 pmol was used; and for all other amplifications, 25 pmol were used. The reaction mixture consisted of 4 μL of chromosomal DNA, 1.5 μL of deoxyribonucleotide triphosphate mixture (10 mM each), 5 μL of 5× PCR buffer (GeneCraft, Lüdinghausen, Germany), 2.5 U of DNA-Polymerase (GeneCraft), and sterile water in a total volume of 50 μL. Amplification program was 10 minutes at 94°C; 32 (estrogen receptor [ER]α) or 36 cycles
<table>
<thead>
<tr>
<th>Gene</th>
<th>Accession no.</th>
<th>For semiquantitative PCR</th>
<th>Product size (bp)</th>
<th>Sequence</th>
<th>For real-time PCR</th>
<th>Product size (bp)</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>b-Actin</td>
<td>NM_001101</td>
<td>3'ACCTTCAACCCACCCAGCCATGTACG 5'TGATCCACATCTGCTGGAAGGTGG</td>
<td>697</td>
<td>3'ACCAACTGCGGACGACATGGGAGAAAA</td>
<td>213</td>
<td>5'TACGCCAGAGCGGTACAGGATAG</td>
<td></td>
</tr>
<tr>
<td>17βHSD-1</td>
<td>NM_000413.1</td>
<td>3'AGGCTTATGCGAGAGTCTGG 5'ATGGCGGTGACGTAGTTGGA</td>
<td>348</td>
<td>3'ATGGCGGTGACGTAGTTGGA</td>
<td>176</td>
<td>5'CACAGCAAGCACTGTTTCG</td>
<td></td>
</tr>
<tr>
<td>17βHSD-2</td>
<td>NM_002153.1</td>
<td>3'GGTGTACGCTTCCTCATGT 5'CTTTATGACCTCACTGTTTC</td>
<td>417</td>
<td>3'GGTGTACGCTTCCTCATGT</td>
<td>157</td>
<td>5'CACAGCAAGCACTGTTTCG</td>
<td></td>
</tr>
<tr>
<td>STS</td>
<td>NM_000351.3</td>
<td>3'CCTTTTCCTGCTGCTCCTTC 5'CCATTACTCCGCGCATGAT</td>
<td>402</td>
<td>3'CCTTTTCCTGCTGCTCCTTC</td>
<td>142</td>
<td>5'CACAGCAAGCACTGTTTCG</td>
<td></td>
</tr>
<tr>
<td>Aromatase</td>
<td>X13589.1</td>
<td>3'TGCTTACCCAGTGAAAGAAAG 5'TCAAGGTCACATTTTTGGGAA</td>
<td>651</td>
<td>3'TGCTTACCCAGTGAAAGAAAG</td>
<td>181</td>
<td>5'TCAAGGTCACATTTTTGGGAA</td>
<td></td>
</tr>
<tr>
<td>ERα</td>
<td>NM_000125.2</td>
<td>3'AGTGTACACATTTTCTGCTCAGCA 5'AGTGTACACATTTTCTGCTCAGCA</td>
<td>227</td>
<td>3'AGTGTACACATTTTCTGCTCAGCA</td>
<td>130</td>
<td>5'GATCTCCACATGCGGTGCTCAGCAG</td>
<td></td>
</tr>
<tr>
<td>PR (A+B)</td>
<td>NM_000926.2</td>
<td>3'GGTCTACCCGCCCTATCTCA 5'GGCTTGGGCTTTCATTTGGGAA</td>
<td>396</td>
<td>3'GGCTTGGGCTTTCATTTGGGAA</td>
<td>171</td>
<td>5'CGATGCGAGCCTTTCGAG</td>
<td></td>
</tr>
</tbody>
</table>

