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Aromatase and cyclooxygenase-2 expression in endometrial polyps during the menstrual cycle

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Abstract

Objectives. To study the changes in aromatase, Ki-67 and cyclooxygenase-2 (COX-2) expression during the menstrual cycle in both endometrial polyps and normal endometria.

Patients and methods. Paraffin-embedded tissue samples from 118 premenopausal patients were submitted to immunohistochemistry for measurement of aromatase, COX-2 and Ki-67 expression. Fifty cases of endometrial polyps and 68 cases of disease-free endometrium were included.

Results. The presence of aromatase expression was significantly higher in endometrial polyps than in disease-free endometria. On the other hand, changes in COX-2 and Ki-67 expression followed a similar pattern during the menstrual cycle in both groups, expression peaking during the proliferative phase and falling during the late luteal phase.

Conclusion. A significantly higher proportion of endometrial polyps express aromatase compared with disease-free endometrium; however, no correlation was found between aromatase expression and changes in either Ki-67 or COX-2 expression during the menstrual cycle.

Introduction

Aromatase activity is detected in several tissues, where it plays a pivotal role in the regulation of local estrogen production from androgen precursors [1]. In the normal cycling endometrium, however, aromatase activity is absent and endometrial proliferation is stimulated by the rising blood levels of estrogens produced by the developing follicle [2]. Although the contribution of local estrogen production to the regulation of endometrial proliferation during the menstrual cycle is negligible, there is evidence that this mechanism plays an important role in the genesis of both benign and malignant endometrial pathologies. In endometriotic lesions, for example, estrogens are produced locally from their androgen precursors, and aromatase activity is expressed mainly in the stromal cells [3]. In addition, the eutopic endometrium of patients with endometriosis is already endowed with the capacity to express aromatase, although levels are somewhat lower than those found in endometriotic foci [4]. However, the presence of aromatase expression in the eutopic endometrium of uteri harboring other gynecological pathologies decreases the specificity of its use as a widespread laboratory test for the diagnosis of endometriosis [5].

The mechanisms involved in the regulation of local estrogen production by endometrial stroma cells and their pivotal role in the development of both malignant and benign endometrial pathologies have not yet been fully clarified. In endometriosis, aromatase activity in stroma cells was induced by prostaglandin E_2 (PGE_2) while in the endometrium of women without the disease this response failed to occur [3,4] despite the fact that cyclooxygenase activity increases during the proliferative phase of the menstrual cycle in parallel with the increase in

proliferation [6]. Moreover, estrogens stimulate cyclooxygenase-2 (COX-2) in endometriosis, thereby creating a local feedback loop that leads to increasing levels of estradiol and prostaglandin that are not present in the endometrium of disease-free women [7]. This suggests that alterations in aromatase activity in the eutopic endometrium in response to prostaglandins may precede the development of peritoneal endometriosis. However, it is not known whether similar alterations occur in the development of other endometrial pathologies such as endometrial polyps.

The observation that endometrial polyps are found much more frequently in patients with pelvic endometriosis than in disease-free women suggests that a similar mechanism may be operating at endometrial level in both conditions [8]. This raises the question of whether or not aromatase expression is present in endometrial polyps and, if so, what its effect would be on their proliferation rates and on cyclooxygenase expression compared with normal endometrium. In the present study, immunohistochemistry was performed to measure the expression of aromatase p450, COX-2 and Ki-67 in both endometrial polyps and normal endometrium.

