Effects of a single post-ovulatory dose of RU486 on endometrial maturation in the implantation phase

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The effect of a single post-ovulatory dose of RU486 on endometrial maturation was studied in the implantation phase. A total of 11 healthy women were followed for one control and one or two treatment cycles. In treatment cycles, a dose of 200 or 400 mg RU486 was administered on day luteinizing hormone (LH)+2. In both control and treatment cycles, an endometrial biopsy was obtained on LH+6 to LH+8. These biopsies were assessed by morphometric and immunohistochemical analyses. The treatment with RU486 did not disturb the normal menstrual rhythm but caused a significant inhibition in the endometrial development. Glandular progesterone receptor staining was significantly more pronounced after RU486 treatment, while there was a reduction in the Dolichos biflorus agglutinin lectin binding, indicating inhibition of the normal secretory transformation of the endometrium. It is likely that these effects on endometrial development and secretory activity represent the basis of the contraceptive effect of post-ovulatory RU486 treatment.

Key words: antiprogestin/endometrium/implantation/mifepristone

Introduction

The mechanism of implantation of the human blastocyst in the endometrium is considered to be initiated by a dialogue between the embryo and maternal tissue (Psychopoulos and Martel, 1985). Studies in animals have shown that, indeed, the developmental stages of both the blastocyst and the endometrium require temporal synchronization for implantation to be possible (Glasser et al., 1991). This permissive stage of the endometrium is sometimes referred to as the ‘implantation window’ (Harper, 1992). According to early work by Hertig et al. (1956, 1959), confirmed recently by others (Bergh and Navot, 1992), human implantation occurs between 5.5 and 6 days after ovulation, which corresponds to 7–8 days after the luteinizing hormone (LH) surge. Although the maturational stages of the endometrium follow well defined histological criteria (Noyes et al., 1950), the clinical usefulness of histology for the assessment of luteal function has been found to be disappointing (Balasch et al., 1992). Moreover, no significant correlation has been found between the histology of endometrial biopsies and the mid-luteal oestradiol or progesterone concentrations in serum (Gerhard et al., 1990). Therefore, it is important to develop new methods of investigating and diagnosing human endometrial function.

Mifepristone (RU486) is a synthetic 19-norsteroid with potent antiglucocorticoid and antigestagenic properties (Baulieu, 1989). It is generally accepted that RU486 acts as a pure progesterone antagonist almost without agonistic properties. The effect of RU486 during the menstrual cycle depends on the time of treatment. Treatment before ovulation will result in a prolongation of the proliferative phase, while treatment during the mid- and late luteal phases will invariably induce bleeding, often followed by a second bleeding episode at or near the expected time of menstruation. The only time when treatment does not upset the bleeding pattern is immediately following ovulation. If RU486 is administered during the first days following ovulation occasional spotting may occur, but the time of menstruation is not influenced. However, the secretory development of the endometrium is significantly inhibited, although the concentration of ovarian steroids seems to remain essentially unchanged (Swahn et al., 1990). In a recent clinical trial, 21 women were treated in 157 cycles with one single dose of 200 mg RU486 2 days after the LH surge as their only contraceptive method (Gemzell-Danielsson et al., 1993). All women had been pregnant previously and were sexually active. Only one pregnancy occurred, illustrating that the blocking of the gestational effect must impair essential functions involved in the implantation process. It is probable that the contraceptive effect of RU486 is due to its action on the endometrium. The aim of the present study was to evaluate further the effect of a single, immediate, post-ovulatory dose of RU486 on endometrial maturation at the time of implantation. The study was approved by the Ethics Committee at the Karolinska Hospital, Sweden, and the patients had given their informed consent.

Materials and methods

Subjects and cycles

A total of 11 healthy women aged 21–40 years with regular menstrual cycles (25–36 days) volunteered for the study [height 167 cm (range 150–167); weight 66 kg (range 53–77)]. All subjects except for three had proven fertility and none of them had used steroidal contraceptives or an intra-uterine device for a minimum of 3 months prior to the study.
The study included one control and one or two treatment cycles, the subjects serving as their own controls. During the first treatment cycle, 200 mg mifepristone (RU486) were given orally between 8 and 10 p.m. on cycle day LH +2. Four subjects participated in a second treatment cycle in which the dose of RU486 was increased to 400 mg.

Hormone determinations

During the control and treatment cycles, morning and evening urine samples were collected from cycle day 10 to cycle day LH +2. The subjects determined the LH levels in their urine samples using a self-test (OvuQuick; Monoclonal Antibodies Inc., Sunnyvale, CA, USA). Values were confirmed by laboratory analyses of LH by automated microparticle enzyme immunoassay (IMX system, Abbott Scandinavia Inc., Kista, Sweden). The coefficient of variation (CV) within each run was <5.6% and between runs <6.8%.

