Estrogen biosynthesis in endometriosis: molecular basis and clinical relevance

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ABSTRACT
Conversion of C19 steroids to estrogens is catalyzed by aromatase in human ovary, placenta and extraglandular tissues such as adipose tissue, skin and the brain. Aromatase activity is not detectable in normal endometrium. In contrast, aromatase is expressed aberrantly in endometriosis and is stimulated by prostaglandin E2 (PGE2). This results in local production of estrogen, which induces PGE2 formation and establishes a positive feedback cycle. Another abnormality in endometriosis, i.e. deficient hydroxysteroid dehydrogenase (17β-HSD) type 2 expression, impairs the inactivation of estradiol to estrone. These molecular aberrations collectively favor accumulation of increasing quantities of estradiol and PGE2 in endometriosis. The clinical relevance of these findings was exemplified by the successful treatment of an unusually aggressive case of postmenopausal endometriosis using an aromatase inhibitor.

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INTRODUCTION
Endometriosis is a chronic disease manifested by pelvic pain and infertility and defined as the presence of endometrial glands and stroma within the pelvic peritoneum and other extra-uterine sites. It is estimated to affect 2–10% of women in the reproductive age group (Vessey et al. 1993, Kjerulf et al. 1996). Endometriosis is viewed to be a polygenically inherited disease of complex multifactorial etiology (Olive & Schwartz 1993). Sampson’s theory of transplantation of endometrial tissue on the pelvic peritoneum via retrograde menstrual efflux from pelvic peritoneal surfaces, possibly involving the immune system (Halme et al. 1988). Alternatively, intrinsic molecular aberrations in pelvic endometriotic implants were proposed to contribute significantly to development of endometriosis. Aberrant expression of aromatase, certain cytokines and tissue metalloproteinases, deficiency of 17β-hydroxysteroid dehydrogenase (17β-HSD) type 2 and resistance to the protective action of progesterone are some of these molecular abnormalities (Khorram et al. 1993, Sharpe-Timms et al. 1995, Noble et al. 1996, Osteen et al. 1996, Bruner et al. 1997, Zeitoun et al. 1998, 1999). Since endometriosis is an estrogen-dependent disorder, aromatase expression and 17β-HSD type 2 deficiency are of paramount importance in the pathophysiology of endometriosis. In this article, aberrant mechanisms of estrogen biosynthesis and metabolism in women with endometriosis are reviewed, with emphasis on identifying targets for new treatment strategies.
DISCUSSION

Estrogen biosynthesis and metabolism in humans

The conversion of androstenedione and testosterone to estrone and estradiol is catalyzed by aromatase, which is expressed in a number of human tissues and cells such as ovarian granulosa cells, placental syncytiotrophoblast, adipose tissue and skin fibroblasts, and the brain. In the reproductive-age woman, the ovary is the most important site of estrogen biosynthesis, and this takes place in a cyclic fashion. Upon binding of follicle-stimulating hormone (FSH) to its G-protein-coupled receptor in the granulosa cell membrane, intracellular cAMP levels rise and enhance binding of two critical transcription factors, i.e. steroidogenic factor-1 (SF-1) and cAMP response element binding protein (CREB), to the classically located proximal promoter II of the aromatase gene (Michael et al. 1995, 1997). This, in turn, activates aromatase expression and consequently estrogen secretion from the pre-ovulatory follicle (Simpson et al. 1994, Michael et al. 1995).

On the other hand, in postmenopausal women, estrogen formation takes place in extra-ovarian tissues such as the adipose tissue and skin (MacDonald et al. 1967, 1978, Ackerman et al. 1981) (Fig. 1). In contrast to cAMP regulation of aromatase expression in the ovary, this is controlled primarily by cytokines (IL-6, IL-11, TNFα) and glucocorticoids via the alternative use of promoter I.4 in adipose tissue and skin fibroblasts (Simpson et al. 1994). The major substrate for aromatase in adipose tissue and skin is androstenedione of adrenal origin. In postmenopausal women, approximately 2% of circulating androstenedione is converted to estrone, which is further converted to estradiol in these extra-ovarian tissues. This may give rise to significant serum levels of estradiol capable of causing endometrial hyperplasia or even carcinoma (MacDonald et al. 1967, 1978).