Real-Time PCR

Samples were further analyzed by quantitative real-time reverse-transcription PCR by using the qPCR MasterMix for SYBR Green I (Eurogentec, Köln, Germany) in a 25-μL volume according to the supplier’s instructions, applying the ABI Prism 5700 Sequence Detection System (Applied Biosystems, Darmstadt, Germany). The amplification of gene targets was accomplished by using 80 ng of chromosomal DNA and 5 pmol gene-specific oligonucleotides per reaction (Table 1). To determine the concentration of the PCR fragments, serially diluted standard chromosomal DNAs generated for each gene were amplified in separate tubes in each run. The amplification program consisted of the following steps: 10 minutes at 95°C, 40 cycles of 15 seconds at 95°C, and 2 minutes at 60°C. Finally, the temperature was raised gradually (0.1°C/s) from 65°C to 95°C for the melting curve analysis to verify the specificity of the amplification. Because of the diversity in the RNA quality, the amplification products were size-fractionated on a 2% agarose gel and detected by ethidium bromide staining. The degree of expression was evaluated densitometrically and correlated to the expression of β-actin.

Immunohistochemistry

Immunostaining was performed on paraffin sections by applying the DAB staining method by using the VECTASTAIN Elite ABC kit (Vector Laboratories, Burlingame, CA) according to the manufacturer’s protocol. Sections were deparaffinized, hydrated, and exposed twice for 5 minutes each in a microwave oven at 250 W in 0.01 M citrate buffer, pH 6 (Merck, Darmstadt, Germany) for antigen retrieval. Endogenous peroxidase activity was quenched by immersion in 1% hydrogen peroxide (Merck) in phosphate-buffered saline for 15 minutes at room temperature, and nonspecific binding was reduced by two 5-minute incubations at 37°C in phosphate-buffered saline containing 0.5% bovine serum albumin, followed by incubation with goat serum (Vectastain; Vector Laboratories, Inc., Peterborough, UK) for 20 minutes. Slides were incubated overnight at 4°C with the following primary antibodies: mouse–anti-human cytochrome P450 aromatase (1:50; Serotec, Düsseldorf, Germany) and rabbit–anti-human 17βHSD-1 (1:500; Solvay Pharmaceuticals, Hannover, Germany), respectively. As positive controls, immunoreactions on adult placenta were performed. To demonstrate specificity of the staining, consecutive sections were stained with the same protocol omitting the primary antibody. Results were recorded with a Zeiss Axiophot photomicroscope (Zeiss, Jena, Germany).

FIGURE 1

Semiquantitative reverse-transcription PCR evaluation of ERα and PR A+B mRNA in human endometrial fragments cultured for ≤28 days in cyclic and hormone-substituted ovariectomized (OVX) mice, respectively. Compared with the corresponding eutopic endometria (C) from day 1 (the day of human tissue collection), the amount of ERα transcripts was lowered during culturing but was maintained generally constant during the culture period. Transcription of PR was maintained in the human endometrial fragments cultured in cyclic mice and showed a significant decrease when cultured in OVX mice. *Significant difference compared with control (P<.05).
The intensity of immunostaining was semiquantitatively divided into strong (+++), moderate (++), low (+), or negative (−).

**Determination of Proliferation Rate**

Immunostaining was performed on paraffin sections as described in the previous section, by using a mouse monoclonal anti–Ki-67 antibody (MIB-1; Dianova, Germany). Sections were counterstained with toluidine blue, and proliferation rate was calculated as percentage of stained cells in relation to the total amount of glandular epithelial cells. For each drug, all glands in one representative section of an endometrial fragment of three different patients were analyzed.

**Statistical Analysis**

Exploratory data analysis and the nonparametric analyses of variances (Kruskal-Wallis and Mann-Whitney tests) were performed by applying the programs SPSS for Windows (version 12; SPSS, Chicago, IL) and Microsoft Excel XP for

**FIGURE 2**

Semiquantitative reverse-transcription PCR of the estrogen-converting enzymes 17βHSD-1, 17βHSD-2, steroid sulfatase (STS), and aromatase transcripts in human endometrial fragments cultured in cyclic and hormone-substituted ovariectomized (OVX) mice at ≤28 days. Compared with the corresponding eutopic endometria (C), in both experimental groups, expression of 17βHSD-1 and 17βHSD-2 mRNA significantly decreased from 14 and 7 days of culture onward, respectively. Amount of STS transcripts was slightly lowered in endometrial transplants in cyclic mice but was significantly decreased during culturing in OVX mice. Aromatase mRNA was expressed in eutopic endometrium as well as in the human endometrial lesions that were cultured in nude mice at ≤28 days. *Significant difference compared with control (P<.05).

