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ENDOMETRIOSIS

The effect of oral contraceptives on aromatase expression in the eutopic endometrium of patients with endometriosis

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Abstract

Objective. To determine the effect of oral contraceptives containing gestodene on aromatase expression in the endometrium of patients diagnosed with endometriosis.

Patients and methods. Endometrial biopsies were taken at the time of laparoscopy in 40 patients with endometriosis, 16 of whom were using an oral contraceptive containing gestodene at the time of laparoscopy. The remaining 24 patients were receiving no form of treatment for endometriosis. Endometrial biopsies taken from 23 patients with normal echographic signs and no symptoms were used as controls. Aromatase expression was evaluated in endometrial samples using immunohistochemistry.

Results. In the untreated, symptomatic endometriosis patients, aromatase expression was detected during the proliferative phase in 92% of cases, while in the symptom-free control patients aromatase was expressed in only 9% of cases. In patients with endometriosis who were using oral contraceptives, there were significantly fewer cases of positive endometria compared with the untreated patients with endometriosis (6%).

Conclusion. Oral contraceptives containing gestodene are effective in decreasing aromatase expression in the eutopic endometrium of patients with endometriosis.

Keywords: *Endometrium, aromatase, gestodene, endometriosis, menstrual cycle*

Introduction

The eutopic endometrium of patients with endometriosis is capable of aberrantly expressing the enzyme aromatase, which stimulates the transformation of androgen precursors into estrogens [1]. In the endometrium, the aromatase gene is activated by inflammatory mediators such as prostaglandin E_2, thus establishing a vicious circle between inflammation and local estrogen production. This mechanism is greatly enhanced during menstruation when endometrial cells capable of expressing aromatase are transported in a retrograde fashion into the peritoneal cavity, thereby triggering an intense inflammatory response that will ultimately augment aromatase activity exponentially [1,2]. The inducing effects of prostaglandin E_2 on the promoter of the aromatase gene is observed only in endometrial cells from patients with endometriosis, being absent in the endometrium of disease-free women [3]. This locally increased estrogen production is thought to play a pivotal role in the mechanism of implantation and in the development of endometriotic lesions, since a hyperestrogenic milieu is known to be a facilitating factor for the development of endometriosis [1]. However, the relationship between aromatase expression in eutopic endometrial cells and the capacity of these cells to implant in the peritoneal cavity, thus escaping the surveillance of the immunological system, is not yet fully understood [4]. The hormonal and inflammatory factors that regulate aromatase expression in the endometrium are therefore important for the survival of endometrial cells in the peritoneal cavity, their subsequent implantation and

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the formation of endometriotic lesions [5]. In patients with myomas, aromatase expression in the endometrium is lower during the luteal phase, thus suggesting an inhibitory effect of progesterone [6]. Similarly in peripheral macrophages, aromatase expression was also found to diminish significantly during the luteal phase compared with the other phases of the menstrual cycle [7]. Oral contraceptives (OCs) and danazol are able to mimic this inhibitory effect of endogenous progesterone on aromatase expression when used in patients with adenomyosis [8,9]. Aromatase inhibitors, either alone or in combination with OCs, are also effective for the treatment of endometriotic lesions including those with aggressive clinical behavior [10,11]. Moreover, when OCs were used in an extended regimen in patients with endometriosis who had been successfully treated by laparoscopic surgery, they were found to be more effective for the treatment of pain recurrence than when the same contraceptives were used cyclically [12,13]. These non-contraceptive health benefits of the pill in the treatment of endometriosis-related pain are probably a consequence of inhibition of cyclooxygenase-2 (COX-2) expression in the endometrium and the consequent reduction in prostaglandin production in this tissue, although other mechanisms may also work synergistically, including the suppression of menstruation itself [14,15].

In the present observational, case–control study, aromatase expression was investigated using immunohistochemical methods in endometrial biopsies obtained from patients with a laparoscopically proven diagnosis of endometriosis, who were either untreated or had been using OCs containing gestodene in an extended regimen prior to surgery.