Aromatase expression in Müllerian-derived tissues

Müllerian tissues are known targets of estrogen action. Until recently, estrogen action has been classically viewed to occur only via an ‘endocrine’ mechanism; in other words, it was thought that only circulating estradiol, whether secreted by the ovary or formed in the adipose tissue, could exert an estrogentic effect after delivery to target tissues via the bloodstream. Studies on aromatase expression in breast cancer demonstrated that paracrine mechanisms play an important role in estrogen action in this tissue (Bulun et al. 1993a). Estrogen produced by aromatase activity in breast adipose tissue fibroblasts was demonstrated to promote the growth of adjacent malignant breast epithelial cells (Yue et al. 1998). Finally, we demonstrated an ‘intracrine’ effect of estrogen in uterine leiomyomas and endometriosis: estrogen produced by aromatase activity in the cytoplasm of leiomyoma smooth muscle cells or endometriotic stromal cells can exert its effects by readily binding to its nuclear receptor within the same cell (Bulun et al. 1994, Noble et al. 1996, 1997). Disease-free endometrium and myometrium, on the other hand, lack aromatase expression (Bulun et al. 1993b, Noble et al. 1997).

The significance of aromatase expression in endometriosis

Among estrogen-responsive pelvic disorders, aromatase expression was studied in greatest detail in endometriosis (Bulun et al. 1993b, Noble et al. 1996, 1997, Zeitoun et al. 1999). Firstly, extremely high
levels of aromatase mRNA were found in extra-ovarian endometriotic implants and endometriomas. Secondly, endometriosis-derived stromal cells in culture incubated with a cAMP analog displayed extraordinarily high levels of aromatase activity comparable to that in placental syncytiotrophoblast (Noble et al. 1997). These exciting findings led us to test a battery of growth factors, cytokines and other substances that might induce aromatase activity via a cAMP-dependent pathway in endometriosis. Prostaglandin E2 (PGE2) was found to be the most potent known inducer of aromatase activity in endometriotic stromal cells (Noble et al. 1997). In fact, this PGE2 effect was found to be mediated via the cAMP-inducing EP2 receptor subtype. Moreover, estrogen was reported to increase PGE2 formation by stimulating cyclo-oxygenase type 2 (COX-2) enzyme in endometrial stromal cells in culture (Huang et al. 1996). Thus, a positive feedback loop for continuous local productions of estrogen and PGs is established, favoring the proliferative and inflammatory characteristics of endometriosis (Fig. 2). Additionally, aromatase mRNA was also detected in the eutopic endometrial samples of women with moderate to severe endometriosis (but not in those of disease-free women) albeit in much smaller quantities compared with endometriotic implants (Noble et al. 1996). This may be suggestive of a genetic defect in women with endometriosis, which is manifested by this subtle finding in the eutopic endometrium. We propose that when defective endometrium with low levels of aberrant aromatase expression reaches the pelvic peritoneum by retrograde menstruation, it causes an inflammatory reaction that exponentially increases local aromatase activity, i.e. estrogen formation, induced directly or indirectly by PGs and cytokines (Noble et al. 1997). It would be rather naive to propose that aberrant aromatase expression is the only important molecular mechanism in the development and growth of pelvic endometriosis. There may be many other molecular mechanisms that favor the development of endometriosis: abnormal expression of proteinase type enzymes that remodel tissues or their inhibitors (matrix metalloproteinases, tissue inhibitor of metalloproteinase-1), certain cytokines (IL-6, RANTES) and growth factors (EGF) represent some of mechanisms (Khorram et al. 1993, Sharpe-Timms et al. 1995, Osteen et al. 1996, Bruner et al. 1997). Alternatively, a defective immune system that fails to clear peritoneal surfaces of the retrograde menstrual efflux has been proposed in the development of endometriosis (Halme et al. 1988, Hill 1992). The development of endometriosis in an individual woman probably requires the coexistence of a threshold number of these aberrations. Nonetheless, aberrant aromatase expression is clinically relevant, since aromatase inhibitors suppress postmenopausal endometriosis (Takayama et al. 1998).