FIGURE 3

(a–d) Immunohistochemical staining of aromatase (a, b) and 17βHSD-1 (c, d) in human endometrium that was cultured in nude mice for 14 and 28 days, respectively. The aromatase protein constantly was expressed mainly in the glandular epithelium at 14 days (a) as well as 28 days (b) after transplantation. In addition to its presence in the glandular epithelium, 17βHSD-1 also was distributed throughout the stromal compartment, showing no obvious change in intensity during the culture period after 14 (c) and 28 (d) days, respectively. (e–h) Immunohistochemical staining of aromatase in human endometrium cultured in mice for 5 days. Staining for aromatase was very weak in the glandular epithelium of endometrial tissue of the vehicle-treated control group (e, g), as well as in the corresponding endometrium of the experimental groups that were treated with finrozole (f) or MPA (h). Immunolabeling of glandular epithelium of human endometrium cultured in MPA-treated mice (h), however, decreased compared with controls (g). In all panels, bar = 20 μm. E = epithelium; S = stroma.

Windows (Microsoft, Redmond, WA). Differences with \(P \leq 0.05\) were regarded as statistically significant.

RESULTS

Expression of Steroid Hormone Receptors

Transcription of ER\(\alpha\), ER\(\beta\), and of both isoforms (A and B) of the P receptor (PR) was investigated in human endometria before and after transplantation into the peritoneal cavity of nude mice. Expression of ER\(\beta\) mRNA could not be detected in eutopic as well as in transplanted endometrial tissue (data not shown), whereas transcripts of ER\(\alpha\) and PR(A+B) were present in the human endometrial tissue during the entire culture period of \(\leq 28\) days (Fig. 1). Reverse-transcription PCR analysis of those endometrial fragments showed an initial decrease in amount of ER\(\alpha\) transcripts compared with the case of the corresponding eutopic endometrium; however, levels stayed widely constant during the culture period (Fig. 1). Levels of PR(A+B) mRNA were decreased in human tissue cultured in mice. Although this was not significant in cyclic mice, PR(A+B) gene transcription decreased significantly from day 3 after transplantation onward in ovariectomized, hormone-supplemented mice (Fig. 1). Taken together, transcripts of steroid hormone receptors were expressed for \(\leq 4\) weeks in human endometrial tissue that was cultured in nude mice.

Expression of Estrogen-Converting Enzymes

Transcripts of the estrogen-converting enzymes 17\(\beta\)HSD-1, 17\(\beta\)HSD-2, steroid sulfatase, and aromatase could be demonstrated in human endometrial tissue that was cultured in the peritoneal cavity of nude mice. Expression of 17\(\beta\)HSD-1 and 17\(\beta\)HSD-2 mRNA decreased in the lesions, starting from day 14 of culture for 17\(\beta\)HSD-1 and from day 7 of culture for 17\(\beta\)HSD-2, respectively (Fig. 2). Transcription of steroid sulfatase was maintained in the human endometrial tissue during culturing in cyclic mice but decreased significantly when cultured in ovariectomized, hormone-substituted mice from day 3 onward, compared with in the corresponding eutopic endometrium. Aromatase, a key enzyme of estrogen biosynthesis, was expressed in eutopic endometrium as well as in the human endometrial lesions that were cultured in nude mice for \(\leq 28\) days (Fig. 2).

Immunohistochemical evaluation revealed consistent staining for aromatase in the glandular epithelium and expression of 17\(\beta\)HSD-1 protein in the glandular epithelium, as well as in the stromal cells of human endometrial tissue for \(\leq 28\) days of culturing (Fig. 3). The decrease in expression shown for 17\(\beta\)HSD-1 transcripts could not be seen on an immunohistochemical level.