Methods

This was a retrospective study carried out on paraffin-embedded endometrial samples obtained from 118 patients who were submitted to hysteroscopy in our institution between December 2004 and December 2005. All patients were premenopausal (mean age 37 years, range 25–50 years). Sixty-eight patients had normal ovulatory cycles and a normal uterus according to transvaginal sonography. They were submitted to diagnostic hysteroscopy with endometrial biopsy as part of their standard diagnostic work-up for *in vitro* fertilization (IVF). The main indications for IVF were, in decreasing order, the male factor, tubal obstruction and unexplained infertility. Diagnostic hysteroscopy was carried out in these patients with the aid of a 3.8 mm Bettocchi hysteroscope, using saline 0.9% as the medium for distention of the uterine cavity. Hysteroscopy revealed a normal uterine cavity in all patients. Endometrial samples were obtained from these patients at the completion of the hysteroscopic examination using a 4 mm Karman curette attached to a 10 ml plastic syringe. The endometria of these patients were used in the study as control samples.

The remaining 50 patients had endometrial polyps that were removed using the Bettocchi hysteroscope and the Versapoint tweezer electrode positioned through the operating channel. All these patients had ovulatory menstrual cycles as inferred by progesterone assays obtained from their medical records. Polypectomy was carried out under para-cervical block associated with intravenous sedation using propofol. The polyp was sectioned at its base with the cutting current at 100 W and either removed intact or after slicing with the Versapoint to facilitate its retrieval. Samples of the surrounding endometrium were obtained with the aid of a 4 mm Karman curette upon completion of the hysteroscopic procedure.

All tissue samples were fixed in 10% formalin and sent to pathology. Routine hematoxylin–eosin staining was carried out in all samples either to confirm the diagnosis of polyp or to date the endometrium. Immunohistochemistry was carried out on all tissue samples following antigen retrieval to detect the presence of aromatase p450, COX-2 and Ki-67 expression. The presence of COX-2 and Ki-67 immunohistochemical staining was detected using commercially available antibodies purchased from Novocastra (clone 4H12; Newcastle-upon-Tyne, UK) and Dako (clone MIB-1; Carpinteria, CA, USA), respectively. The reaction was revealed by the streptavidin–biotin method. The percentage of cell nuclei positive for Ki-67 was measured in the endometrial glands in ten random microscopic fields ($\times 25$) and expressed as a percentage of positive nuclei. COX-2 expression was evaluated semi-quantitatively by two certified pathologists (T.M.C.S. and L.A.R.F.) and scored according to the intensity of the staining reaction in the endometrial glandular cells. Tissue slices showing no staining reaction were labeled as negative and graded as (0); those showing a weakly positive staining reaction in at least 10% of the cells were graded as ($+1$), a moderate reaction as ($+2$), while those with a strongly positive staining reaction were graded as ($+3$).

Aromatase expression was investigated using a commercially available monoclonal antibody (MCA2077, clone H4; Serotec, Raleigh, NC, USA) in 14 endometrial polyps and in 24 normal endometrial samples used as controls. Antigen retrieval was carried out using Tris–ethylenediamine-N,N,N', N'-tetraacetic acid buffer at pH 8.0. 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 Mann–Whitney test was used to compare variables between the two groups and the Kruskal–Wallis test for comparison between three or more groups, followed by the Dwass–Steel–Chritchlow–Fligner test for paired comparisons. The χ^2 test was used to compare differences in the percentage of aromatase-positive cases. Significance was established as $p < 0.05$.

Results

Aromatase expression

The frequency of positivity for aromatase p450 did not differ significantly between the luteal and proliferative phases of the menstrual cycle, and these cases were therefore analyzed together. In endometrial polyps, aromatase p450 immunohistochemical expression was rated as positive in 9/14 (64%) of the samples. In these polyps, aromatase p450 expression was always expressed in the stromal cells, although it was also detected in the glandular epithelium in 4/9 (44%) of the stroma-positive cases. The percentage of positive glands was, however, always <10%, whereas in the stroma the staining reaction was homogeneous in all cells. A similar staining pattern for this enzyme was also found in the stromal cells of the endometrium surrounding the aromatase-positive polyps (Figure 1).

In patients with no endometrial pathology in the cycling endometrium, aromatase expression was detected in only 4/24 cases (17%). The percentage of aromatase-positive cases in the pathology-free endometrium was significantly lower than that observed in endometrial polyps ($p < 0.01$). These results are summarized in Table I.