**Figure 2.** Origin of estrogen in endometriotic lesions: estradiol (E2) that affects an endometriotic lesion arises from several body sites. In an ovulatory woman, E2 is secreted directly from the ovary in a cyclic fashion. In the early follicular phase and after menopause, extra-ovarian tissues (adipose and skin) are the most important sources to account for the circulating E2. Estradiol is also produced locally in the endometriotic implant itself in both ovulatory and postmenopausal women. The most important precursor, androstenedione (A) of adrenal and ovarian origins, becomes converted to estrone (E1) that is in turn reduced to E2 in these tissues and endometriotic implants. We demonstrated significant levels of 17β-hydroxysteroid dehydrogenase type 1 expression in endometriosis, which catalyzes the conversion of E1 to E2 (Zeitoun et al. 1998). Estradiol and cytokines (IL-1β, TNFβ), which are increased in endometriosis, induce cyclo-oxygenase-2 (COX-2) giving rise to elevated concentrations of PGE2 in this tissue (Huang et al.). PGE2 in turn, is the most potent known stimulator of aromatase in endometriotic stromal cells (Noble et al. 1997). This establishes a positive feedback loop in favor of continuous estrogen formation in endometriosis.

**Regulation of aromatase expression in endometriotic stromal cells**

As emphasized earlier, PGE2 was found to be the most potent known inducer of aromatase activity by increasing cAMP levels via cell surface EP2 receptors in endometriotic stromal cells (Noble et al. 1997).
et al. 1997). On the other hand, neither cAMP analogs nor PGE$_2$ was capable of stimulating any detectable aromatase activity in eutopic endometrial stromal cells in culture. The obvious question became: what are the molecular differences that give rise to aromatase expression in endometriosis and its inhibition in eutopic endometrium? To address this, we first determined that the cAMP-inducible promoter II was used for in vivo aromatase expression in endometriotic tissue (Zeitoun et al. 1999). Then, a stimulatory transcription factor, SF-1, and an inhibitory factor, chicken ovalbumin upstream promoter transcription factor (COUP-TF), were found to compete for the same binding site in aromatase promoter II. COUP-TF was ubiquitously expressed in both eutopic endometrium and endometriosis, whereas SF-1 was expressed, specifically in endometriosis but not in eutopic endometrium, and binds to aromatase promoter more avidly than COUP-TF (Zeitoun et al. 1999). Thus, SF-1 and other transcription factors (e.g. CREB) activate transcription in endometriosis, whereas COUP-TF, which occupies the same DNA site in eutopic endometrium, inhibits this process (Zeitoun et al. 1999) (Fig. 3). In summary, one of the molecular alterations leading to local aromatase expression in endometriosis but not in normal endometrium is the aberrant production of SF-1 in endometriotic stromal cells, which overcomes the protective inhibition maintained normally by COUP-TF in the eutopic endometrium.

Interconversions of estrone and estradiol in endometriosis

The primary substrate for aromatase activity in endometriosis is androstenedione of adrenal and ovarian origins in premenopausal women. The major product of aromatase activity in endometriosis, namely estrone, is only weakly estrogenic and must be converted to the potent estrogen estradiol to exert a full estrogenic effect. We demonstrated that the enzyme 17β-HSD type 1, which catalyzes the conversion of estrone to estradiol, is expressed in endometriosis (Andersson & Moghrabi 1997, Zeitoun et al. 1998). In contrast, the enzyme 17β-HSD type 2 (encoded by a separate gene)
inactivates estradiol by catalyzing its conversion to estrone in eutopic endometrial glandular cells during the luteal phase (Andersson & Moghrabi 1997). Progesterone actually induces the activity of this enzyme in endometrial glandular cells in culture, making inactivation of estradiol to estrone one of the anti-estrogenic properties of progesterone (Satyaswaroop et al. 1982). The expression of 17β-HSD type 2 is absent from endometriotic glandular cells, as demonstrated in paired samples of eutopic endometrium and pelvic endometriosis obtained simultaneously during the luteal phase (Zeitoun et al. 1998). Consequently, this protective mechanism that lowers estradiol levels is lost in endometriotic tissue (Zeitoun et al. 1998). The aberrant expression of aromatase, the presence of 17β-HSD type 2 is absent from endometriotic glandular cells, as demonstrated in paired samples of eutopic endometrium and pelvic endometriosis obtained simultaneously during the luteal phase (Zeitoun et al. 1998). Consequently, this protective mechanism that lowers estradiol levels is lost in endometriotic tissue (Zeitoun et al. 1998). The aberrant expression of aromatase, the presence of 17β-HSD type 2 in endometriosis collectively give rise to elevated local levels of estradiol compared with eutopic endometrium. Additionally, 17β-HSD type 2 deficiency may also be viewed as a defective action of progesterone, which fails to induce this enzyme in endometriotic tissue (Fig. 4).

