Iron overload enhances epithelial cell proliferation in endometriotic lesions induced in a murine model

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BACKGROUND: Iron deposits are characteristic of endometriotic lesions, and pelvic iron concentrations are higher in endometriosis patients than in women without endometriosis. In this study, the effect of iron overload and iron chelation on the development of endometriosis in a murine model was investigated. METHODS: Human menstrual endometrium was injected i.p. into nude mice, either alone (controls) or supplemented with erythrocytes or desferrioxamine (DFO), an iron chelator. After 5 days, the iron load of endometriosis-like lesions and peritoneal macrophages and fluid was evaluated. Lesions were quantified by immunohistochemical morphometry, and their proliferative activity was assessed. RESULTS: Injection of erythrocytes into the pelvic cavity caused iron overload in lesions (P < 0.025) and peritoneal macrophages (P < 0.01) and fluid (P < 0.05), whereas DFO effectively reduced iron status in lesions (P < 0.05) and macrophages (P < 0.01) compared with controls. No difference was observed in the number or surface area of lesions between the three groups. Erythrocytes increased (P < 0.05) and DFO significantly decreased (P < 0.01) the proliferative activity of lesions. CONCLUSIONS: Iron overload does not appear to affect lesion establishment but may contribute to the further growth of endometriosis by promoting cell proliferation of lesions. Iron chelator treatment could therefore be beneficial in endometriosis to prevent iron overload in the pelvic cavity and decrease cellular proliferation of lesions.

Key words: desferrioxamine/endometriosis/iron/nude mouse model/proliferation

Introduction

Endometriosis is a gynaecological disorder affecting about 10–15% of women in their reproductive years. This pathology is characterized by the proliferation of endometrial glands and stroma outside the uterine cavity. Despite an increasing number of studies on its physiopathology, the aetiology of endometriosis remains elusive because of, in part, its multifactorial characteristics. Indeed, a growing body of evidence indicates that a combination of genetic, hormonal, environmental, immunological and anatomical factors plays a role in the pathogenesis of this disorder (Giudice and Kao, 2004).

Our hypothesis is that iron overload observed in the peritoneal cavity of endometriosis patients may be involved in the pathogenesis of endometriosis.

Indeed, several studies have demonstrated the presence of iron overload in the peritoneal cavity in endometriosis cases (Van Langendonckt et al., 2002b,c). In patients developing the disease, increased iron levels were found in peritoneal tissue, macrophages and pelvic fluid, as well as ectopic endometrial tissue. In the peritoneum and stroma of endometriotic lesions, cytologic and histochemical data revealed the presence of iron conglomerates (Moen and Halvorsen, 1992; Petrozza et al., 1993) and macrophages heavily laden with ferric pigment (Gaulier et al., 1983). In endometriotic cysts too, iron concentrations in cystic fluid are considered to be an indicator of endometriosis (Sugimura et al., 1992; Takahashi et al., 1996; Iizuka et al., 1998). In the peritoneal fluid of patients with endometriosis, higher levels of iron were detected, the levels correlating with the severity of the disease (Arunugam, 1994; Arunugam and Yip, 1995).

This iron could originate from the lysis of erythrocytes carried into the pelvic cavity by retrograde menstruation (Van Langendonckt et al., 2004). This phenomenon, by which menstrual fluid is transported through the Fallopian tubes into the peritoneal cavity and body iron content is preserved, results from uterine peristalsis of the non-pregnant uterus (Kunz and Leyendecker, 2001). According to Sampson’s implantation theory, retrograde menstruation, peritoneal adhesion of shed endometrial tissue and outgrowth of endometrial cells are essential steps in the pathogenesis of endometriosis (Sampson, 1927). Retrograde menstruation is a very common physiologic event in all menstruating women with patent tubes (Halme et al., 1984). However, its occurrence is often increased in endometriosis patients because of certain anatomical dispositions.
Iron, together with apoptotic endometrial fragments and activated macrophages, may induce oxidative stress within the pelvic cavity of patients with endometriosis, as reviewed by Murphy et al. (1998) and Van Langendonck et al. (2002b). Although iron is essential for living cells, free iron plays a key role in the formation of deleterious reactive oxygen species (ROS) (Crichton, 2001; Crichton et al., 2002), and iron-induced oxidative stress has been implicated in numerous pathologic processes (Iancu, 1992; Crichton, 2001).

