Effects of luteal phase administration of mifepristone (RU486) and prostaglandin analogue or inhibitor on endometrium in the rhesus monkey

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Introduction

Progesterone is essential for the secretory differentiation of the endometrium, successful implantation and the establishment of pregnancy. Thus, the use of anti-progestins during the pre- and peri-implantation period has been suggested as a new approach to fertility regulation (Van Look and von Hertzen, 1994). Administration of mifepristone (RU486), a potent anti-progestin, on day 2 after ovulation has been shown to inhibit implantation and the establishment of pregnancy without marked changes in menstrual cyclicity and ovarian steroid hormone profiles (Danielsson et al., 1993; Ghosh and Sengupta, 1993). Also, post-coital treatment of women with RU486 inhibited implantation when given within 72 h of unprotected intercourse (Glasier et al., 1992; Webb et al., 1992); RU486 also inhibited implantation when given on cycle days 16, 17 and 18 of mated monkeys (Nayak et al., 1997). However, the underlying mechanism of the anti-gestatory action of luteal phase mifepristone is not very clear.

Luteal phase administration of mifepristone has been reported to induce several changes in the progesterone dominated endometrium, such as desynchronization of endometrial maturation, delay in endometrial glandular secretory differentiation, inhibition of glandular leukaeimia inhibitory factor (LIF) expression and changes in vascular compartments in women and in monkeys (Li et al., 1988a; Swahn et al., 1990; Danielsson et al., 1994, 1997; Ghosh et al., 1996). It has been suggested that prostaglandins (PG) may be involved in the anti-gestatory action of mifepristone (Smith, 1991; Psychoyos et al., 1994, 1995; Ghosh and Sengupta, 1996). Generally, it is considered that endometrial PG play an important role in the process of implantation, as treatment with a PG biosynthesis inhibitor prevents or delays implantation (Smith, 1991). However, the nature of its involvement is not clear. In-vitro exposure of human endometrial cells to anti-progestin has been shown to stimulate endogenous PG production and to inhibit PG catabolism (Kelly et al., 1986; Smith and Kelly, 1987; Norman et al., 1991). Danielsson and Hamberg (1994), on the contrary, have suggested that early luteal phase administration of RU486 inhibits the release of endometrial PGF_2α. Furthermore, we have recently reported that the anti-nidatory action of RU486 not be accentuated or attenuated with co-administration of PGE or diclofenac, nor could these be mimicked by these agents alone.

Key words: endometrium/mifepristone/oestrogen receptor/progesterone receptor/prostaglandin

Early luteal phase administration of a potent anti-progestin like mifepristone (RU486) inhibits blastocyst implantation and the establishment of pregnancy without marked changes in menstrual cyclicity and ovarian steroid hormone profiles; however, the underlying mechanism is not very clear. In the present study, a hypothesis that prostaglandins (PG) are involved in the anti-gestatory action of luteal phase mifepristone was tested. Endometrial changes in rhesus monkeys were examined following luteal phase administration of mifepristone, a prostaglandin synthesis inhibitor (diclofenac) and a prostaglandin analogue (misoprostol) either alone or in combination. Twenty-five monkeys were randomly assigned to six groups: group 1 (n = 4), normal control group; group 2 (n = 4), mifepristone (2 mg, daily, s.c.) treated group; group 3 (n = 4), diclofenac (25 mg, daily, i.m.) treated group; group 4 (n = 4), misoprostol (100 µg, daily, oral) treated group; group 5 (n = 5), mifepristone and diclofenac (same dosages as for groups 2 and 3) treated group; group 6 (n = 4), mifepristone and misoprostol (same dosages as for groups 2 and 4) treated group. All treatments were given to monkeys on days 16–18 of mated cycles and endometrial tissue samples were collected on day 20. With diclofenac alone (group 3), marginal changes were observed in glandular, stromal and vascular compartments, and there were few apoptotic bodies in gland cells; partial inhibition and delay in implantation was earlier reported. Significantly higher oestrogen receptor expression in glandular epithelial cells as compared with all other treatment groups was found after treatment with misoprostol alone (group 4) and was associated with normal fecundity. The anti-nidatory action of luteal phase antiprogestin treatment alone or in combination with diclofenac or misoprostol was associated with altered endometrial histometric features characterized by glandular apoptosis, regression in secretory functions, decreased oedema, extravasation and a higher degree of stromal leukocytic infiltration. In these three groups (groups 2, 5 and 6) receptors for oestrogen and progesterone receptors were significantly higher in stromal cells, and lower in vascular cells, while glandular cells showed significantly higher progesterone receptors compared with the control group. The anti-nidatory activity of mifepristone and associated endometrial changes could

