The Effect of Oral Contraceptives Combined With Pycnogenol (Pinus Pinaster) On Aromatase and VEGF Expression in the Eutopic Endometrium of Endometriosis Patients

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Abstract

Oral contraceptives are effective in curbing down endometrial inflammation and decreasing endometriosis-related dysmenorrhea. This antiinflammatory action is mediated by the progestin component of the pill and it involves the blockade of the NF-kappa.B inflammatory cascade. One of the genes activated by NF-kappa.B is the one that codes for angiogenic factors such as VEGF. In this respect, oral contraceptives would be expected to block VEGF expression in the endometrium. However, the use of oral contraceptives alone failed to significantly decrease the percentage of endometria testing positive for VEGF. Introducing pycnogenol to the treatment regimen, on the other hand, significantly reduced the percentage of VEGF-positive cases in oral contraceptive users. Since pycnogenol blocks NF-kappa.B gene transcription after its binding to DNA, the combination of oral contraceptives with pycnogenol would be expected to have a synergistic effect on the eutopic endometrium of endometriosis patients. The expression of other inflammatory-induced genes in the endometrium, such as aromatase, is also significantly diminished with the use of oral contraceptives alone. However, this inhibitory effect was significantly enhanced with the concomitant use of pycnogenol. These results suggest that the concomitant use of pycnogenol with oral contraceptives is more effective in reducing VEGF and aromatase expression in the eutopic endometrium of endometriosis patients than the use of oral contraceptives alone.

Keywords: Endometriosis; Pycnogenol; Progestin

Introduction

When used in extended regimens, oral contraceptives effectively reduce pain in patients with endometriosis and this is accomplished through a combination of several pathways including the diminution of inflammation [1]. This analgesic effect is caused by the progestin component of the pill and is mediated not only by the blockade of NF-kappa.B activation but also by the subsequent inhibition of enzymes necessary for the progression of endometriosis such as aromatase p450 and Cox-2, both in the lesions and in the eutopic endometrium [1-3]. However, one common side effect of this treatment is the occurrence of breakthrough bleeding, which may occur unpredictably throughout treatment and is usually accompanied by the resumption of pain. In patients with idiopathic menorrhagia using oral contraceptives in extended regimens, the occurrence of breakthrough bleeding is associated with reactivation of NF-kappa.B transcription factor [1]. Persistent Cox-2 and aromatase expression was also detected in the eutopic endometrium of endometriosis patients using oral contraceptives continuously and experiencing breakthrough bleeding [3,4]. When this occurs, local estrogen production remains unabated, thus fomenting a vicious cycle of increased inflammation in which the persistent activation of NF-kappa.B plays a pivotal role [1]. The concomitant use of natural NF-kappa.B inhibitors with oral contraceptives, on the other hand, may be beneficial in decreasing the occurrence of these adverse events during the use of oral contraceptives without compromising their efficacy [1,4]. One promising compound that is currently attracting attention is pycnogenol, a complex mixture of procyanidins and polyphenol components harvested from the bark of the French Maritime Pine (Pinus pinaster). Preliminary studies have shown that the addition of pycnogenol to oral contraceptive regimens increases their efficacy in relieving endometriosis-related pain and decreasing the incidence of breakthrough bleeding [5]. Pycnogenol blocks the activating effect of NF-kappa.B on the inflammatory cascade at transcription level, hence downstream from its binding to DNA [6]. This may explain why pycnogenol is able to potentiate the anti-inflammatory effects of progestins, since the latter block NF-kappa.B action in a step prior to its translocation to cell nuclei [7]. If this hypothesis is correct, the combination of oral contraceptives with pycnogenol may diminish the expression of aromatase and NF-kappa.B-induced genes such as VEGF in endometrios is more effectively than progestin therapy alone [8].

In the present paper, aromatase and VEGF expression were investigated in the eutopic endometrium of endometriosis patients using extended regimens of oral contraceptives either alone or in combination with pycnogenol.