Furthermore, it is well known that iron may have an influence on proliferation (Le and Richardson, 2002; Pham et al., 2006). The importance of iron for cell cycle progression has led to the use of iron chelators as anti-proliferative agents in the treatment for cancer (Simonart et al., 2002; Pahl and Horwitz, 2005; Richardson, 2005; Brard et al., 2006), but it is not yet known whether iron may also affect endometriotic lesion proliferation. However, the high proliferative activity of endometriotic lesions suggests that mechanisms underlying proliferation control differ from those observed in eutopic endometrium (Nisolle et al., 1997).

This study was designed to investigate the effect of iron overload and iron chelation on the development (adhesion and proliferation) of pelvic endometriosis induced in a nude mouse model. For this purpose, endometriosis was induced by injection of human menstrual endometrium either alone or supplemented with erythrocytes, as a source of iron, or desferrioxamine (DFO), a current therapeutic iron-chelating agent, into the pelvic cavity of nude mice. After 5 days, endometriosis-like lesions, peritoneal fluid and macrophages were recovered. Owing to technical problems, peritoneal macrophages were recovered. Owing to technical problems, peritoneal fluid and macrophages were collected from five experiments (15 mice).

Materials and methods

Experimental design

This study aimed to investigate and evaluate the effect of iron overload and iron chelation on the development of pelvic endometriosis induced in a nude mouse model, developed in our laboratory (Nisolle et al., 2000; Defrère et al., 2006). To this end, endometriosis was induced in nude mice by i.p. injection of human menstrual endometrium, either alone or in combination with erythrocytes or DFO.

Mesutritional endometrium collected from a single donor was used per experiment involving three mice:

(i) one ‘control’ mouse, injected i.p. with 100 μl of phosphate-buffered saline (PBS; Gibco, Scotland, UK) every 6 h for 3 days;

(ii) 500 million erythrocytes resuspended in 100 μl PBS corresponds to the erythrocytes concentration in blood. The final i.p. concentration should be of about 1 billion erythrocytes/ml which is in the same range as the concentration measured in the peritoneal fluid from patients during menstruation (unpublished data). PBS injections (100 μl) were administered every 6 h between erythrocyte injections to ensure that any differences observed between the groups did not result from a different number of injections;

(iii) one ‘DFO’ mouse, injected i.p. with the iron chelator, DFO (100 mg/kg; Desferal®, Novartis, Zurich, Switzerland), diluted in 100 μl of PBS, every 6 h for 3 days. DFO chelates Fe³⁺ from free iron, ferritin or haemosiderin. Chelated iron is then eliminated through urine.

The experiment was replicated eight times. Twenty-four mice were used. Two mice died during surgery (one control mouse and one DFO mouse).

After 5 days, endometriosis-like lesions, peritoneal fluid and pelvic macrophages were recovered. Owing to technical problems, peritoneal fluid and macrophages were collected from five experiments (15 mice). For each recovered lesion, the histological characteristics of endometriosis were confirmed. Ferric iron deposits were stained and quantified in both lesions and peritoneal macrophages. Iron concentrations in peritoneal fluid were evaluated. The occurrence, the surface area and the proliferative activity of lesions were analysed, as detailed below.

Collection of human endometrial tissue and erythrocytes

The use of human tissue for this study was approved by the Institutional Review Board of the Université Catholique de Louvain.

Mesutritional endometrium was obtained, by aspiration with a syringe without any anticoagulants, from eight women aged 31–45 years (mean age = 38.14 ± 4.53 years) undergoing surgery for benign conditions. A sample of the biopsy was fixed in 4% buffered formaldehyde and embedded in paraffin for histological confirmation of the menstrual phase using established criteria (Noyes et al., 1950) and for immunohistochemical controls. The rest of the tissue was minced into microfragments able to pass through a 19-G needle (maximal size = 750 μm) and injected into nude mice, as described below.

