REVIEW

Estrogen biosynthesis in endometriosis: molecular basis and clinical relevance

S E Bulun[1], K M Zeitoun[2], K Takayama[3] and H Sasano[3]

[1]Departments of Obstetrics and Gynecology and Molecular Genetics, University of Illinois at Chicago, 820 S. Wood St. M/C 808, Illinois 60612, USA

[2]Department of Obstetrics and Gynecology, Columbia University College of Physicians and Surgeons, 622 W. 168th St., New York, New York 10032–3702, USA

[3]Departments of Pathology and Obstetrics and Gynecology, Tohoku University School of Medicine, 2–1 Seiryo Machi, Sendai-Shi 980, Japan

(Requests for offprints should be addressed to S E Bulun; Email: sbulun@uic.edu)

ABSTRACT

Conversion of C_{19} 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 E_2 (PGE_2). This results in local production of estrogen, which induces PGE_2 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 PGE_2 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, Kjerulff *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 menstruation is the most widely accepted explanation for the development of pelvic endometriosis because of convincing circumstantial and experimental evidence (Sampson 1927). Since retrograde menstruation is observed in almost all cycling women, endometriosis is postulated to develop as a result of the coexistence of a defect in clearance of the 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.

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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 estrogenic effect after delivery to target tissues via the bloodstream. Studies on aromatase expression in breast cancer demonstrated that paracrine
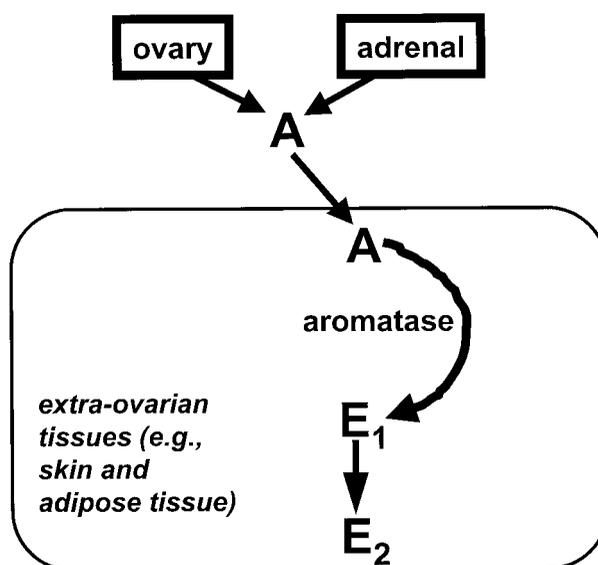

FIGURE 1.   Extra-ovarian estrogen formation in women. Estradiol (E_2) in women is either directly secreted by the ovary or produced in extra-ovarian sites (adipose tissue and skin). The principal substrate for extra-ovarian aromatase activity in women is androstenedione (A) of adrenal and ovarian origins. Androstenedione is converted by aromatase to estrone (E_1) in adipose tissue and skin fibroblasts. Estrone is further converted to E_2 by 17β-HSD type 1 activity in these peripheral tissues. Thus, extra-ovarian aromatization is the major source for circulating E_2 in the postmenopausal period or during ovarian suppression.

mechanisms play an important role in estrogen action in this tissue (Bulun *et al*. 1993*a*). 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*. 1993*b*, 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*. 1993*b*, 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 E_2 (PGE_2) was found to be the most potent known inducer of aromatase activity in endometriotic stromal cells (Noble *et al*. 1997). In fact, this PGE_2 effect was found to be mediated via the cAMP-inducing EP_2 receptor subtype. More-over, estrogen was reported to increase PGE_2 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 endo-metriosis (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 devel-opment 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 metalloprotein-ases, 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
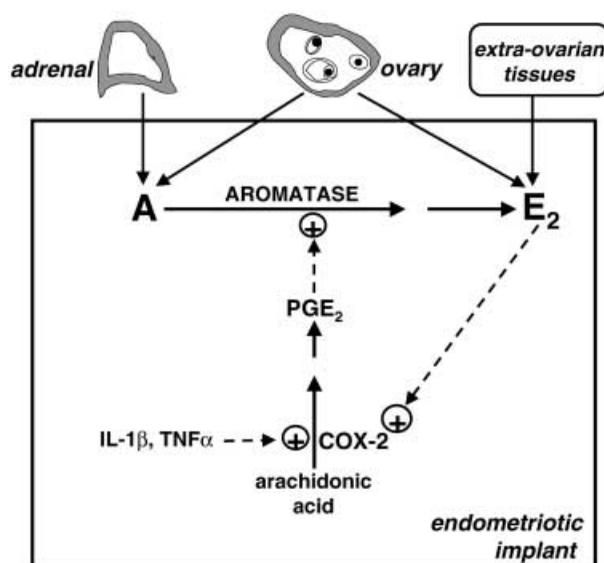