COX-2 and Ki-67 expression

The intensity of COX-2 expression in the glandular epithelium varied during the menstrual cycle. In endometrial polyps, the median COX-2 scores in the proliferative phase were significantly greater than in the late luteal and menstrual phases of the menstrual cycle, although they were similar to those found in the early luteal phase (Figure 2). The reduction in COX-2 immunostaining scores during the late luteal phase, on the other hand, was statistically significant only when compared with the proliferative phase but not the other phases of the menstrual cycle (Tables II and III). In normal endometrium, COX-2 expression decreased significantly in the late luteal phase compared with the menstrual, proliferative and early luteal phases (Tables IV and V). Median COX-2 expression in the endometrium during the late luteal phase was also significantly lower than that observed in endometrial polyps during this phase of the menstrual cycle. However, in the other phases of the cycle, median COX-2 scores in the glandular epithelium were not significantly different in endometrial polyps compared with normal endometrium. In contrast to the glands, immunohistochemical staining for COX-2 was always present in the superficial epithelium throughout the menstrual cycle and showed no decrease in intensity of staining during the late luteal phase (Figure 3).

There were similar variations in Ki-67 expression in both endometrial polyps and normal endometrium
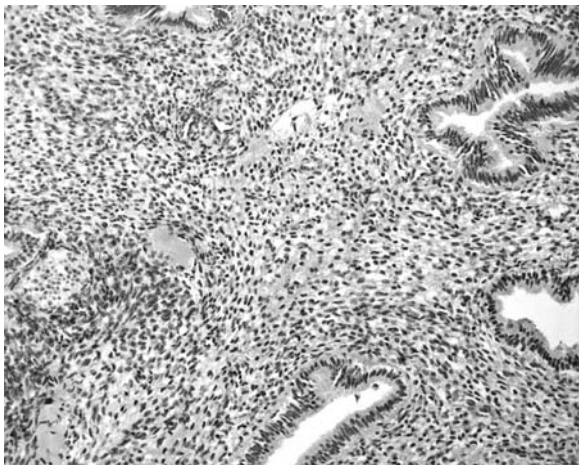

Figure 1. Aromatase expression in the stromal cells of the surrounding endometrium in a patient with an endometrial polyp.

Table I. Aromatase activity in disease-free endometrium and endometrial polyps.

Disease-free endometrium	4/24 (17%)
Endometrial polyp	9/14 (64%)*

*Significant difference vs. disease-free endometrium: $p < 0.01$.

Figure 2. Strong COX-2 expression (+3) in the glandular epithelium of an endometrial polyp during the proliferative phase of the menstrual cycle.

Table II. Changes in the expression of Ki-67 and COX-2 in polyps during the menstrual cycle.

Phase of the menstrual cycle	Ki-67	COX-2
Menstrual	4.8 ± 3.32 (4.5)	1.8 ± 1.03 (2)
Proliferative	56.92 ± 22.18 (60)	2.72 ± 0.67 (3)
Early luteal	39.87 ± 19.49 (40)	2.62 ± 0.51 (3)
Late luteal	2.75 ± 1.66 (3)	1.37 ± 1.3 (1.5)
	$p < 0.001$*	$p < 0.0108$*

Data are expressed as mean ± standard deviation (median); *Kruskal–Wallis test.

Table III. Comparison of the variations in Ki-67 and COX-2 in endometrial polyps during the menstrual cycle.

Comparison of the different phases of the menstrual cycle	Ki-67	p*	COX-2	p*
Menstrual × proliferative	4.5 × 60	<0.0001	2 × 3	0.0179
Menstrual × early luteal	4.5 × 40	0.0032	2 × 3	0.2701
Menstrual × late luteal	4.5 × 3	0.5243	2 × 1.5	0.8835
Proliferative × early luteal	60 × 40	0.192	3 × 3	0.8042
Proliferative × late luteal	60 × 3	0.0001	3 × 1.5	0.0106
Early luteal × late luteal	40 × 3	0.004	3 × 1.5	0.1833

Data are expressed as median × median; *Kruskal–Wallis tests: all paired comparisons (Dwass–Steel–Chrichtlow–Fligner).