Patient and methods

This was a retrospective, observational study carried out in paraffin-embedded endometrial tissue to detect aromatase expression in both untreated and OC-treated patients with endometriosis. Endometrial biopsies were performed using a 4 mm Karman catheter (IPS, USA) in 40 patients submitted to laparoscopy and hysteroscopy for the treatment of endometriosis in our institute between January 2005 and January 2007. All patients submitted to this procedure had a history of pelvic pain associated or not with abnormal ultrasonographic findings. In this group, 17 patients had a history of pelvic pain and a previous diagnosis of ovarian endometrioma made following transvaginal sonography (TVS) and confirmed by laparoscopy. In the remaining patients ($n = 23$) preoperative TVS findings were normal but peritoneal foci of endometriosis were detected in the Douglas pouch, bladder, peritoneum and broad ligament during laparoscopy. Patients with normal uteri at sonography but who were referred to our unit for hysteroscopic evaluation of the uterine cavity, including endometrial biopsy, as part of the diagnostic work-up prior to *in vitro* fertilization procedures because of male-factor infertility, served as symptom-free controls ($n = 23$). These patients had no history of pelvic pain or abnormal uterine bleeding, and the uterine cavity was normal at hysteroscopy. Laparoscopy was carried out in all 40 patients not only to confirm the diagnosis of endometriosis but also to coagulate peritoneal lesions or to drain and excise ovarian endometriotic cysts. Hysteroscopy and endometrial biopsies were carried out concomitantly. All patients were included in the present study retrospectively and had progesterone levels compatible with ovulatory cycles and normal thyroid function as inferred by their admission medical records. At the time of laparoscopy and endometrial biopsy, 16 patients with endometriosis were using an OC containing 30 μg of ethinyl estradiol and 75 μg of gestodene (Gestinol®; Libbs Farmacêutica, Sao Paulo, Brazil) in a continuous regimen as prescribed by their attending physician to ameliorate pelvic pain and uterine bleeding. The duration of OC treatment varied between 2 and 6 months in 15 patients, while one patient with an ovarian endometrioma had been using the pills for 24 months. In the untreated patients with endometriosis, laparoscopy was carried out in the proliferative phase of the menstrual cycle. All patients were premenopausal and were in the 22- to 40-year age bracket.

The endometrial samples were fixed in formalin 10% before being sent to pathology. Immunohistochemistry was carried out following antigen retrieval to detect the presence of aromatase p450. Aromatase expression was investigated using a commercially available monoclonal antibody (MCA2077, clone H4; Serotech, Raleigh, NC, USA). Antigen retrieval was carried out using the Tris–ethylenediaminetetraacetic acid buffer at pH 8.0. The reaction was revealed using the streptavidin–biotin method. The presence of aromatase expression was rated either as positive if there was any detectable staining reaction or negative when no reaction was observed. Placental tissue and an atrophic endometrial sample were used as positive and negative controls, respectively, in all immunostaining reactions for aromatase p450. Statistical analysis was carried out using the StatsDirect software program, version 2.3.8 (StatsDirect Ltd, Cheshire, UK, 2004). The χ^2 and Fisher's exact tests with significance established at $p < 0.05$ were used to compare proportions of aromatase expression in the endometrium among the three groups: untreated, symptomatic endometriosis patients ($n = 24$); OC users with endometriosis ($n = 16$); and asymptomatic patients with normal findings at TVS ($n = 23$). Patients included in the present study

gave their informed consent for the immunohistochemical studies to be performed on the biopsy specimens.

Results

The proportion of patients with peritoneal and ovarian forms of endometriosis was the same in both OC-treated and untreated patients, as shown in Table I. However, in all patients previously treated with gestodene there was a visible reduction in inflammation and lesion vascularization, which was observed during the laparoscopic procedure. Endometrial biopsies taken at the time of hysteroscopy showed proliferative endometrium in the untreated patients with endometriosis. In patients using an OC containing 75 μg of gestodene with 30 μg of ethinyl estradiol, the most common histological feature of the endometrium was either decidual stroma in 12/16 (75%) of the biopsies or atrophy in the remaining cases.

Aromatase expression was detected by immunohistochemistry in the eutopic endometrium of untreated, symptomatic patients with endometriosis in 22/24 cases (92%) during the proliferative phase of the menstrual cycle, but was seldom detected in the endometrium of symptom-free control women (2/23, 9%). This difference was statistically significant ($p < 0.001$). In endometriosis patients not using OCs, aromatase expression in the eutopic endometrium was present solely in the stroma in 17/24 (71%) of endometrial biopsies, while the glandular epithelium was positive in 3/24 (13%) (Figure 1). In the remaining cases, aromatase expression was either detected in both gland and stroma or the endometrium was negative (Table II). The difference between stroma and glands in the percentage of positive aromatase expression was significant ($p < 0.001$).

In endometriosis patients using an OC containing gestodene at the time of laparoscopy, aromatase expression in the eutopic endometrium was detected in only one case (1/16) (6%), being negative in the rest of the patients. This aromatase-positive patient had used OCs for 4 weeks and at the time of laparoscopy she had a 7 cm hemorrhagic functional ovarian cyst and multiple scattered endometriotic lesions in her pelvis. There were also thick adhesions between the colon and the posterior part of the uterus, obliterating the Douglas pouch. The difference in endometrial aromatase expression between untreated and OC-treated patients with endometriosis was statistically significant ($p < 0.01$). However, there was no significant difference between OC users and symptom-free controls during the proliferative phase. These results are summarized in Table III.

