Erythropoietin concentrations are elevated in the peritoneal fluid of women with endometriosis

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Erythropoietin (Epo) is an important regulator of erythropoiesis and stimulates the proliferation of early erythroid precursors as well as the differentiation of late erythroid precursors of the erythroid lineage. However, recent studies have indicated that Epo also has angiogenic properties and plays an important role in the oestrogen-dependent cyclical angiogenesis within the mouse uterus. It was therefore postulated that Epo may be an important angiogenic factor in endometriosis. In order to address this hypothesis the concentration of Epo in peritoneal fluid (PF) was determined in patients with or without endometriosis. PF was collected from patients with endometriosis (n = 42) or without endometriosis (n = 18). Detectable concentrations of Epo were found in all PF samples analysed. The concentration of Epo in PF from patients with endometriosis was significantly higher than that in the control group (13.1 ± 1.2 mIU/ml versus 7.2 ± 0.7 mIU/ml, mean ± SE respectively, P < 0.01). Furthermore, in patients with endometriosis the Epo concentrations in PF from patients with stage I disease (n = 17, 16.6 ± 3.0 mIU/ml) were significantly higher than those with stage II (n = 8, 10.7 ± 1.2 mIU/ml, P < 0.03), III (n = 13, 8.4 ± 1.0 mIU/ml, P < 0.01), IV disease (n = 7, 7.5 ± 1.0 mIU/ml, P < 0.01). These data suggest that Epo may play a role in the pathogenesis of endometriosis particularly in the initiation of the disease.

Key words: angiogenesis/endometriosis/erythropoietin/peritoneal fluid

Introduction

Endometriosis is a common gynaecological disorder of unknown aetiology and accounts for infertility and pelvic pain in 10–15% of women of reproductive age. Angiogenesis is the formation of new capillaries from pre-existing vessels and it is now apparent that this process plays an important role in the pathogenesis of this condition (Healy et al., 1998).

Studies have demonstrated that angiogenic factors may be localized to endometriotic tissues and were also found to be increased in the peritoneal fluid (PF) of women with endometriosis (Oosterlynck et al., 1993; Ferriani et al., 1993; Oosterlynck et al., 1994; Ryan et al., 1995; McLaren et al., 1996a,b; Shifren et al., 1996; Donnez et al., 1998; Gazvani et al., 1998; Iwabe et al., 1998; Osuga et al., 1999). The release of angiogenic factors into the peritoneal cavity may well stimulate increased neovascularization of endometriotic tissues (Ramey and Archer, 1993; Konincx et al., 1998). Therefore, the detection and evaluation of potent angiogenic factors in endometriosis is important and could potentially lead to the design of novel therapeutic strategies aimed at both preventing and treating endometriosis.

Recent studies have indicated that several cytokines and interleukins (IL) including granulocyte colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), IL-6 and IL-8, which were formerly thought to be predominantly specific for the haematopoietic cells, are also capable of affecting certain endothelial cell functions including angiogenesis (Bikfalvi and Han, 1994). Erythropoietin (Epo) is a key factor in the regulation of erythropoiesis and stimulates the proliferation of early erythroid precursors and the differentiation of late erythroid precursors of the erythroid lineage (Krantz, 1991; Jelkmann, 1992). However, important recent studies have demonstrated the angiogenic potential of Epo (Carlini et al., 1995; Yasuda et al., 1998; Masuda et al., 1999; Ribatti et al., 1999). In addition, the Epo receptor has been demonstrated in endothelial cells both in vitro and in vivo (Anagnostou et al., 1994). Furthermore, Epo stimulation of endothelial cells may elicit an angiogenic response in vitro and in vivo (Carlini et al., 1995; Ribatti et al., 1999). Lastly, physiological angiogenesis occurs within the female reproductive organs of the adult and a recent study demonstrated that Epo is involved in the oestrogen-dependent cyclical angiogenesis occurring within the mouse uterus (Yasuda et al., 1998; Masuda et al., 1999).

It was therefore postulated that Epo is one of the angiogenic factors involved in endometriosis. This hypothesis was addressed by initially determining if Epo was present in PF.
and then comparing the concentrations of Epo in PF from patients with and without endometriosis.