FIGURE 2.   Origin of estrogen in endometriotic lesions: estradiol (E_2) that affects an endometriotic lesion arises from several body sites. In an ovulatory woman, E_2 is secreted directly from the ovary in a cyclic fashion. In the early follicular phase and after menopause, extra-ovarian tissues (adipose and skin) are the most important sources to account for the circulating E_2. 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 (E_1) that is in turn reduced to E_2 in these tissues and endometriotic implants. We demonstrated significant levels of 17β-hydroxysteroid dehydrogenase type 1 expression in endometriosis, which catalyzes the conversion of E_1 to E_2 (Zeitoun *et al*. 1998). Estradiol and cytokines (IL-lα, TNFβ), which are increased in endometriosis, induce cyclo-oxygenase-2 (COX-2) giving rise to elevated concentrations of PGE_2 in this tissue (Huang *et al*.). PGE_2 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.

these aberrations. Nonetheless, aberrant aromatase expression is clinically relevant, since aromatase inhibitors suppress postmenopausal endometriosis (Takayama *et al*. 1998).

Regulation of aromatase expression in endometriotic stromal cells

As emphasized earlier, PGE_2 was found to be the most potent known inducer of aromatase activity by increasing cAMP levels via cell surface EP_2 receptors in endometriotic stromal cells (Noble
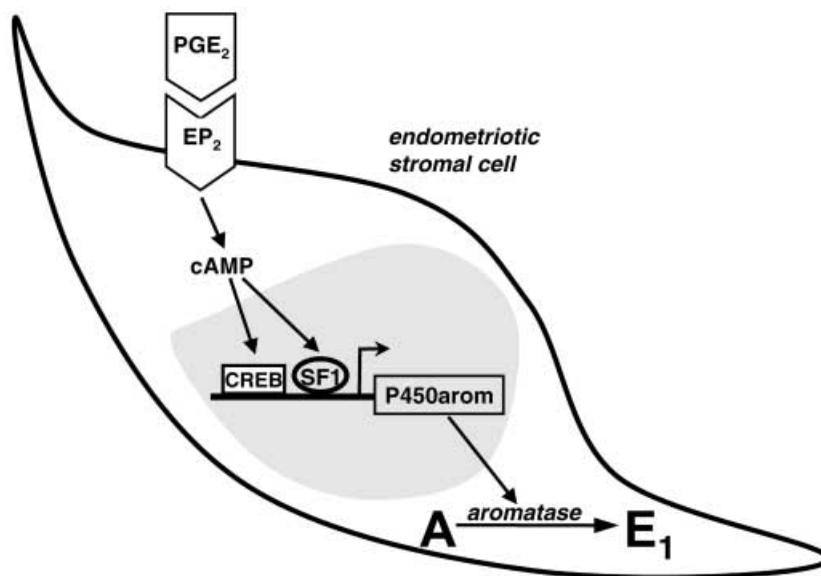
FIGURE 3. Proposed mechanism of regulation of aromatase (P450arom) expression by SF-1 and CREB in endometriosis. Upon binding of PGE_2 to its cell surface EP_2 receptor, intracellular cAMP levels increase. This gives rise to binding of cAMP response element binding protein (CREB) and SF-1 to specific motifs upstream of aromatase promoter II. The stimulatory type transcription factor SF-1 binds as a monomer to a nuclear receptor half-site with a higher affinity compared with that of the inhibitory factor COUP-TF (not shown in the figure), which binds to the same site relatively loosely as a dimer. SF-1 then synergizes with CREB (bound to upstream CRE) and possibly other factors to activate transcription of the P450arom gene in response to cAMP (Zeitoun *et al.* 1999).