Patients and Methods

This clinical observational study was conducted to retrospectively evaluate the effect of pycnogenol on aromatase and VEGF expression in the eutopic endometrium of endometriosis patients using oral contraceptives in extended regimens to treat this pathology. A total of 122 patients of reproductive age (range 22-37 years) with a previous laparoscopic diagnosis of endometriosis, who were referred to the Itaigara Memorial Day Hospital for a hysteroscopy with biopsy, were invited to participate in this study. The main inclusion criteria were a laparoscopic diagnosis of endometriosis and a medical history of associated infertility and/or dysmenorrhea. The indications for the diagnostic hysteroscopy with endometrial biopsy were dysmenorrhea,
menorrhagia, infertility or for evaluating theuterine cavity prior to in vitro fertilization or intrauterine insemination. At the time of hysterectomy, 65 patients had not been in use of any form of hormonal treatment for at least the preceding three months (Group I). In these patients, the procedures were carried out either in the proliferative (n=30) or luteal phase (n=35) of the menstrual cycle according to the availability of the surgical theater. The remaining 57 patients had been using oral contraceptives in extended regimens either alone (Group II; n=31) or in association with 100 mg of pycnogenol (Group III; n=26) for a mean period of 3 months. The oral contraceptives used were either an association of 75 mcg of gestodene with 30 mcg of ethinylestradiol (Gestinol 28, Libbs, Brazil) (n=42) or an association of drospirenone 3 mg with ethinylestradiol 30 mcg (Elani 28, Libbs, Brazil) (n=15). Pycnogenol was prescribed at the dose of 100 mg/day and was obtained either from a compound pharmacy (Pinus pinaster extract, Fagron, The Netherlands) or in the form of a commercially available product (Flebon®, Farmoquimica, Brazil). The indication for the prescription of oral contraceptives in extended regimens for these endometriosis patients by their attending physicians was to suppress ovulation and menstruation prior to initiating an IVF cycle, since this not only increases pregnancy rates but also ameliorates dysmenorrhea. Pycnogenol was used since it potentiates the pain-relieving effects of oral contraceptives.

The stage of endometriosis was determined according to the guidelines defined by the American Society for Reproductive Medicine (ASRM) classification system and data were obtained from the patient’s medical records. An endometrial biopsy was taken during hysterectomy using a 4 mm Karman curette attached to a 20 cc plastic syringe to produce vacuum. The tissue was fixed in formalin 4% and sent to pathology. Immunohistochemistry was performed in an automated system (DAKO Autostainer). Aromatase expression was investigated using a commercially available monoclonal antibody, MCA2077, clone H4 (SeroTech, Raleigh, NC). Antigen retrieval was performed using the Tris-EDTA buffer at pH 8.0. The reaction was revealed using the DAKO EnVision Flex detection system+Linker followed by DAB+substrate chromogen mix (DAKO). The presence of aromatase expression was rated either as positive if there was any detectable staining reaction in the glandular epithelium or negative when no reaction was observed. VEGF expression was similarly determined in the endometrium by immunohistochemistry using the same methodology. For VEGF, the monoclonal antibody used was obtained from Dako (clone VG1). VEGF expression was rated as positive if any staining reaction was detected in the endometrium. In the statistical analysis, the chi-square test was used to detect differences in percentages and significance was established at p<0.05.

This observational study was approved by the internal review board of the Itaigara Memorial Day Hospital, and the Instituto da Mulher and all the patients gave written informed consent allowing the tissue from endometrial biopsies to be used in immunohistochemical studies. As these patients had been referred to our institution for laparoscopy or hysterectomy, they were already under treatment at admission to the Itaigara Memorial Day Hospital. Neither pycnogenol nor the oral contraceptive pills used in extended regimens are new investigational drugs. Pycnogenol, either in the form of a commercially available product (Flebon®) or in the form of pinus pinaster extract prepared by a compound pharmacy in Salvador (Formula) (Fagron, the Netherlands), has been approved by the Brazilian regulatory authorities (ANVISA) for the treatment of varicose veins, melasma and to prevent thrombosis in long haul intercontinental flights. Its concomitant use with oral contraceptives is not contraindicated. Both contraceptives used prior to hysterectomy have already been approved by ANVISA for use in extended regimens to suppress menstruation whenever amenorrhea is desired, as mentioned previously.