**Rationale for using aromatase inhibitors to treat endometriosis**

Endometriosis is successfully suppressed by estrogen deprivation with GnRH analogs or the induction of surgical menopause. Control of pelvic pain with GnRH agonists is usually successful during and immediately after the treatment, whereas pain associated with endometriosis returns in up to 75% of these women (Henzl et al. 1988, Waller & Shaw 1993). There may be multiple reasons for the failure of GnRH agonist treatment of endometriosis. One likely explanation is the presence of significant estradiol production that continues in the adipose tissue, skin and endometriotic implant per se during the GnRH agonist treatment. Therefore, blockage of aromatase activity in these extra-ovarian sites with an aromatase inhibitor may keep larger number of patients in remission for longer periods of time (Fig. 5). The most striking evidence for the significance of extra-ovarian estrogen production is the recurrence of endometriosis after successfully completed hysterectomy and bilateral salpingo-oophorectomy in a
number of women (Metzger et al. 1991, Takayama et al. 1998). Endometriotic tissue in one such aggressive case was found to express much higher levels of aromatase mRNA compared with premenopausal endometriosis (Takayama et al. 1998). We recently reported the treatment of a 57-year-old overweight woman who had recurrence of severe endometriosis after hysterectomy and bilateral salpingo-oophorectomy. Two additional laparotomies were performed owing to persistent severe pelvic pain and bilateral ureteral obstruction leading to left renal atrophy and right hydrenephrosis. Treatment with megestrol acetate was ineffective. A large (3 cm) vaginal endometriotic lesion contained unusually high levels of aromatase mRNA. The patient was given anastrozole (an aromatase inhibitor) for 9 months. Despite the addition of calcium and alendronate (a nonsteroidal inhibitor of bone resorption), bone density in the lumbar spine decreased by 6.2%. The occurrence of significant bone loss in this particular case should be studied further. Dramatic relief of the pain and regression of the vaginal endometriotic lesion were observed within the first month of treatment. At the same time, circulating estradiol levels were reduced to 50% of the baseline value. Markedly high pretreatment levels of aromatase mRNA in the endometriotic tissue became undetectable in a repeat biopsy 6 months later, and the lesion nearly disappeared after 9 months of therapy. Two potential mechanisms may have accounted for this strikingly successful result. Firstly, there was evidence of suppression of extra-ovarian (i.e. skin and adipose tissue) aromatase activity, giving rise to a significant decrease in serum estradiol level (Fig. 5). Secondly, unusually high levels of aromatase expression in the endometriotic lesion disappeared after treatment with the aromatase inhibitor, anastrozole (Fig. 5). Besides the expected direct inhibition of aromatase activity in endometriosis by anastrozole, the disappearance of aromatase mRNA expression in the lesion may be explicable by denial of estrogen that is known to stimulate local biosynthesis of PGE2, which, in turn, stimulates aromatase expression (Fig. 2).

In summary, the recently developed potent aromatase inhibitors are candidate drugs in the treatment of endometriosis that is resistant to standard regimens. In fact, the use of aromatase inhibitors may be the only available treatment for aggressive postmenopausal endometriosis. It remains to be seen whether aromatase inhibitors alone or together with present lines of therapy in premenopausal women will increase the pain-free interval and time to recurrence after discontinuation (Fig. 5). Studies are under way to address these questions.
SUMMARY

The development and growth of endometriotic lesions are estrogen-dependent. The mechanisms and effectiveness of hormonal treatments for endometriosis should be re-evaluated in view of the new advances that increased our understanding of the body sites of estrogen production in a woman with endometriosis. In addition to ovarian secretion, estradiol is also produced in peripheral sites such as skin, adipose tissue and endometriotic lesions per se. We suggest that the intracrine and paracrine effects of estradiol produced in the target tissue amplify the estrogenic action of steroid hormones delivered via the circulation. Additionally, defective inactivation of estradiol in endometriosis in contrast to eutopic endometrium may further enhance this local effect. Aberrant aromatase activity and defective estradiol metabolism in endometriosis are consequences of specific molecular aberrations such as inappropriate expression of a stimulatory transcription factor or progesterone resistance in this tissue. The clinical relevance of these findings was recently exemplified by the successful treatment of a severe case of recurrent postmenopausal endometriosis with an aromatase inhibitor. Future treatment strategies may be designed to target the signal transduction for aromatase expression in endometriosis or to enhance progesterone action in this tissue.

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