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Healthy, adult female and male rhesus monkeys (Macaca mulatta) of proven fertility were used in this study. The details of animal selection and management have been described elsewhere (Nayak et al., 1993; Ghosh et al., 1996; Nayak et al., 1997). Briefly, animals showing at least two consecutive cycles of normal length (28–32 days) were used in this study, and were cohabited with males during cycle days 8–19. Daily blood samples were collected to determine the peripheral serum levels of oestrogen and progesterone by radioimmunoassay, as described previously, using antisera and chemicals obtained from the WHO Matched Assay Reagents Programme (Sufi et al., 1984). The study was performed with the approval of the Ethics Committee on Use of Non-Human Primates in Biomedical Research, AIIMS.

**Tissue collection and processing**

Endometrial tissue samples were collected on cycle day 20 from all monkeys by performing laparotomy and fundal hysterotomy following ketamine (12 mg/kg, Ketlar; Parke-Davis, Mumbai, India) immobilization. The presence of a corpus luteum was checked before tissue collection. The procedural details of tissue collection and processing have been described elsewhere (Ghosh et al., 1993b, 1996). Briefly, tissue samples were fixed in phosphate-buffered neutral formaldehyde and embedded in paraffin wax by a routine procedure. Tissue sections (5 μm) were stained with haematoxylin and eosin for histological evaluation and were also used in immunohistochemistry. Endometrial samples were also fixed in 3% glutaraldehyde, post-fixed in osmium tetroxide and embedded in epoxy resin (EMBed 812). Semi-thin (0.5 μm) and thin (70–80 nm) sections were cut and stained as detailed previously (Ghosh et al., 1993b, 1996). The supplies were obtained from Electron Microscopy Sciences (Fort Washington, PA, USA) and Sigma Chemical Company (St Louis, MO, USA). Light and transmission electron microscopy were performed using a Leica microscope and a Phillips transmission electron microscope.

**Morphometric analysis**

Morphometric measurements for different indices in the functionalis zone were performed on at least two blocks from each animal. Five sections from each block were obtained at different planes of the tissue, and five to 10 fields from each section were used for histometric analyses as described by Johannisson et al. (1987), Li et al. (1988b) and Ghosh et al. (1993b, 1996). The indices measured in glandular epithelial and stromal compartments are given in Tables II and III. Evaluation of different indices were made by counting (G1, G6, G7, G8, G12, S3), linear measurement (G3, G4, S1), semi-quantitative measurement (G9, G11, S2, S4, S5), and quantitative two-dimensional measurement (G2, G5, G10) in semi-thin sections. Semi-quantitative measurements were carried out with a four point scoring system from 0 to 3 (0, absent; 1, mild; 2, moderate; and 3, marked). Linear and quantitative two-dimensional measurements were performed using a Leica microscope and a Quantimet-500 C+ image processing and analysis system (Leica, Cambridge, UK).

**Immunohistochemistry of receptors for oestrogen and progesterone**

Immunohistochemical staining of oestrogen and progesterone receptors was performed using a method described by Szekeres et al. (1994) and Cameron et al. (1996). Briefly, tissue sections were deparaffinized and hydrated through graded alcohols to phosphate-buffered saline (PBS). Following quenching of endogenous peroxidase activity, tissue sections were subjected to trypsin digestion (0.05% for 10 min at 4°C) before microwave heating in 0.1 M sodium citrate buffer (pH 6.0) for retrieval of oestrogen receptors. For progesterone receptors, trypsin digestion was performed after microwave heating for antigen retrieval. Mouse monoclonal antisera were used to detect oestrogen receptors (ER1D5; Immunotech, Cedex, France) and progesterone receptors (PR10A9; Immunotech, Cedex, France). Sections were incubated overnight with the primary antibody at a pre-calibrated dilution (1:50) at 4°C. Late proliferative endometrial tissue sections were also included as positive control and mouse IgG was substituted for the primary antibody in sections, which were used as negative controls. Final visualization was achieved using the ABC kit (Vector Laboratories, Burlingame, CA, USA) and freshly made diaminobenzidine hydrochloride (Sigma) with hydrogen peroxide as described previously (Ghosh et al., 1993b). All immunostaining procedures were performed in a single run. Duplicate sections were lightly counterstained with haematoxylin to facilitate the identification of cellular elements. Morphometric assessments of immunopositive areas for oestrogen receptors and progesterone receptors were performed using an image processing and analysis system (Quantimet-500 C+; Leica, Cambridge, UK) and an improved quantification technique described by Esteban et al. (1993). Briefly, total areas of positively stained nuclei and total areas of all nuclei within glandular, stromal, and vascular cells, were recorded separately, and positivity was expressed as a percentage of nuclear area (Esteban et al., 1993).