Materials and methods

Patients undergoing laparoscopic surgery for infertility or investigation of pelvic pain or a pelvic mass were recruited for the study. All patients had a normal menstrual cycle and had received no hormonal treatment during the 6 month period prior to the surgery. PF and a serum sample were collected during the laparoscopy. Fluid was aspirated from the posterior cul-de-sac immediately after the insertion of the trocar. The volume of peritoneal fluid was noted. Samples were centrifuged at 800 g for 10 min at 4°C in order to remove cells and debris and the supernatants were stored at −80°C until analysis. Operative findings were recorded regarding the presence of endometriosis and its location, extent and degree. The degree of endometriosis was scored according to the latest revision of the American Society for Reproductive Medicine classification (American Society for Reproductive Medicine, 1997), and biopsy specimens were taken to confirm the diagnosis histologically. Fourteen patients had stage I disease, eight patients stage II, 13 patients stage III, and seven patients stage IV. Of the 22 patients with stage I or stage II, four patients had red peritoneal lesions only, five patients black lesions only and the remaining 13 patients had both red and black lesions. All patients with stage III or IV had ovarian endometriotic lesions. PF collected from 18 women with no visible evidence of pelvic pathology served as controls. There were no significant differences in the clinical characteristics between the patients with or without endometriosis (Table I). All peritoneal fluid and serum samples were obtained with the patient’s full informed consent whilst the human research board and then comparing the concentrations of Epo in PF from patients with and without endometriosis.

Table I. Erythropoietin concentrations in peritoneal fluid of women with and without endometriosis (control)

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Agea</th>
<th>Parityb</th>
<th>Epo concentration (mIU/ml)c</th>
<th>Cyclic specific Epo concentration (mIU/ml)cc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proliferative</td>
<td>Secretary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endometriosis</td>
<td>23</td>
<td>19</td>
<td>30.3 ± 4.7d</td>
<td>13.1 ± 1.2e</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>8</td>
<td>30.3 ± 6.6</td>
<td>7.2 ± 0.7</td>
</tr>
</tbody>
</table>

aMean ± SD.
bMedian (range).
cMean ± SE.
dIn comparison with the control group, no significant difference was observed.
eIn comparison with the control group, a significant difference was observed (over whole cycle: P < 0.01, proliferative phase: P < 0.05, secretory phase: P < 0.01).
fIn comparison with secretory phase, a significant difference was observed.
fIn comparison with secretory phase, no significant difference was observed.

Results

Erythropoietin was detectable in all samples of PF analysed. However, the concentration of EPO in the PF of patients with endometriosis was significantly higher than that in the control group (13.1 ± 1.2 versus 7.2 ± 0.7 mIU/ml respectively, P < 0.01, Table I). Furthermore, Epo concentrations in PF from patients with stage I disease (16.6 ± 3.0 mIU/ml), stage II, III, IV endometriosis and those in the control group (10.7 ± 1.2 mIU/ml, P < 0.03; 8.4 ± 1.0 mIU/ml, P < 0.01; 7.5 ± 1.0 mIU/ml, P < 0.01; 7.2 ± 0.7 mIU/ml, P < 0.001 respectively, Figure 1). Of the patients with stage I or stage II, Epo concentrations in PF from patients with red lesions only was significantly higher than those with black lesions only (21.7 ± 4.0 versus 7.3 ± 1.6 mIU/ml respectively, P < 0.02). Epo concentrations in PF from patients with red and black lesions were significantly higher than those with black lesions only (16.3 ± 2.7 versus 7.3 ± 1.6 mIU/ml respectively, P < 0.03).