In contrast to the expression of steroid hormone receptors, transcription especially of the HSDs decreased significantly when cultured in nude mice for \(> 1\) week. For this reason, in following experiments, the effect of drugs on the transcription of estrogen-converting enzymes in ectopic endometrial lesions was analyzed in human tissue after 5 days of culture.

Effects of Therapeutic Drugs on the Expression of Estrogen-Converting Enzymes and on Proliferation of the Ectopic Tissue

Effects of therapeutic drugs on transcription of steroid hormone receptors and estrogen-converting enzymes were analyzed by quantitative real-time reverse-transcription PCR analysis of transcription of ER\(\alpha\) and PR and of the estrogen-converting enzymes 17\(\beta\)HSD-1, 17\(\beta\)HSD-2, steroid sulfatase (STS), and aromatase in human endometrial fragments cultured in the peritoneal cavity of cyclic nude mice. Mice were SC injected with 50 \(\mu\)g of MPA, or finrozole, or of dydrogesterone or with 500 \(\mu\)g of danazol daily, for 5 days from transplantation of human tissue onward. Animals in the control group were treated with vehicle only. Expression in the corresponding human endometria cultured in control animals was set at one. Application of all substances tested resulted in a significant reduction of aromatase mRNA. Treatment with finrozole in addition led to a significant decrease in transcription of 17\(\beta\)HSD-1, and treatment with danazol, to a reduction of STS mRNA. *Significant difference compared with control (\(P < 0.05\)).

PCR in human endometrial tissue that was cultured for 5 days in the peritoneal cavity of nude mice. Subcutaneous application of the progestational drugs MPA and dydrogesterone and the aromatase inhibitor finrozole in doses that were related to those used for the treatment of endometriosis in patients had no significant effect on transcription of ERα and PR(A+B). However, all substances tested caused a significant reduction of aromatase mRNA levels within the endometrial fragments (MPA, 48%; dydrogesterone, 67%; and finrozole, 59%) compared with controls (Fig. 4). In addition, application of the aromatase inhibitor finrozole resulted in a decrease in transcription of all enzymes investigated, leading to a significant reduction of 17βHSD-1 mRNA (53%) in addition to the inhibition of aromatase transcription (Fig. 4).

These short-time effects on transcription of aromatase occurring after 5 days of treatment hardly could be detected at the protein level. Immunostaining for aromatase was very weak in the vehicle-treated control group (Fig. 3e and g), as well as in the experimental groups that were treated with finrozole (Fig. 3f) or MPA (Fig. 3h). Thus, transcription of estrogen-converting enzymes can be regulated in ectopic endometrial lesions by systemic application of therapeutic drugs.

A reduction of proliferation of human endometrial epithelial cells of 38% was observed after application of dydrogesterone; a reduction of 36% after application of MPA; and a statistically significant reduction of 80.4%, after application of finrozole (Fig. 5).

**DISCUSSION**

Estradiol is the most important factor that is known to be involved in the development of endometriosis. It has been reported that estrogen is produced locally within endometriotic lesions as a result of an aberrant expression of estrogen-converting enzymes (9, 11). Here, we have demonstrated that ectopic human endometrial fragments cultured in the peritoneal cavity of nude mice express transcripts of estrogen and P receptors as well as of the estrogen-converting enzymes 17βHSD-1, 17βHSD-2, aromatase, and E1 sulfatase. Moreover, transcription of enzymes can be regulated within the human tissue by application of therapeutic drugs, leading to an inhibition of local estrogen synthesis. As a functional parameter, epithelial proliferation was decreased within the ectopic endometrial fragments in parallel to inhibition of aromatase and 17βHSD-1.

Because the development of endometriosis is restricted to human beings and subhuman primates, transplantation of human endometrial tissue into nude mice represents one of the most widely used and well-established models for the investigation of mechanisms that are involved in the development of endometriosis (22). In this mouse model, lesions were shown to share morphological and histological appearance with the native human endometrial tissue (16, 23). The presence of estrogen and P receptors at the protein level in these lesions, as well as angiogenesis, which guarantees the transport of systemically applied drugs to the human tissue, already has been demonstrated in this model (16). Human endometrial tissue cultured in ovariectomized mice substituted with constant levels of estrogen and P showed a significant decrease of PR over time when compared with tissue cultured in cyclic mice. This change had no obvious impact on the gene expression of steroid-converting enzymes in these tissue fragments, but should be taken into consideration when using hormone-supplemented mice as a model.