Human erythrocytes were isolated from the peripheral blood of alternative patients by density gradient centrifugation. Ficoll-Paque™ PLUS was used according to the manufacturer’s instructions (Amersham Pharmacia Biotech AB, Uppsala, Sweden). The resulting erythrocyte pellet was washed three times in PBS. The washed red blood cells were counted using a Bürker slide (Marienfield Superior, Marienfield Laborato- ry Glassware, Lauda-Königshofen, Germany) before injecting into the pelvic cavity of mice.

Transplantation of menstrual endometrium into nude mice

The guidelines for animal welfare were approved by the Committee on Animal Research of the Université Catholique de Louvain, and the mice were operated on under the supervision of an experienced veterinarian.

Twenty-four nude (Swiss nu/nu) 6- to 10-week-old female mice (Charles River Laboratories, Wilmington, NC, USA) were used for this study. Handling and maintenance were implemented as previously described (Nisolle et al., 2000).

The mice were anaesthetized with an i.p. injection of ketamine (75 mg/kg; Anesketin®, Eurovet, Heusden-Zolder, Belgium) and medetomidine (1 mg/kg; Domitor®, Pfizer, Cambridge, MA, USA). After surgery, the anaesthesia was reversed by injection of atipamezole (1 mg/kg; Antisedan®, Pfizer).

To mimic retrograde menstrual discharge, we injected menstrual endometrium i.p. A ventral incision of 0.5 cm was made in the skin, keeping the abdominal wall intact, followed by i.p. injection of the menstrual endometrium under visual control. A thin needle (19 G) was used to avoid i.p. organ injury or damage to the peritoneal layer. The skin was then sutured with one or two stitches.

Peritoneal fluid, macrophage and lesion recovery

After 5 days, the mice were euthanized by cervical dislocation, according to the standard euthanasia guidelines for rodents from the
Staining of iron deposits
Ferric iron deposits were evidenced in recovered lesions and macrophages from all three groups (control, iron and DFO) using Prussian blue staining according to Perls’ reaction (Perls, 1867). Briefly, dewaxed 5-μm thick sections of lesions and permeabilized macrophages were stained for 20 min in a solution of kalium hexacyanoferrate 1% and HCl 2%, v/v, and nuclei were counterstained with nuclear fast red (Gabe, 1968).

Seventy-eight lesions were stained, observed under a microscope (Zeiss, Munich, Germany) at ×100 magnification and classified as lesions with or without iron deposits. The presence or absence of iron deposits was recorded in the stroma and interface area between the endometrial implant and murine tissue. Results were expressed as the percentage of lesions of each group showing iron deposits.

Macrophages from peritoneal fluid were distinguished from other cell types by their cytological characteristics (Koss, 1992). The presence or absence of iron deposits was also recorded in the cytoplasm of macrophages. At least 100 macrophages per mouse were observed and classified as with or without iron deposits. For each group, results were expressed as the mean ± SD of the percentage of macrophages with iron deposits.

Iron concentrations in peritoneal fluid
Total iron concentrations in peritoneal fluid were measured by electrothermal graphite furnace atomic absorption spectrometry at 248.3 nm (Perkin-Elmer 5100PC) with Zeeman correction. Samples of peritoneal lavage fluid were diluted in 0.01 M nitric acid. External quality controls (bovine liver, European Commission Bureau of Reference) were included in each batch. All measurements were performed in duplicate. For each group, results were expressed as the mean ± SD of pelvic iron concentrations (expressed in μg/l).

Quantification of endometriosis-like lesions by immunohistochemical morphometric analysis
Immunohistochemical morphometric analysis allows measurement of specific constituents of endometriosis-like lesions—epithelial glands and stroma—as previously described (Defrère et al., 2006).

To this end, paraffin-embedded lesions were cut into semi-serial sections of 5 μm. Every fifth slide was stained with haematoxylin–eosin, and the section with the largest endometriotic lesion surface area was selected. The next two serial sections were immunostained (Figure 1).