Blood samples were obtained three times weekly during the entire study period and were analysed for oestradiol and progesterone by radioimmunoassay (Coat-a-Count, Diagnostic Products Corporation Inc., Los Angeles, CA, USA) with CV values <8.4% intra-assay and <10% interassay.

The geometric means and 95% confidence limits were calculated for all hormone concentrations and the values were normalized around the day of the serum LH surge. For statistical calculations, the Wilcoxon’s matched pairs test, signed-ranks test and the Student’s paired t-test were used.

Endometrial biopsies

One endometrial tissue specimen was obtained in both the control and the treatment cycle(s) from the anterior and lateral walls of the uterine cavity using a Randall curette. This excludes the need for cervical dilatation and/or anaesthesia. The day of biopsy varied from LH +6 to LH +9. One part of each specimen was fast-frozen and stored in liquid nitrogen for immunohistochemical analysis. Another part of each specimen was fixed immediately in Bouin’s solution for light microscopic examination after paraffin embedding and haematoxylin staining. The material prepared for light microscopy was assessed by morphometric analysis, as described earlier (Johannisson et al., 1987). Microscopic evaluation of the samples was performed at the end of the study by one person who was unaware of the precise cycle day and if the biopsy was taken in the control or treatment cycle.

Immunohistochemistry

The endometrial biopsy specimens were mounted in an embedding medium, which beside non-reactive ingredients contained polyvinyl alcohol 10.24% and polyethylene glycol 4.26% (O.T.C. compound, Miles Inc., Elkhart, IN, USA), at −17°C and sectioned to 8–10 µ using a Reichert-Jung Cryocut 1800 (Cambridge Instruments GmbH, Nussloch, Germany). The sections were placed on glass slides and air dried for 15–20 min before a 10 min fixation in acetone. Thereafter, the mounted sections were wrapped in parafilm and stored at −70°C until processed for immunohistochemistry.

Human progesterone receptors were detected in the endometrial sections by using the Abbott PgR-ICA monoclonal assay system (Abbott Laboratories Inc., North Chicago, IL, USA). Positive and negative control labelling was performed using Abbott control slides. The secretory components of endometrial glands were detected by lectin cytochemistry using biotinylated Dolichos biflorus agglutinin (DBA) at a concentration of 5 mg/ml and the Vectastain Elite ABC immunoperoxidase detection system (Vector Laboratories Inc., Burlingame, CA, USA). DBA binds to N-acetylgalactosamine and galactose residues present in the glandular secretions of the mid-luteal phase endometrium in normal women (Mazur et al., 1989). As a negative control, DBA was co-incubated with the corresponding carbohydrate ligand at 200 mM concentration, which produced a complete inhibition of the binding.

Immunohistochemical staining was evaluated blindly by two independent persons, using a Zeiss light microscope at ×200 magnification. To avoid errors due to uneven staining, three sections of each endometrial biopsy specimen were evaluated in two or three different labelling experiments and included both epithelial and stromal cells. For each section, nine microscopic fields were randomly selected and evaluated. In each field, stromal and glandular cells were assigned a score of 0–4 based on the intensity of the staining as follows: 0 = negative (0% positive cells), 1 = very weak (<5% positive cells), 2 = weak (5–25% positive cells), 3 = moderate (25–75% positive cells), and 4 = strong (>75% positive cells). A similar scoring system has been used by Press et al. (1988). Results are mean values of the fields evaluated.

Results

Effects on cycle length

The mean length of the menstrual cycle as well as the duration of the bleeding were the same during the control and treatment periods. The amount of blood loss after RU486 administration was subjectively evaluated to correspond to a normal menstrual period. However, two of the 11 women experienced slight bleeding on the second day after treatment with 200 mg RU486 (Table I) and the bleeding continued for 2 days. A bleeding for 2 or 3 days was also noted for two of the four women receiving 400 mg RU486. However, in none of these women was the length of the menstrual cycle influenced.

Hormone concentrations

The timing of urinary LH peaks was found to be accurate in most cycles. In five subjects, the LH peak measured by enzyme immunoassay coincided with the urinary self-test in both the control and treatment cycles. In the remaining cycles, the difference between the LH surges was ±1 day. The LH peak determined by enzyme immunoassay was used in calculating the correct day for the endometrial biopsy.