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)
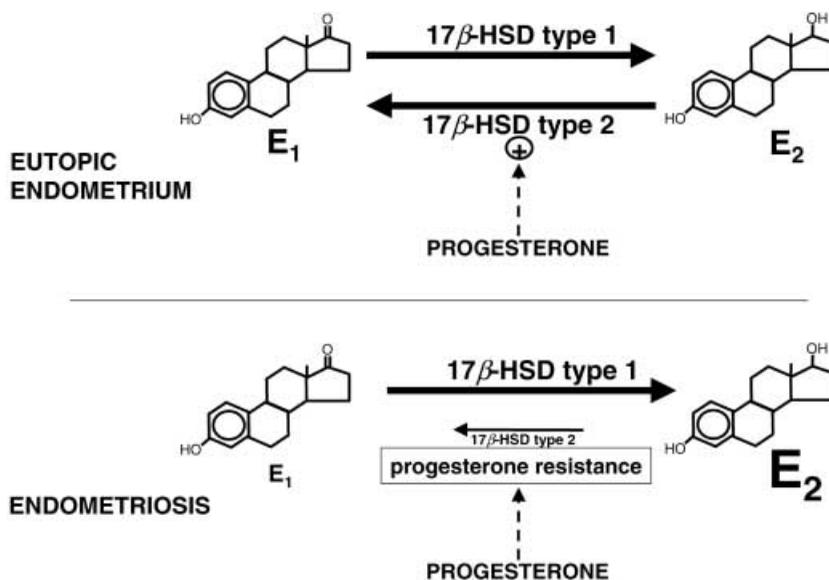
FIGURE 4. Defective inactivation of estradiol (E_2) in endometriosis: E_2 reaches the endometriotic lesions via the blood stream. Additionally, estrone (E_1) is produced in the stromal cell via aberrant aromatase activity. Estrone is further reduced to E_2 by 17β-HSD type 1 in the endometriotic tissue. Estradiol is normally inactivated by conversion to E_1 by 17β-HSD type 2 in epithelial cells of the eutopic endometrium in response to progesterone during the secretory phase. In endometriotic tissue, however, E_2 is not metabolized owing to the lack of 17β-HSD type 2, giving rise to increased local concentration of this potent estrogen. The absence of 17β-HSD type 2 expression in endometriosis despite high levels of progesterone during the secretory phase is indicative of selective progesterone resistance in this tissue.

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 1 and the absence of 17β-HSD type 2 from 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
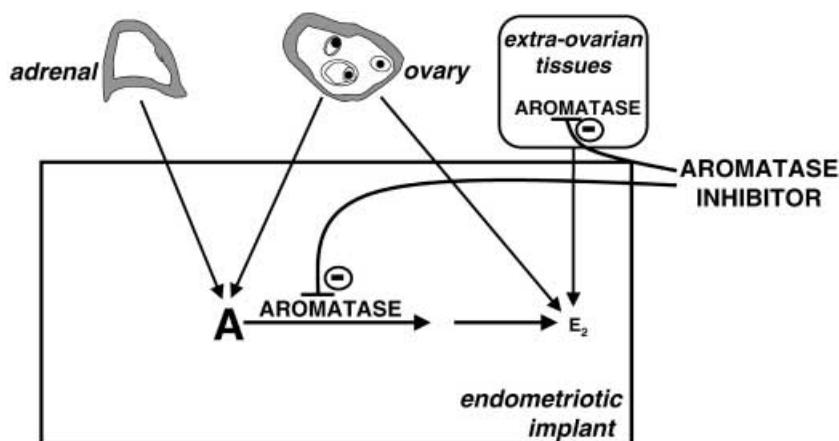
FIGURE 5.   Sites of action of aromatase inhibitors to treat endometriosis. In cases resistant to treatment with GnRH agonists or in postmenopausal endometriosis, the use of aromatase inhibitors to block estrogen formation in the skin and adipose tissue as well as in endometriotic tissue may be critical in inhibiting the growth of endometriotic tissue. Recurrent endometriosis, especially after surgical removal of the ovaries, may represent lesions that are sensitive to extremely low levels of estradiol (E_2). Thus, suppression of E_2 production in the extra-ovarian sites (adipose tissue/skin) and in endometriotic tissue may be mandatory for successful treatment of endometriosis.

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 pre-menopausal 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 laparoto-mies were performed owing to persistent severe pelvic pain and bilateral ureteral obstruction leading to left renal atrophy and right hydronephrosis. 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 in-hibitor) 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 disappear-ance of aromatase mRNA expression in the lesion may be explicable by denial of estrogen that is known to stimulate local biosynthesis of PGE_2, 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.

ACKNOWLEDGEMENTS

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