Results

VEGF expression

The staining reaction for VEGF in the endometrium was present mainly in the stroma, while no staining reaction was found in the glands irrespective of any previous treatment or the phase of the menstrual cycle (Figure 1). In Group I, the percentage of endometria showing a positive staining for VEGF was 76% and there was no statistically significant difference between the proliferative and luteal phases of the menstrual cycle. In patients using oral contraceptives alone in extended regimens for a mean time of 3 months prior to hysterectomy (Group II), endometrial biopsies showed the presence of a strong decidual reaction in the stroma in 80% of the patients. VEGF expression was detected by immunohistochemistry in 80% of the cases, and the stained reaction was always present in the decidual stroma, while the glandular epithelium was negative. There was no statistically significant difference between Group I and Group II with respect to the percentage of endometria that tested positive for VEGF. In Group III, which consisted of patients who had been using pycnogenol associated with oral contraceptives for a mean of 3 months, the percentage of endometrial with a decidual reaction in the stroma was not significantly different from that found in Group II. However, 30% of endometria tested positive for VEGF, a percentage that was significantly lower than that found both for the untreated patients (Group I) and for the users of oral contraceptives alone (Group II). These results are summarized in Table 1.

Aromatase expression

In the untreated group of endometriosis patients (Group I), 81% tested positive for aromatase expression in the endometrium and there was no statistically significant difference between the

<table>
<thead>
<tr>
<th>Group I</th>
<th>Untreated patients</th>
<th>VEGF-Positive</th>
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<tbody>
<tr>
<td>Group II</td>
<td>OC Users</td>
<td>20/26 76%</td>
</tr>
<tr>
<td>Group III</td>
<td>Users of OC+pycnogenol</td>
<td>6/20 30%</td>
</tr>
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Table 1: The effect of oral contraceptives (OC) alone or in association with pycnogenol on VEGF expression in the eutopic endometrium of endometriosis patients.

Figure 1: VEGF expression in the stroma of the eutopic endometrium of an endometriosis patient.
Table 2: The effect of oral contraceptives (OC) alone or association with pycnogenol on aromatase expression in the eutopic endometrium of endometriosis patients.

<table>
<thead>
<tr>
<th>Group</th>
<th>Aromatase-Positive</th>
</tr>
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<tbody>
<tr>
<td>Untreated patients</td>
<td>59/65</td>
</tr>
<tr>
<td>OC Users</td>
<td>12/31</td>
</tr>
<tr>
<td>Group I</td>
<td>2/26</td>
</tr>
<tr>
<td>Group II</td>
<td>59/65</td>
</tr>
<tr>
<td>Group III</td>
<td>12/31</td>
</tr>
<tr>
<td>Group I+Group II</td>
<td>2/26</td>
</tr>
<tr>
<td>Group I+Group III</td>
<td>59/65</td>
</tr>
</tbody>
</table>

Group I: p<0.05
Group I: p<0.01
Group I: p<0.001

The present study showed that a combination of oral contraceptives (OC) alone or association with pycnogenol on aromatase expression in the eutopic endometrium of endometriosis patients. Pycnogenol is able to block NF-kappa.B expression, which may explain its efficacy in decreasing VEGF expression in the endometrium, since expression of the VEGF gene is induced by NF-kappa.B upon its activation and translocation to cell nuclei [8]. The greater reduction in VEGF expression in the eutopic endometrium during the combined use of oral contraceptives with pycnogenol when compared to the use of oral contraceptives alone is therefore a consequence of a more effective block of NF-kappa.B induction of the VEGF gene caused by synergism between the blockade of NF-kappa.B translocation to cell nuclei by the progestin and the suppression of its transcriptional activity following DNA binding by pycnogenol [1,7,8]. The decrease in VEGF expression in pycnogenol users did not affect the decidual reaction in the endometrial stroma induced by oral contraceptives.