Discussion

This study demonstrates that Epo is detectable in PF and that women with endometriosis have significantly higher concentrations of Epo in the PF than women without endometriosis, suggesting that Epo may be involved in the pathogenesis of endometriosis. A recent study demonstrated that the PF concentration of IL-8, which is a potent angiogenic factor, was significantly higher in women with early endometriosis compared to women with late stage disease (Gazvani et al.,
different stages of the spontaneous evolution of endometriotic implants; the first stage red lesions having increased stromal vascularization as compared with black and white lesions (Donnez et al., 1992; Nisolle et al., 1993, 1997; Nisolle and Donnez, 1997; Donnez et al., 1998; Matsuzaki et al., 1998). Although our sample size was too small, the present findings suggest that PF Epo concentrations from patients with red lesions only may be higher than those with black lesions only. Further studies are needed to find a relationship between PF Epo concentrations and the presence of red peritoneal lesions only (Calhaz-Jorge et al., 2000).

The angiogenic response of the chick embryo chorioallantoic membrane blood vessels to Epo is comparable with that elicited by the prototypic angiogenic cytokine basic fibroblast growth factor (Ribatti et al., 1999). Fibroblast growth factor is one of the potent angiogenic factors in endometriosis (Ferriani et al., 1993). Several other angiogenic factors are also considered to be involved in the pathogenesis of endometriosis (Oosterlynck et al., 1993, 1994; Ryan et al., 1995; McLaren et al., 1996a,b; Shifren et al., 1996; Donnez et al., 1998; Gazvani et al., 1998; Iwabe et al., 1998; Osuga et al., 1999). Therefore, the relative strength of the action of Epo should be investigated in endometriotic tissues.

The cellular source of origin of PF Epo remains to be investigated. The major site of erythropoietin production in the adult is the kidney although extrarenal sites of Epo production may include bone marrow macrophages (Vogt et al., 1989), early colony-forming cells (Hermine et al., 1991), brain astrocytes (Masuda et al., 1994) and trophoblast cells (Conrad et al., 1996). Diffusion rates between PF and the blood stream decreases with increasing molecular weight to become extremely slow for molecules with a molecular weight >100 kDa (Dunselman et al., 1988). Prolactin (molecular weight: 20 kDa) and albumin (molecular weight: 60 kDa) concentrations are 67% of those in serum (Koninckx et al., 1980; Pattinson et al., 1981). The molecular weight of Epo is approximately 30 kDa (Tilbrook and Klinken, 1999). If the origin of the PF Epo is from the serum, theoretically, Epo concentrations in the paired serum should be higher than those in PF. However, Epo concentrations of PF were significantly higher than those of the paired serum. In addition, no correlations were detected between the paired serum and PF Epo concentrations. Therefore, extrarenal sites of Epo production should be considered as a potential source. A recent in-vitro study demonstrated that the mouse uterus expressed Epo mRNA and produced Epo protein in an oestrogen dependent manner (Yasuda et al., 1998; Masuda et al., 1999). This suggests that refluxed endometrial tissues may be direct candidates for a source of Epo production and secretion into PF. However, in the present study differences in PF Epo concentrations according to disease severity were detected. In addition, refluxed endometrial tissues are not specific to patients with endometriosis. Therefore, it is likely that endometrial tissues are not the sole source of Epo in PF. Endometriosis is associated with increased concentrations of activated macrophages, which may produce many cytokines, growth factors and angiogenic factors (Koninckx et al., 1998). Indeed, it has been demonstrated that activated macrophages are a major

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Figure 1. Erythropoietin concentrations in the peritoneal fluid (PF) of 18 patients without endometriosis (control) and 42 patients with endometriosis at various stages (stage I: n = 14, stage II: n = 8, stage III: n = 13, stage IV: n = 7). The circle represents the erythropoietin concentration, and the horizontal line represents the median value. Epo concentrations in PF from patients with stage I were significantly higher than those with stage II, stage III, stage IV and without endometriosis (control) (P < 0.03, P < 0.01, P < 0.01 and P < 0.001 respectively). C: control.
source of VEGF in endometriosis (McLaren et al., 1996b) and it may be that they are also a potential source of Epo in PF. Furthermore, red peritoneal lesions have significantly higher VEGF content as compared with black lesions (Donnez et al., 1998). Thus, a potential source of Epo may also include endometriotic tissues.

In conclusion, this study suggests that Epo may play a role in the pathogenesis of endometriosis, particularly during the early stages of the disease. Further studies to clarify a role of Epo in PF may provide a better understanding of the pathogenesis of endometriosis.

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References


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