Immunostaining was performed on these sections with a labelled immunoperoxidase method using mouse monoclonal antibody to human CD10 (clone 56C6; Novocastra, Newcastle, UK) and to cytokeratin CK22 (Biomedca, Foster City, CA, USA). CD10 is an immunohistochemical marker of endometrial stroma, which has been used to confirm a diagnosis of endometriosis, whereas endometrial glands selectively express CK22. Briefly, endogenous peroxidase activity quenching, heat epitope retrieval and blocking of non-specific staining were performed. The specimens were then incubated overnight at 4°C with primary antibodies (dilutions: 1 : 800 for CK22 and 1 : 200 for CD10), followed by incubation with secondary antibody conjugated to peroxidase (EnVision+™, Dakocytomation, Glostrup, Denmark). The presence of peroxidase was revealed using 3,3′-diaminobenzidine (Dakocytomation) and specimens were counterstained with Mayer’s hemalum solution (Merck, Darmstadt, Germany).

Figure 1. Immunohistochemical staining with mouse monoclonal antibodies to CD10 (A and B) and to human cytokeratin cocktail CK22 (C and D). As shown in sections of normal human endometrium, endometrial stromal cells selectively express CD10 (A), and endometrial epithelial cells selectively express CK22 (C). On lesion sections from the mouse model (B and D), this staining allows accurate identification and measurement of specific constituents of endometriosis-like lesions: epithelial glands and stroma (original magnification: ×100).

CD10- and CK22-stained slides were examined under a microscope (Zeiss) at ×100 magnification and all fields were digitalized using a Leica DFC320 camera and IM50 program (Leica Microsystems, Wetzlar, Germany).

ImageJ, a freely available image processing and analysis program developed at the National Institutes of Health (http://rsb.info.nih.gov/ij/), was used to delimit all glandular and stromal structures in the lesions and measure their surface area.

Menstrual endometrium collected from a single donor was used per experiment involving three mice. For each mouse, the total surface area of a cross section of the lesions was measured. Owing to the heterogeneity between menstrual endometrium samples, the surface areas of DFO and iron lesions were normalized relative to the control lesions for each experiment.

Measurement of proliferative activity
Ki67 immunostaining was performed on sections with a labelled immunoperoxidase method using rabbit antibody to human Ki67 (Dakocytomation). In short, endogenous peroxidase activity quenching, heat epitope retrieval and blocking of non-specific staining were performed. The specimens were then incubated overnight at 4°C with the primary antibody (dilution: 1 : 500), followed by incubation with secondary antibody conjugated to peroxidase. The presence of peroxidase
was revealed using 3,3′-diaminobenzidine, and specimens were counterstained with Mayer’s hemalum solution.

Ki67-stained slides were examined under a microscope (Zeiss) at \( \times100 \) magnification. Proliferative activity was evaluated by counting Ki67-positive and -negative nuclei of epithelial cells of each lesion (average number of cells per lesion: 512.9 ± 329.1, range: 19–1498 cells per lesion). The proliferative activity of stromal cells was not reported because, as shown in our previous studies, the rate of positive stromal cells was <1% (Nisolle et al., 2000; Van Langendonckt et al., 2004).

Results were normalized as described above.

Statistical analysis

Results were expressed as means ± SD. To evaluate the statistical significance of differences between the three groups, we applied the two-tailed Student’s \( t \)-test to test for mean comparisons. A \( P \) level of ≤0.05 was considered statistically significant. All statistical analyses were performed using the Statistical Package for the Social Sciences 11.5 for Windows package (SPSS Inc., Chicago, IL, USA).

Results

In this study, we mimicked the ectopic implantation of human menstrual endometrium. The histological characteristics of endometriosis were confirmed after haematoxylin–eosin staining. Immunohistochemical labelling with anti-human CD10 antibodies, specific for stromal cells, and anti-human CK22 antibodies, specific for glandular epithelial cells, confirmed the human origin of the endometrial tissue.