The present results also show that pycnogenol potentiates the inhibitory effects of oral contraceptives on aromatase expression in the eutopic endometrium of endometriosis patients; however, it is not known whether this is the consequence of a direct effect on the aromatase gene or an indirect action resulting from greater suppression of the NF-kappa.B-induced inflammatory cascade. Pycnogenol is able to suppress cyclooxygenase activity, thus interrupting the production of prostaglandin E2, which is a potent aromatase gene inducer in the endometrial stroma cells from endometriosis patients [1,9]. This may explain the greater suppression of aromatase expression in the eutopic endometrium of endometriosis patients using oral contraceptives in association with pycnogenol, since the blockade of the NF-kappa.B-induced inflammatory cascade at both pre- and post-transcriptional level would halt the transcription of the cyclooxygenase gene. Pycnogenol has already been shown to exert anti-inflammatory and antithrombotic effects by inhibiting both Cox-1 and Cox-2 enzymatic activity [9,10]. Since prostaglandin E2 plays a pivotal role in the induction of the aromatase gene, the effects of pycnogenol may be indirect and a consequence of the reduction in inflammation rather than a direct effect on the aromatase gene promoter [1,9].

The greater efficacy of oral contraceptives when combined with pycnogenol in reducing aromatase and VEGF expression in the eutopic endometrium may also explain the better pain-relieving effects of this combination therapy in endometriosis patients. It should be emphasized that this was accomplished without any increase in the number of adverse events, which could have compromised its enhanced efficacy. Pycnogenol alone has been reported to be as effective as GnRH anlogs for the treatment of endometriosis-related pain after the second month of use, which is in accordance with the results of the present study, and it may constitute a useful adjuvant in the arsenal of options for hormone therapy [11]. The blockade of Cox-2 and Cox-1 enzymatic activity also explains the efficacy of pycnogenol in treating dysmenorrhea [12].

In conclusion, although both the progestins present in oral contraceptives and pycnogenol are able to reduce inflammation by modulating NF-kappa.B-induced gene transcription, they act on different steps in this mechanism, thus explaining the greater efficacy of the combination therapy in suppressing both VEGF and aromatase expression in the eutopic endometrium of endometriosis patients. When given together with an oral contraceptive, there is a reduction not only in DNA binding activity but also in the transactivation capacity of the bound NF-kappa.b [1,6,7]. This distinctive effect on the NF-kappa.B activation pathway may provide a plausible explanation at the molecular level for the greater inhibition of both VEGF and aromatase expression in the eutopic endometrium of endometriosis patients when pycnogenol is used together with oral contraceptives in extended regimens. However since the amount of procyanidins and polyphenols is not known whether this is the consequence of a direct effect on the aromatase gene or an indirect action resulting from greater suppression of the aromatase gene in the eutopic endometrium of endometriosis patients; however, it is not known whether this is the consequence of a direct effect on the aromatase gene or an indirect action resulting from greater suppression of the NF-kappa.B-induced inflammatory cascade. Pycnogenol is able to suppress cyclooxygenase activity, thus interrupting the production of prostaglandin E2, which is a potent aromatase gene inducer in the endometrial stroma cells from endometriosis patients [1,9]. This may explain the greater suppression of aromatase expression in the eutopic endometrium of endometriosis patients using oral contraceptives in association with pycnogenol, since the blockade of the NF-kappa.B-induced inflammatory cascade at both pre- and post-transcriptional level would halt the transcription of the cyclooxygenase gene. Pycnogenol has already been shown to exert anti-inflammatory and antithrombotic effects by inhibiting both Cox-1 and Cox-2 enzymatic activity [9,10]. Since prostaglandin E2 plays a pivotal role in the induction of the aromatase gene, the effects of pycnogenol may be indirect and a consequence of the reduction in inflammation rather than a direct effect on the aromatase gene promoter [1,9].

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References