Following the treatment with 200 or 400 mg RU486, a slight increase in the serum progesterone levels was observed for the period LH +3 to LH +10 in comparison with the control cycle (P < 0.05) (Figure 1). No differences in the serum oestradiol values were found (Figure 2). The oestradiol/progesterone ratio was essentially the same during the control and treatment cycles for both the 200 and 400 mg RU486 treatments.
Table I. Morphometric dating of endometrial biopsies and bleeding characteristics in patients receiving RU486

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Controls</th>
<th>RU486 200 mg</th>
<th>RU486 400 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>biopsy</td>
<td>morphometric</td>
<td>biopsy</td>
</tr>
<tr>
<td></td>
<td>day*</td>
<td>dating</td>
<td>day*</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>6</td>
<td>7</td>
</tr>
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<td>3</td>
<td>8</td>
<td>8</td>
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<td>6</td>
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<td>6/7</td>
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<td>7</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>IS</td>
<td>8</td>
</tr>
<tr>
<td>9</td>
<td>7/8</td>
<td>IS</td>
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<tr>
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<td>7/8</td>
<td>IS</td>
<td>8</td>
</tr>
<tr>
<td>11</td>
<td>7</td>
<td>7</td>
<td>8</td>
</tr>
</tbody>
</table>

P = proliferative phase; IS = irregular secretory phase.

*According to urinary luteinizing hormone (LH) peak.

Slight bleeding for 2 days after treatment LH+4, LH+5.

Slight bleeding for 3 days after treatment LH+5, LH+6, LH+7.

Slight bleeding for 2 days after treatment LH+5, LH+6.

**Morphometric analysis**

In 10 of the 11 subjects the control cycles showed an excellent correspondence between the calculated cycle day, based on the LH peak in urine, and the dating, based on morphometric analysis. In one subject the difference was 2 days.

The treatment with RU486 produced profound endometrial changes in all subjects (Table I). A histological pattern corresponding to the proliferative phase was seen in seven subjects, whereas two others demonstrated irregular secretory activity and two biopsies showed a pattern corresponding to LH+4.

Statistical evaluation of the morphometric analyses revealed a significantly decreased glandular diameter (P < 0.01) and an increased number of glandular (P < 0.01 and stromal (P < 0.01) mitoses in comparison with biopsies taken in the control cycle. The number of vacuolated cells was unaffected (Table II). Treatment with RU486 at the higher dose gave the same changes as the lower dose. However, the material was too small for statistical evaluation.

**Immunohistochemistry**

Enough material for immunohistochemical scoring of the endometrial biopsies was obtained for all but one subject. All four subjects that received two doses (400 mg) of RU486 were also analysed. The scoring data of progesterone receptors in cellular compartments of the endometrium are summarized in Table III. In the biopsy specimens obtained from normal cycles, the progesterone receptors (PgR) observed were located in the stroma, with absent or very faint staining in the glandular cells (Figure 3, left panel). In RU486-treated cycles, the staining pattern of the stroma was similar to control, although the glandular staining was entirely different, exhibiting a moderate to strong epithelial cell staining (Figure 3, right panel). The pattern of PgR staining corresponded well with the pattern seen at the time of ovulation, when progesterone receptors are maximally expressed in the human endometrium (Lessey et al., 1988). These results indicate that the normal down-regulation of PgR in epithelial cell nuclei is inhibited when RU486 binds to the PgR of endometrial epithelial cells.

![Fig. 1. Serum concentrations of progesterone in control and treatment cycles. RU486 (200 mg) was given on day luteinizing hormone (LH)+2 in the treatment cycle (geometric mean, 95% confidence limits).](https://academic.oup.com/humrep/article-abstract/9/12/2398/685774)

![Fig. 2. Serum concentrations of oestradiol in control and treatment cycles. RU486 (200 mg) was given on day luteinizing hormone (LH)+2 in the treatment cycle (geometric mean, 95% confidence limits).](https://academic.oup.com/humrep/article-abstract/9/12/2398/685774)
Table II. Results of morphometric analyses of the endometrium in a control cycle and following treatment with RU486 (200 or 400 mg) administered on cycle day LH+2*