Number of recovered lesions

On day 5, endometriosis-like lesions were recovered. In this series of eight experiments, 78 endometriotic lesions were collected from 22 mice in total (mean number = 3.59 ± 2.34 lesions per mouse; range: 1–10 lesions per mouse). As summarized in Table I, in the control group, 24 endometriotic lesions were recovered from seven mice (mean number = 3.43 ± 1.40 lesions per mouse; range: 1–5 lesions per mouse); in the iron group, 30 endometriotic lesions were recovered from eight mice (mean number = 3.87 ± 2.94 lesions per mouse; range: 1–10 lesions per mouse); in the DFO group, 24 endometriotic lesions were recovered from seven mice (mean number = 3.43 ± 2.64 lesions per mouse; range: 1–9 lesions per mouse).

No significant difference in lesion number was observed between the three groups.

<table>
<thead>
<tr>
<th>Table I. Effect of iron and desferrioxamine (DFO) treatment on the number and surface area of recovered lesions</th>
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<tbody>
<tr>
<td>Control</td>
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<tr>
<td>------------------------</td>
</tr>
<tr>
<td><strong>Number of lesions</strong></td>
</tr>
<tr>
<td>(( n = 7 ))(^a)</td>
</tr>
<tr>
<td>(average number of lesions per mouse)</td>
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<tr>
<td>3.43 ± 1.40</td>
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<tr>
<td><strong>Surface area of pooled lesions per mouse</strong></td>
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<td>(range: 1–5)</td>
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<td>1 (( n = 7 ))(^a)</td>
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\(^a\)number of mice. One control and one DFO mouse died during surgery.

Quantification of endometriosis-like lesions by immunohistochemical morphometric analysis

All 78 lesions were quantified by morphometry (results expressed as \( \mu \text{m}^2 \)). The results summarized in Table I were obtained as follows: surface areas of pooled lesions from the iron or DFO mice were normalized relative to the pooled control mouse lesions.

As summarized in Table I, the surface area of pooled lesions per mouse was similar in both the iron group (2.04 ± 1.83-fold increase) and the DFO group (1.54 ± 0.94-fold decrease) compared with the control mice. No significant difference was observed in the surface area of lesions between the three groups.

Staining of iron deposits in lesions and macrophages

Ferric iron deposits of endometriosis-like lesions and peritoneal macrophages were evidenced using Prussian blue staining. Before injection, most menstrual endometrium was devoid of iron deposits.

In endometriosis-like lesions, as illustrated in Figure 2, deposits were particularly numerous in the interface area between the implanted endometrial tissue and the murine tissue. Iron deposits were never observed in glandular epithelium. As summarized in Table II, iron deposits were detected in 62.5% (15/24) of lesions from the control group. A significant increase in lesions with iron deposits [86.7% (26/30)] was observed in the iron group compared with the control group. On the contrary, a significant decrease in lesions with iron deposits [37.5% (9/24)] was noted in the DFO group compared with the control group.

In peritoneal fluid cells, iron deposits were mainly observed in macrophages and mesothelial cells, whereas lymphocytes

![Figure 2. Perls' staining in recovered endometriosis-like lesions (A and B) and peritoneal macrophages (C and D) from control mouse; nuclei were counterstained with nuclear fast red. A and B, interface area between the stroma of the lesion and the murine peritoneum (original magnification: A, \( \times200 \) and B, \( \times400 \). C and D, photomicrographs of one positive-stained macrophage (C) and one negative-stained macrophage (D) (original magnification: \( \times1000 \)).](https://academic.oup.com/humrep/article-abstract/21/11/2810/2939259)
were never found stained (Perls' reaction). The percentage of peritoneal macrophages showing iron deposits is summarized in Table II. A significant increase in the percentage of iron-loaded macrophages (70.40 ± 17.06%) was noted in the iron group compared with the control group (22.05 ± 22.48%). On the contrary, a significant decrease in the percentage of iron-loaded macrophages (8.84 ± 12.72%) was observed in the DFO group compared with the control group.