Table I. Peritoneal and ovarian endometriosis in patients treated or not with oral contraceptives (OCs).

	Untreated	OC users
Peritoneal	14/24 (58)	9/16 (56)
Ovarian	10/24 (42)	7/16 (44)

Data are expressed as *n*/*N* (%).

Discussion

The present study confirmed previous observations that aromatase expression is present in the eutopic endometrium of endometriosis patients, while it is absent in the endometrium of disease-free women. These results are in agreement with recent observations that aromatase expression is detected

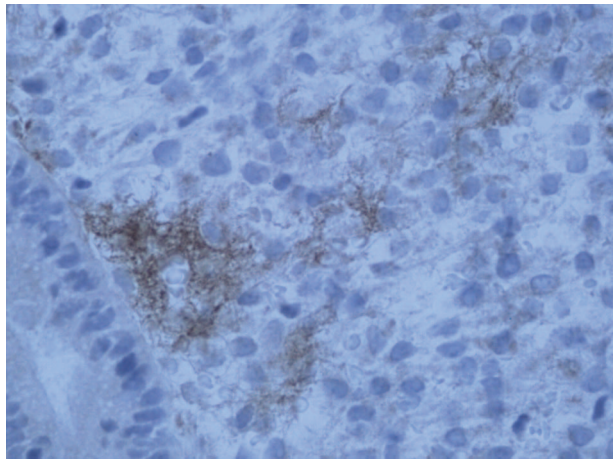

Figure 1. Aromatase expression in the endometrial stroma of a patient with endometriosis during the proliferative phase of the menstrual cycle.

Table II. Cellular distribution of aromatase expression in the endometrium of endometriosis patients during the proliferative phase of the menstrual cycle.

Stroma positive–gland negative	17/24 (71)*
Gland positive–stroma negative	3/24 (13)
Gland and stroma positive	2/24 (8)
Gland and stroma negative	2/24 (8)

Data are expressed as *n*/*N* (%); *significantly more than in the glandular epithelium ($p < 0.001$).

Table III. Aromatase expression in the endometrium of patients with endometriosis and disease-free patients, and the effect of oral contraceptives (OCs).

Untreated patients with endometriosis	22/24 (92)
Patients with endometriosis using OCs	1/16 (6)*
Symptom-free patients	2/23 (9)*

Data are expressed as *n*/*N* (%); *significantly less than untreated patients ($p < 0.001$).

by immunohistochemistry in endometriotic tissue and that it correlates positively with the severity of the disease [16]. Our findings that aromatase expression is detected mainly in the stroma of the eutopic endometrium is in agreement with previous studies showing a direct relationship between the amount of stroma present in endometriotic lesions and their histological appearance and hormonal responsiveness [17]. Similar to the eutopic endometrium, aromatase expression in the endometriotic foci is also present mostly in the stroma and is greatly stimulated by prostaglandin E_2 [1,2]. The stimulation of aromatase expression by inflammatory mediators is one of the key mechanisms that lead to the enhanced estrogen production in endometriosis, since aromatase expression is exponentially increased in endometrial cells upon their arrival in the peritoneal cavity and their exposure to the ensuing inflammation and its mediators [18]. One of the intriguing features of the pathogenesis of endometriosis is why macrophages have an impaired scavenger capacity to phagocyte these cells, although they continue to produce cytokines, prostaglandins and growth factors that will ultimately promote more estrogen production and angiogenesis in the endometriotic lesions [19,20]. It is possible that excessive local estrogen production may play a pivotal role in suppressing the phagocytosis of endometrial cells by activated macrophages [20]. Despite the impaired scavenger function, macrophage-conditioned media have the capacity to stimulate endometrial stromal cell proliferation *in vitro* [21]. The exponential rise in aromatase expression in the endometrial cells when they reach the peritoneal cavity through retrograde menstruation will greatly stimulate estrogen production in these cells, thereby inhibiting the scavenger function of the macrophages [1,2,4,19,20].