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 11)</th>
<th>( p^b )</th>
<th>RU486 200 mg (n = 11)</th>
<th>RU486 400 mg (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glands/mm(^2) (n)</td>
<td>18.73 ± 6.1</td>
<td>NS</td>
<td>12.79 ± 5.33</td>
<td>17.9 ± 1.2</td>
</tr>
<tr>
<td>Glandular mitoses/1000 cells (n)</td>
<td>0.25 (0-2)</td>
<td>&lt;0.01</td>
<td>3.9 (0-8)</td>
<td></td>
</tr>
<tr>
<td>Stromal mitoses/1000 cells (n)</td>
<td>0.17 (0-2)</td>
<td>&lt;0.01</td>
<td>4.00 (0-10)</td>
<td>2.75 (0-10)</td>
</tr>
<tr>
<td>Vacuolated cells/1000 cells (n)</td>
<td>151.7 (0-800)</td>
<td>NS</td>
<td>133.9 (0-560)</td>
<td>225 (0-900)</td>
</tr>
<tr>
<td>Glandular diameter (µm)</td>
<td>74.65 ± 14.3</td>
<td>&lt;0.01</td>
<td>50.4 ± 18.7</td>
<td>54.0 ± 16.0</td>
</tr>
<tr>
<td>Glandular epithelial height (µm)</td>
<td>20.3 ± 4.4</td>
<td>NS</td>
<td>18.6 ± 5.5</td>
<td>21.8 ± 3.4</td>
</tr>
</tbody>
</table>

NS = not significant.

*Endometrial biopsies were obtained 4–7 days later on LH+6 to LH+9 in both cycles.

Statistical significance for comparisons between control and RU486 200 mg values only.

Table III. Progesterone-receptor staining intensity in the human endometrium*

<table>
<thead>
<tr>
<th>Cellular location</th>
<th>Type of cycle and labelling score (mean ± SD, and probability)</th>
<th>Control (n = 10)</th>
<th>RU486 200 mg (n = 10)</th>
<th>RU486 400 mg (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal epithelium</td>
<td>0.3 ± 0.4 P &lt; 0.01</td>
<td></td>
<td>2.7 ± 1.5</td>
<td>ND</td>
</tr>
<tr>
<td>Glandular epithelium</td>
<td>0.4 ± 0.4 P &lt; 0.01</td>
<td></td>
<td>2.8 ± 1.2</td>
<td>ND</td>
</tr>
<tr>
<td>Stroma</td>
<td>3.6 ± 0.5 NS</td>
<td></td>
<td>3.7 ± 0.6</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND = not determined; NS = not significant.

*Type of treatment and the day of biopsy was the same as in Table II.

Fig. 3. Progesterone receptor concentration located in the stroma, with faint staining in the glandular cells in a control cycle (left), and intense following treatment with 200 mg RU486 on day luteinizing hormone (LH)+2 (right). After treatment, the staining pattern of the stroma was similar to control, while the glandular staining was different, exhibiting a moderate to strong epithelial cell staining. The endometrial specimens were obtained on cycle day LH+7 in both cycles. Microscope magnification X200.
Table IV. *Dolichos biflorus* agglutinin (DBA) staining intensity in the human endometrium

<table>
<thead>
<tr>
<th>Cellular location</th>
<th>Type of cycle and labelling score (mean ± SD, and probability)</th>
<th>RU486 200 mg</th>
<th>RU486 400 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n = 10)</td>
<td>RU486 200 mg (n = 10)</td>
<td>RU486 400 mg (n = 4)</td>
</tr>
<tr>
<td>Luminal epithelium</td>
<td>2.4 ± 1.1, P &lt; 0.01</td>
<td>1.3 ± 0.7, ND</td>
<td>1.5 ± 1.0, ND</td>
</tr>
<tr>
<td>Glandular epithelium</td>
<td>3.6 ± 0.5, P &lt; 0.01</td>
<td>1.5 ± 0.5, ND</td>
<td>1.0 ± 0.8, ND</td>
</tr>
<tr>
<td>Stroma</td>
<td>1.7 ± 0.5, P &lt; 0.01</td>
<td>1.1 ± 0.6, ND</td>
<td>1.4 ± 0.8, ND</td>
</tr>
</tbody>
</table>

ND = not determined.

*Type of treatment and day of biopsy was the same as in Table II.

Fig. 4. Staining of glandular secretory components with *Dolichos biflorus* agglutinin (DBA) lectin in a control cycle (left) and following treatment with 200 mg RU486 on day luteinizing hormone (LH)+2 (right). After treatment there was a significant reduction in the DBA staining (*P* < 0.01). Both endometrial specimens were obtained on cycle day LH+7. Microscope magnification ×200.

The immunohistochemical scoring of DBA staining in cellular compartments of the endometrium is summarized in Table IV. Staining of glandular secretory components with DBA lectin in biopsy specimens obtained in the control cycle revealed a strong staining, indicating a normal secretory activity of the endometrium (Figure 4, left panel). In all endometrial specimens examined after RU486 treatment there was a reduction in the DBA staining (Figure 4, right panel). This indicates that a dose of 200 mg RU486 administered at LH+2 causes an inhibition of the secretory transformation of the endometrium.