Iron concentrations in peritoneal fluid
Iron concentrations in peritoneal fluid were measured by atomic absorption. As summarized in Table II, there was a significant increase in peritoneal lavage fluid iron levels in the iron group (246.46 ± 79.33 μg/l) compared with the control group (135.42 ± 67.39 μg/l), whereas no significant difference was observed between the DFO group (74.7 ± 53.28 μg/l) and the control group.

Measurement of proliferative activity in endometriosis-like lesions
Proliferative activity was assessed immunohistochemically in recovered endometriotic lesions using an antibody specific for human Ki67. The proliferation index was <1% in menstrual endometrium before injection (day 0) in both stromal and glandular epithelial cells. As shown in Figure 3, 5 days after injection, a dramatic increase was observed in the proliferative activity of glandular epithelial cells of endometriotic lesions. By contrast, the proliferative activity of stromal cells of lesions was in the same range as noted before injection, in accordance with our previous findings (Nisolle et al., 2000; Van Langendonckt et al., 2004).

As presented in Figure 4, cell proliferation was significantly higher in epithelial glands from lesions in the iron group (1.31 ± 0.35-fold increase) but lower in the DFO group (0.71 ± 0.15-fold decrease), compared with the control mice.

Discussion
Iron deposits are considered to be typical features of endometriotic lesions, and increased concentrations of iron are found in the pelvic cavity of endometriosis patients. The objective of this study was to investigate the impact of pelvic iron overload and iron chelation on the development (adhesion and proliferation) of peritoneal endometriosis induced in a nude mouse model, in conditions mimicking menstrual effluent discharge.

### Table II. Effect of iron and desferrioxamine (DFO) treatment on pelvic iron load

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Iron</th>
<th>DFO</th>
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<tbody>
<tr>
<td>Lesions with iron deposits</td>
<td>62.5% (15/24) (n = 7)a</td>
<td>86.7%b (26/30) (n = 8)a</td>
<td>37.5%c (9/24) (n = 7)a</td>
</tr>
<tr>
<td>Macrophages with iron deposits</td>
<td>22.05 ± 22.48% (n = 5)a</td>
<td>70.40 ± 17.06%c (n = 5)a</td>
<td>8.84 ± 12.72%c (n = 5)a</td>
</tr>
<tr>
<td>Iron concentrations in peritoneal lavage fluid (μg/l)</td>
<td>135.42 ± 67.39 (n = 5)a</td>
<td>246.46 ± 79.33 (n = 5)a</td>
<td>74.7 ± 53.28 (n = 5)a</td>
</tr>
</tbody>
</table>

*a, number of mice. One control and one DFO mouse died during surgery.

bp < 0.025.

cp < 0.05.

dp < 0.01. P-values expressed relative to controls.
For this purpose, endometriosis was induced in nude mice by i.p. injection of human menstrual endometrium, either alone or supplemented with erythocytes or DFO. As previously shown, endometrium is able to implant onto intact mesothelium and reorganize itself into structured glands and stroma, forming endometriosis-like lesions on peritoneal and organ surfaces in athymic mice within 5 days (Nisolle et al., 2000; Van Langendonckt et al., 2004; Eggermont et al., 2005; Defrère et al., 2006).

After 5 days, pelvic iron load was markedly greater in mice injected with erythrocytes than in control mice. This was highlighted by the presence of ferric iron deposits in lesions and pelvic macrophages and by a significant increase in iron concentrations in peritoneal fluid. The iron overload observed in the tissue and soluble compartments of the murine pelvic cavity is similar to that encountered in the pelvic cavity of endometriosis patients (Gaulier, cavity is similar to that encountered in the pelvic cavity of endometriosis patients (Gaulier et al., 1983; Moen and Halvorsen, 1992; Petrozza et al., 1993; Arumugam, 1994; Arumugam and Yip, 1995; Van Langendonckt et al., 2002a,c). Our murine model, mimicking conditions of retrograde menstrual discharge, clearly suggests that peritoneal iron overload encountered in endometriosis patients may well originate from the lysis of erythrocytes from retrograde menstrual effluent. As in most tissue, activated macrophages probably play an important role in the degradation of erythrocytes, as indicated by the presence of numerous siderophages, known as iron-storing macrophages, in the peritoneal fluid of patients with endometriosis (unpublished data) and mice injected with erythrocytes. Macrophages usually internalize and lyse senescent erythrocytes, releasing haemoglobin and ensuring its degradation by haeme oxygenase. The iron released is then returned to the iron store by transferrin (Van Langendonckt et al., 2002b), resulting in increased peritoneal fluid iron concentrations in both patients and our murine model. Lesions and adjacent peritoneum also show typical features of iron-overloaded tissue in patients and our experimental model.