One may hypothesize that under these circumstances the ensuing inflammatory response triggered by the presence of menstrual debris in the peritoneal cavity, instead of being detrimental to the shed of endometrial cells, will promote their survival by stimulating an exponential rise in aromatase activity [1]. However, this will only occur in endometrial cells already displaying aromatase expression, since prostaglandin E_2, an inflammatory mediator, does not stimulate aromatase expression in normal endometrial cells [2]. It is important to emphasize that endometriosis is a disease with high recurrence rates following both medical and surgical treatment, and this may not only be due to the reactivation of preexisting lesions [22]. One additional explanation for this may lie in the fact that the eutopic endometrium of these patients is somewhat different from the endometrium of disease-free women, since it constitutively expresses the enzyme aromatase as demonstrated in this and other previous studies [1–3]. The presence of aromatase will facilitate the implantation of new cells in the peritoneum as soon as menstruation resumes, thus initiating a new cycle of inflammation and *de novo* formation of endometriotic lesions [1,2]. This explains the discrepancy between the almost universal occurrence of retrograde menstruation and the development of endometriosis in a much smaller proportion of cases [23]. The inflammation in the peritoneum triggered by the presence of menstrual debris will exacerbate aromatase expression solely in endometrial cells already expressing this enzyme, since prostaglandin E_2 is devoid of any effect in the negative endometrial stroma [2]. Therefore, the expression of aromatase will determine whether endometrial cells will be spared or destroyed in the pelvis by the immunological system in the days following menstruation. This is in agreement with our present findings that there is a high incidence of positive aromatase expression in the eutopic endometria of symptomatic women with active endometriosis lesions that is not found in the endometrium of disease-free women.

The estrogen concentration inside the endometriotic foci correlates positively with the severity of the disease, since high levels of aromatase expression are found in particularly aggressive forms of the disease [10,11,16]. The enhanced estrogen production may facilitate the progression of endometriosis through several mechanisms, although interference with macrophage phagocytosis is probably one of the most important [4,18–20]. In patients with endometriosis, there is indeed an increase in the number of activated non-adherent macrophages with a reduced surface expression of scavenger receptors and diminished capacity to destroy ectopic endometrial cells [19].

If this explanation is correct, then aromatase activity in the eutopic endometrium may be a major risk factor for the development of endometriosis, since locally produced estrogens may play a pivotal role not only in preventing macrophages from destroying these cells in the peritoneal cavity, but also in allowing their implantation and survival [1,3,5]. In this respect, the role of stroma in the development of endometriosis is crucial [17], since aromatase expression was mainly detected in this tissue not only in the eutopic endometrium but also in the endometriotic lesions [2]. Because aromatase was rarely found in eutopic endometrial glands, it is possible that stroma cells are the ones that actually implant in the peritoneum, inducing the formation of glandular-like epithelium in the peritoneal mesothelium by metaplasia. This may explain the important differences in histological features and hormonal responsiveness between the underdeveloped glands in endometriotic lesions and the fully functional ones in the ectopic endometrium [17,23]. However, this assumption cannot be proved or refuted by the

present data since this a clinical observational study that was not designed to test this hypothesis.

Our findings that OCs containing gestodene are able to suppress aromatase expression in the eutopic endometrium of patients with endometriosis may explain their effectiveness in the treatment of endometriosis [24]. Progestins may inhibit aromatase expression through several mechanisms including suppression of ovarian steroidogenesis, although a local effect on the eutopic endometrium diminishing aromatase gene transcription may be the most important one [8,25]. The ability of progestins and danazol to suppress aromatase expression in the endometrium suggests that they may play an important role in the prevention and treatment of endometriosis [8,9,25]. If aberrant aromatase expression in the eutopic endometrium plays a key role in the progression of endometriosis, then OCs should be an important line of therapy to prevent the recurrence of endometriosis [12,13,25]. OCs, when used continuously, are effective in preventing recurrences following surgical treatment. Epidemiological studies have also shown that the risk of developing endometriosis is lower during OC use; however, this protective effect disappears following discontinuation of treatment [25]. This suggests that as long as OCs are taken continuously, aromatase expression will be suppressed in the endometrium, and this may be the mechanism by which these compounds are effective in preventing the progression or recurrence of endometriosis. OCs containing gestodene also inhibit COX-2 expression in the endometrium in a similar fashion to that of endogenous progesterone, and this constitutes an additional factor contributing towards a reduction in inflammation and associated pain [14,15]. Endometriosis is an inflammatory disease and the blockade of both COX-2 and aromatase expression by OCs may explain the effectiveness of these compounds in controlling the disease [25].

One possible explanation for the failure of both medical and surgical treatments of endometriosis in the long term may be the resumption of menstruation, which in a retrograde fashion will carry aromatase-positive cells to the peritoneum. If this proves to be correct, then patients with endometriosis should not be allowed to menstruate, and one of the most cost-effective ways of accomplishing this is through the use of OCs in extended regimens.

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