Table IV. Changes in Ki-67 and COX-2 expression in disease-free endometrium during the menstrual cycle.

Phase of the menstrual cycle	Ki-67	COX-2
Menstrual	3.2 ± 2.04 (3)	2.4 ± 0.54 (2)
Proliferative	50.62 ± 19.38 (60)	2.34 ± 0.85 (3)
Early luteal	21.42 ± 25.44 (10)	1.71 ± 0.95 (2)
Late luteal	0.08 ± 0.04 (0)	0.51 ± 0.70 (0)
	$p < 0.0001$*	$p < 0.0001$*

Data are expressed as mean ± standard deviation (median); *Kruskal–Wallis test.

throughout the menstrual cycle. During menstruation, the number of Ki-67-positive nuclei was low, but this number increased significantly during the proliferative phase only to decrease again after ovulation. Median percentages of Ki-67 expression, both in endometrial polyps and normal endometrium, were significantly lower during the late luteal phase than during the proliferative and early luteal phases, but were not significantly different from the median found during menstruation (Tables II–V). There were no statistically significant differences between the median values found in the endometrial polyps and the endometrium during any phase of the menstrual cycle.

Discussion

The present study shows that p450 aromatase expression was present in the stromal cells of both endometrial polyps and the surrounding endometrium in a significantly greater number of samples compared with disease-free endometrium. These findings are in agreement with data from other studies published in the literature on endometriosis [5] and reinforce the hypothesis that endometrial polyps and the adjacent endometrium may have the capacity to produce estrogens locally from androgen precursors. It is not yet known how this may affect polyp development, although in rhesus monkeys the systemic administration of estradiol induces the development of endometrial polyps, thereby suggesting an association between hyperestrogenism and polyp formation [9]. The presence of increased aromatase expression in the eutopic endometrium of women with other gynecological pathologies, such as myoma and adenomyosis, suggests that the local production of estrogens may also play an important role in the growth of these lesions [10,11]. Likewise, its presence in the endometrium bearing polyps suggests that some of the symptoms attributed to these neoplasms may indeed be a consequence of this locally augmented aromatase expression in the endometrium, leading to an increase in angiogenesis and irregular bleeding. Aromatase inhibitors, on the other hand, are effective in suppressing the *in vitro* proliferation of endometrial cells harvested from patients with endometriosis, indicating that local estrogen production may play a role in the regulation of endometrial proliferation in pathological conditions, at least under *in vitro* conditions [12]. The presence of aromatase expression in endometrial polyps and in the surrounding endometrium suggests that the primary defect may be located in the endometrium, thus creating a local hyperestrogenism that may contribute to the development of these neoplasms and to their recurrence following conservative treatment. The presence of aromatase expression in endometrial stromal cells in the case of endometriosis may indeed augment the local production of estrogens from C19 androgens, which in turn may stimulate PGE_2 production by upregulation of COX-2, thus establishing a positive feedback cycle in these lesions stimulating their growth [2–4]. An aberrant response of the endometrium to prostaglandins resulting in aromatase induction rather than a primary increase in their production is the most likely mechanism in the pathogenesis of endometriosis [4]. Molecular studies have shown that an altered response of the promoter gene for aromatase due to an aberrant production of SF-1 transcription factor is probably the underlying mechanism for the activation of aromatase in response to PGE_2 in endometriosis [13,14]. The development of an aberrant response of the endometrium to prostaglandins rather than an increase in their production is thus the probable mechanism for the enhanced aromatase expression found in endometriotic lesions. However, it is not yet known whether similar mechanisms are responsible for the enhancement of aromatase expression in endometrial polyps and in the surrounding endometrium. The

Table V. Comparison of the variations in Ki-67 and COX-2 in disease-free endometrium during the menstrual cycle.