The drug classes tested here in cyclic mice are used in the treatment of endometriosis and are known to affect estrogen metabolism. Dydrogesterone, MPA, as well as the aromatase inhibitor finrozole had no significant effect on transcription of steroid hormone receptors; however, all led to a significant reduction of proliferation of glandular epithelium in human endometrial fragments cultured in mice treated SC with 50 μg of MPA, of finrozole, or of dydrogesterone, for 5 days from transplantation of human tissue onward. Animals in the control group were treated with vehicle only. Application of MPA and dydrogesterone led to a slight decrease, whereas treatment with finrozole resulted in a significant reduction of proliferation rate. *Significant difference compared with control (P<.05).

![FIGURE 5](image-url)
decrease in aromatase transcription compared with the case of control animals. Progestational drugs like MPA and dydrogesterone frequently are used primarily to reduce pain that is associated with endometriosis (24, 25). Our studies reveal for the first time that those progestins inhibit aromatase transcription in ectopic human endometrial lesions. With regard to their influence on aromatase enzyme activity, different effects are described in literature. Although it could be shown that aromatase activity was stimulated by P and MPA in adenomyosis (26) and in stromal cells of human uterine endometrium (27), no effect of high doses of MPA could be seen on the peripheral conversion of androstenedione to E₁ as a precursor for E₂ biosynthesis (29), and it has been discussed that locally enhanced estrogen biosynthesis may be a result of increased expression of aromatase (9). The clinical significance of local aromatase activity in endometriotic tissue was exemplified recently by the successful use of aromatase inhibitors in the treatment of endometriosis (15, 30, 31). A role for aromatase enzyme activity for the growth of endometriotic implants could be shown by Fang and coworkers (32), who demonstrated that uterine mouse tissue autotransplanted to peritoneal surfaces of aromatase knockout mice or of wild-type mice treated with letrozole led to a significant reduction in lesion size. However, aromatase inhibitors may cause unwanted side effects because of the high expression of aromatase in the ovary (33) and interference with the hypothalamic-pituitary-gonadal axis in premenopausal patients.

The major product of aromatase activity in endometriosis is E₁, which is only weakly estrogenic and must be converted to the potent estrogen E₂ to exert a full estrogenic effect. Expression of 17βHSD-1, which catalyzes the conversion of E₁ to E₂, could be shown in endometriotic lesions (11, 12), whereas 17βHSD-2, which inactivates estrogen, is absent from endometriotic cells, favoring an increase of E₂ levels in endometriotic tissue (11). Because 17βHSDs show tissue-specific expression and play a pivotal role in the local intracrine modulation of steroid hormones in target tissues (34), inhibitors of these enzymes are predicted to cause tissue-specific responses to steroid hormones. This new class of therapeutics is called selective intracrine modulators and exhibits an enzyme-mediated effect in contrast to receptor-mediated effects (35). In ongoing experiments, newly developed 17βHSD-1 inhibitors are tested in this animal model of endometriosis, which offers the advantage of investigating the effect of systemically applied drugs on peritoneal endometrial lesions. Effects of systemic application on human endometrial tissue have been described before for antiangiogenic agents by Hull and coworkers (20) and for application of ERβ antagonists by Harris and colleagues (36) in the nude-mouse model. Here we extend this model in terms of estrogen metabolism and interaction with therapeutic drugs, as a convincing experimental tool for testing locally restricted, tissue-specific inhibition of estrogen synthesis.

Acknowledgments: The authors thank Hormos Medical Ltd, Turku, Finland for providing finrozole and thank the staff of the central animal unit of the University Hospital Essen for breeding and maintaining of nude mice.

REFERENCES