This peritoneal iron overload might have numerous cytotoxic effects in the peritoneal environment (Arumugam, 1994; Murphy et al., 1998; Skowron, 2000; Van Langendonckt et al., 2002a,c). In this study, we were able to significantly decrease the number of lesions with iron deposits, iron concentrations in peritoneal fluid and the percentage of iron-loaded pelvic macrophages by DFO treatment. An iron chelator could thus be beneficial in endometriosis to reduce the iron content of the peritoneal cavity and moderate its deleterious effect.

This model allows us to postulate that iron overload does not alter the initial steps of lesion formation because, on day 5, we did not observe any significant effect of iron overload or iron chelation on the number or surface area of lesions, as precisely recorded by immunohistochemical morphometry. However, our results suggest that iron overload may contribute to the further growth of endometriosis by promoting epithelial cell proliferation. Indeed, we demonstrated that erythrocyte injection increased the proliferative activity of epithelial cells in endometriotic lesions, whereas DFO administration significantly decreased it.

Iron is an absolute requirement for proliferation, as iron-containing proteins catalyse key reactions involved in oxygen sensing, energy metabolism, respiration, folate metabolism and DNA synthesis (e.g. ribonucleotide reductase that catalyses the conversion of ribonucleotides into deoxyribonucleotides for DNA synthesis). It has been shown that subunit R2 of the ribonucleotide reductase gene contains iron-specific regulatory elements and is up-regulated by blood feeding (Pham et al., 2006). In fact, deprived of iron, cells are unable to proceed from the G1 to the S phase of the cell cycle (Le and Richardson, 2002). Iron chelators have proved to be efficient anti-proliferative agents for the treatment for cancer (Simonart et al., 2002; Pahl and Horwitz, 2005; Richardson, 2005; Brard et al., 2006). However, to our knowledge, this is the first time that the impact of iron overload and iron chelation has been evaluated with respect to endometriotic lesion proliferation.

After implantation onto the mesothelium, proliferation and vascularization of lesions are essential steps in the further development of endometriosis (Nisolle and Donnez, 1997; Groothuis et al., 2005). Proliferation of epithelial cells and their differentiation into glandular structures are key events, likely to be under the control of factors in the local environment. Mitogens produced by stromal cells, like hepatocyte growth factor and estradiol (E2) (Giudice and Kao, 2004), or growth factors and inflammatory cytokines present in peritoneal fluid, have indeed been shown to promote epithelial cell proliferation and ectopic endometrial cell growth. As shown in the present study, iron could be one of the factors promoting further growth of implanted ectopic endometrial tissue.

Several studies indicate that the amount of retrograde menstruation appears greater in patients with endometriosis than in women without endometriosis (Salamanca and Beltran, 1995; Kunz and Leyendecker, 2001; Vinatier et al., 2001; Bulleiti et al., 2002). This may result in increased reflux of erythrocytes into the pelvic cavity in such patients (Halme et al., 1984), leading to iron overload in the different compartments (lesions, peritoneal fluid and peritoneal macrophages), as suggested by the results of this study. Although iron overload does not appear to affect lesion establishment, our study strongly suggests that it may contribute to the further growth of endometriosis by promoting epithelial cell proliferation of lesions. Treatment with an iron chelator could thus be beneficial in endometriosis to prevent iron overload in the pelvic cavity, thereby diminishing its possible deleterious effect, and reduce cellular proliferation of lesions.

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