Comparison of the different phases of the menstrual cycle	Ki-67	p^*	COX-2	p^*
Menstrual × proliferative	3 × 60	0.002	2 × 3	0.9964
Menstrual × early luteal	3 × 10	0.9107	2 × 2	0.5181
Menstrual × late luteal	3 × 0	0.0001	2 × 0	0.0023
Proliferative × early luteal	60 × 10	0.0283	3 × 2	0.2483
Proliferative × late luteal	60 × 0	0.0001	3 × 0	0.0001
Early luteal × late luteal	10 × 0	0.0026	2 × 0	0.0201

Data are expressed as median × median; *Kruskal–Wallis tests: all paired comparisons (Dwass–Steel–Chrichtlow–Fligner).
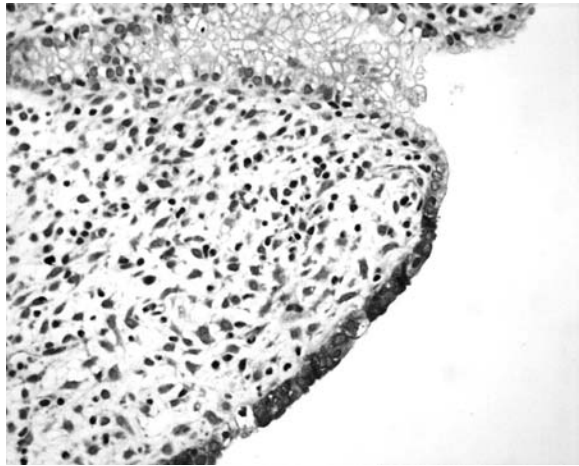

Figure 3. Persistence of COX-2 expression in the superficial epithelium of an endometrial polyp during the luteal phase.

observation reported in the present paper that cyclooxygenase expression detected by immunohistochemistry displays menstrual cycle-related changes in both endometrial polyps and the normal endometrium is in accordance with the suggestion that an altered response of the endometrial cells to prostaglandins rather than an augmentation in their production is the most likely mechanism involved in the stimulation of aromatase p450 expression. However, it is merely speculative to say that a similar mechanism occurs in endometrial polyps. Nevertheless, the high incidence of endometrial polyps in patients with endometriosis is in agreement with the assumption that there are probably common pathogenic mechanisms operating at endometrial level in both pathologies, although this evidence is only circumstantial [8].

The role played by aromatase p450 in the regulation of local estrogen production in the endometrium may lead to the development of new treatment modalities for several endometrial pathologies including polyps through the use of aromatase inhibitors [15]. Oral contraceptives containing gestodene 75 μg/ethinylestradiol 30 μg, on the other hand, have been found to be efficacious in decreasing COX-2 expression in the endometrium, thus explaining their therapeutic efficacy in reducing the occurrence of dysmenorrhea and menorrhagia

[6,16]. The reduction in COX-2 expression in the endometrium of oral contraceptive users may, in principle, lead to a reduction in inflammation in this tissue, thereby contributing to the prevention of endometrial pathologies, including carcinoma [17]. However, it is not yet known whether the decrease in COX-2 expression at endometrial level induced by oral contraceptives can ultimately suppress aromatase expression in the endometrium, further contributing to the treatment of endometrial pathology. There is a paucity of data on the effect of oral contraceptives on aromatase expression, although other steroids such as danazol are effective in suppressing aromatase activity at endometrial level in cases of adenomyosis and endometriosis [11].

In conclusion, the presence of aromatase expression in both endometrial polyps and the surrounding endometrium suggests a common pathogenetic pathway with other uterine pathologies, and this may suggest that the interaction between inflammatory mediators such as COX-2 and aromatase expression in the tissue, leading to local estrogen production, may play a pivotal role in the development of endometrial pathology.

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