Discussion

The aim of this study was to evaluate further the effect of immediate post-ovulatory treatment with RU486 on the endometrial maturation in healthy women at the time of implantation. The results of this study confirm that treatment with RU486 immediately after ovulation may, in a few women, cause slight bleeding for 2–3 days but does not influence the length of the menstrual cycle or the duration of bleeding even if the dose of RU486 is increased to 400 mg (Swahn et al., 1990; Gemzell-Danielsson et al., 1993).

Traditionally, the criteria of Noyes et al. (1950) have been the standard method for histological dating of the endometrium. However, the clinical usefulness of histology for the assessment of luteal function has been found to be disappointing (Balasch et al., 1992). More recently, morphometric analysis, a quantitative and objective histological technique, has been used to study the receptive endometrium and endometrial function (Johannisson et al., 1982; Dockery et al., 1988a,b, 1990; Li et
Also, immunohistochemistry of PgR and oestradiol receptors (ER) has been found to be of value for endometrial dating. García et al. (1988) suggested that the disappearance of both PgR and ER in the mid- and late luteal phase represents the effect of progesterone and may therefore be used as an index of the cumulative activity of progesterone during the luteal phase.

We have reported previously that following treatment with 200 mg RU486 on day LH+2, the mean values for progesterone and oestradiol in the secretory phase were slightly but not significantly elevated in comparison with values during the control cycle (Swahn et al., 1990). An increase could also be observed in the present study. Moreover, it seemed slightly more pronounced for progesterone and also reached statistical significance at one point. Elevated progesterone concentrations have been reported previously by Garzo et al. (1988) when RU486 was administered in the mid-luteal phase of the menstrual cycle.

As we and others have shown, post-ovulatory treatment with RU486 will significantly inhibit endometrial development. In this study, a significant decreased glandular diameter and an increased number of glandular and stromal mitoses were found in the endometrial biopsies taken on days LH+6 to LH+8. A desynchronization of the endometrium has also been reported by Berthois et al. (1991) when 10 mg RU486 was given daily for 4 days starting on the day of ovulation, and by Greene et al. (1992) who gave the same dose of RU486 (10 mg) on days LH+5 and LH+8 with biopsies taken 24 h later. Together, these studies and that of Swahn et al. (1990), in which endometrial biopsies were taken at different times following a single treatment with RU486, indicate that the effect of a single dose of RU486 can be observed already after 12 h and persists for up to 4–6 days.

The significance of the unopposed oestriol effect on the endometrium, as expressed by the increased number of glandular mitoses, has to be evaluated further. However, the undisturbed rhythm of the menstrual cycle with monthly shedding of the endometrium might prevent endometrial hyperstimulation and carcinogenesis.

In this study, the glandular PgR staining was significantly more pronounced in the treatment than in the control cycle. These results are in agreement with Berthois et al. (1991) and indicate that the normal progesterone-dependent down-regulation of PgR in epithelial cell nuclei during the luteal phase is inhibited by RU486.

The staining of secretory components with DBA lectin in the control cycle revealed a strong staining of the glands, indicating secretory activity of the endometrium. The proliferative phase endometrium shows no DBA staining (Wu et al., 1993). After treatment with RU486, the DBA labelling was decreased significantly, indicating an inhibition of the normal secretory transformation that occurs in the endometrium in response to progesterone. Graham et al. (1991) studied the effect of RU486 (5–200 mg) on the appearance of a specific secretory glycan detected by a monoclonal antibody, D9B1. A single dose of RU486 was administered on days LH+2 to LH+6, an endometrial biopsy was obtained on the same day, and a second biopsy 3 days later. Their tissues were fixed in glutaraldehyde and embedded in JB4 resin, while we used frozen sections and acetone fixation to ensure a maximum antigen preservation.

Graham et al. (1991) concluded that the inhibitory effect of RU486 on the endometrial secretory capacity was more pronounced if RU486 was administered early in the secretory phase. That RU486 does inhibit the secretory transformation is supported further by the decrease reported previously in the plasma concentration of placenta protein (PP14) observed following administration of RU486 in the luteal phase of the cycle (Swahn et al., 1993).

We conclude that the profound effects of RU486 on endometrial development and secretory activity are most likely the underlying reason for the contraceptive effect of RU486 when administered immediately following ovulation. The exact mechanism responsible for the disturbed progesterone-dependent embryo–endometrial interaction awaits further clarification.

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References


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