Differential expression of steroidogenic enzymes according to endometriosis type

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Objective: To evaluate, in peritoneal, ovarian, and rectovaginal endometriotic lesions, expression of steroidogenic enzymes involved in the activation and inactivation of estrogens: 17β-hydroxysteroid dehydrogenase type 1 (HSD17B1) and 2 (HSD17B2), estrone sulfotransferase (EST), and steroid sulfatase (STS).
Setting: Academic gynecology research unit.
Design: Retrospective study.
Patient(s): Disease-free (n = 41) patients and patients with endometriosis (n = 79) were included for quantitative polymerase chain reaction (q-PCR) (15 disease-free, 33 endometriosis) and immunohistochemistry (26 disease-free, 46 endometriosis) studies.
Intervention(s): Q-PCR and immunohistochemistry.
Main Outcome Measure(s): Evaluation of mRNA and protein expression.
Result(s): Glandular HSD17B1, HSD17B2, and STS protein expression were demonstrated. HSD17B2 mRNA values were higher in the secretory phase of the menstrual cycle in the endometrium of disease-free women, but not in the eutopic endometrium of patients with endometriosis. HSD17B1 mRNA was equally expressed in the various tissues investigated, and EST mRNA was expressed at low levels in the different lesion types. HSD17B2 mRNA expression was decreased in ovarian and rectovaginal endometriosis compared with eutopic endometrium, while STS mRNA was increased in rectovaginal lesions compared with ovarian lesions. Ratios between pro- and antiestrogenic enzymes (STS/EST and HSD17B1/HSD17B2) were more in favor of estrogens in ovarian and rectovaginal endometriosis.
Conclusion(s): In endometriosis development, local activation of estrogens appears to be important. STS and HSD17B1 inhibitors may therefore prove useful to treat the disease. (Fertil Steril® 2013;100:1642–9. ©2013 by American Society for Reproductive Medicine.)
Key Words: Peritoneal endometriosis, ovarian endometriosis, rectovaginal endometriosis, steroid enzymes

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Endometriosis is one of the most common benign gynecological conditions, characterized by proliferation of endometrial glands and stroma outside the uterine cavity. Growing evidence suggests that endometriosis can present in three different forms, each with its own pathogenesis: peritoneal, ovarian, and rectovaginal (1–5). Endometriosis is known to be an estrogen-dependent disease and primarily a disease of the reproductive years, only rarely observed in teenage girls and postmenopausal women. It does affect postmenopausal women treated with estrogen replacement therapy, and histological endometriosis has even been found in men undergoing high-dose estrogen therapy for prostate cancer (6–8). Suppression of estrogen with GnRH agonists leads to regression of lesions, while discontinuation of therapy results in their rapid recurrence (9–11).

Steroidogenesis is the biological process by which steroids are generated from cholesterol and transformed into other steroids (12). The aromatase enzyme is implicated in the conversion of androgens (androstenediol and T) to

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estrogens (estrone and E_2, respectively). Estrone can be converted to biologically active E_2 by the 17β-hydroxysteroid dehydrogenase type 1 (HSD17B1) enzyme, with inverse conversion catalyzed by 17β-hydroxysteroid dehydrogenase type 2 (HSD17B2). Much of the estrogen formed can be converted to estrogen sulfate by the action of estrogen sulfotransferase (EST). Sulfation of estrogens converts them from hydrophobic to hydrophilic molecules and renders them biologically inactive, since they are unable to bind to estrogen receptors (ERs) (13). The steroid sulfatase (STS) enzyme catalyzes reactivation of these sulfated estrogens but also plays an important role in steroidogenesis by virtue of its ability to convert dehydroepiandrosterone sulfate (DHEA-S) to DHEA.

The role of aromatase in endometriosis is still controversial (14–17).

Human HSD17B1 is expressed only in a limited number of normal tissues, such as placenta, ovarian follicles, mammary glands, and the uterus (18, 19). Expression of HSD17B1 was shown to be elevated and have prognostic significance in hormone-dependent breast cancer (20, 21), as well as leiomyomas (22) and endometriosis (23, 24). HSD17B1 mRNA expression was found to be up-regulated in ovarian endometriomas (23) and deep-infiltrating endometriosis (25).

HSD17B2 is an important enzyme implicated in the inactivation of E_2 in disease-free endometrium, whose expression is normally up-regulated in early secretory endometrium (26, 27). In endometriotic tissues, HSD17B2 expression and activity levels were found to be lower than in disease-free tissues (25, 28).

EST was shown to be equally expressed in eutopic endometrium from disease-free patients (29), and eutopic and ectopic endometrium from patients with endometriosis (23, 25, 30). In a previous study, it was found to be expressed in the secretory phase only, suggesting that it is regulated by progestins (29).

STS is known to be widely distributed throughout the body and present in eutopic endometrium, endometriotic implants, and uterine adenomyotic lesions (25, 29, 31–33). STS mRNA expression was notably reported to be higher in ovarian endometriosis than in disease-free endometrium (23). Moreover, Purohit et al. found STS activity in endometriotic lesions to be correlated with endometriosis severity (34). No difference in STS activity was identified in a recent study, however, with all tested samples (eutopic and ectopic endometrial tissues) showing high levels of STS activity (28).

While the estrogen dependence of endometriosis has long been known, only a few studies have focused on enzymes potentially implicated in local estrogen biosynthesis. These studies often concentrated on analyzing expression of one enzyme (mainly aromatase) in one type of endometriotic lesion (or in endometriosis, without specifying the lesion type).

The objective of the present study was to analyze, at both transcriptional (quantitative polymerase chain reaction [q-PCR]) and post-transcriptional (immunohistochemistry [IHC]) levels, expression of the four steroid enzymes downstream of aromatase in estrogen biosynthesis (i.e., STS, EST, HSD17B1, and HSD17B2) in the three types of human endometriotic lesions (peritoneal, ovarian, and rectovaginal) and to compare these levels with those observed in their matched eutopic endometrium.

MATERIALS AND METHODS

Use of human tissue for this study was approved by the Institutional Review Board of the Université Catholique de Louvain (B403201213871).

Biopsies for q-PCR and IHC studies were recovered from two different patient pools (Table 1).

Q-PCR Study

Biopsy collection. Biopsies of endometriotic lesions (15 peritoneal, 15 ovarian, and 15 rectovaginal) and their matched eutopic endometrium (n = 33) were taken from 33 patients (mean age, 29.3 ± 4.6 years) with laparoscopically proven endometriosis (Table 1). Among these 33 women, eight were in the proliferative phase, nine in the secretory phase, and 16 on oral contraceptives. Eutopic endometrium was also collected from 15 patients (mean age, 39.2 ± 10.2 years) without endometriosis (as confirmed by laparoscopy at the time of biopsy) using an endometrial biopsy curette (Table 1).

All the biopsies were dissected by an expert gynecological surgeon. A small sample of each was fixed in 4% formaldehyde, embedded in paraffin, and serially sectioned (5 μm) for histologic confirmation of endometriosis (presence of

TABLE 1

Patient characteristics

	Q-PCR		IHC	
	Disease-free	Endometriosis	Disease-free	Endometriosis
No. of patients	15	33	26	46
Proliferative phase	7	8	17	15
Secretory phase	5	9	8	18
Oral contraception	3	16	1	9
GnRH agonist treatment	—	—	—	4
No. of patients with one lesion		21		43
No. of patients with more than one lesion		12		3

Colette. Steroid enzymes in endometriosis. Fertil Steril 2013.