**Statistical analysis**

Statistical analyses of quantitative measurements were performed using a modified t-test with the inclusion of a correction for continuity when required (Samuels, 1991), and the Kruskal–Wallis Test followed by multiple comparison test (Steel and Torrie, 1980) as appropriate. The probability level of $P = 0.05$ was taken as the limit of significance. The data are shown as means ± SEM.
Ru486 and PG modulators on endometrium

### Results

A total of 25 monkeys was used in six treatment groups in this study. Ovulation occurred in all animals. Retrospective analysis of serum concentrations of oestradiol and progesterone revealed that ovulation occurred between cycle days 9–13, and there was no significant change in the profiles of serum oestradiol and progesterone in different treatment groups (Table I). No significant differences from control were noted in tissue recoveries among control (group 1, 334.6 ± 31.0 mg/animal), diclofenac (group 3, 329.2 ± 69.4 mg/animal), misoprostol (group 4, 269.3 ± 22.9 mg/animal), and mifepristone plus diclofenac (group 5, 195.3 ± 48.1 mg/animal) treated monkeys. However, the amounts of tissue recovered from mifepristone (group 2, 114.0 ± 27.4 mg/animal) and mifepristone plus misoprostol (group 6, 66.0 ± 2.3 mg/animal) treated monkeys were significantly lower than treatment groups 1 (P < 0.01), 3 (P < 0.05), and 4 (P < 0.01). The amount of tissue recovered from the Ru486 plus misoprostol treatment (6) was also significantly (P < 0.05) less than Ru486 plus diclofenac treatment group (5).

Table I shows the morphometric analyses and Figures 1 and 2 show characteristic morphological features of the endometrial glandular and stromal compartments on cycle day 20 from different treatment groups. In the glandular compartment, there was (i) decreased vacuolation following diclofenac and misoprostol treatments as compared with the control group, (ii) a higher degree of supranuclear vacuole migration in misoprostol treatment group relative to the control and diclofenac treatment groups, (iii) decreased secretion in the diclofenac treatment group compared with control and misoprostol treatment groups, and (iv) a marginal increase in glandular apoptosis following diclofenac treatment. However, no marked changes were observed in the histology and electron microscopic morphology of stroma and blood vessels following different treatments. A high degree of oedema, vessel congestion, and moderate rounding up of stromal cells in endometria from control and misoprostol treatments relative to diclofenac treatment were also observed.

Mifepristone administration resulted in significant decreases in G2, G3, G8, and G10, as well as significant increases in G5, G9, and G12 compared to control, diclofenac and misoprostol treatments (Table II). The amount of vacuolated cells and the amount of secretion were also reduced following Ru486 treatment compared to the control. Co-administration of diclofenac and Ru486 did not induce any additional change in glandular histology as compared to Ru486 treatment alone, except for a significant increase in the number of apoptotic bodies in glandular epithelium. In contrast, co-treatment of PGE analogue and Ru486 (group 6) induced discernible changes (increases in G2, G3, G8, and G10 and decreases in G5 and G7) in glands compared with Ru486 alone (group 2) or Ru486 plus diclofenac treatment (group 5), and numbers of apoptotic bodies were significantly lower as compared to Ru486 plus diclofenac treatments (group 5).

As shown in Table III, the amounts of leukocytic infiltration (S4) and extravasation (S5) were significantly increased with a reduction in the amount of oedema (S2) in endometrial samples from all groups exposed to Ru486 (groups 2, 5 and 6) compared with control, diclofenac, and misoprostol treated endometria. Inhibition of PG synthesis by diclofenac following Ru486 treatment significantly reduced the amount of leukocytic infiltration and extravasation. On the other hand, treatment with a PGE analogue following mifepristone treatment resulted in decreases in venous diameter, and the amount of oedema compared with Ru486 treatment alone. When comparing Ru486 plus diclofenac (group 5) and Ru486 plus misoprostol (group 6) treatment groups, venous capillary diameter and amount of oedema were found to be significantly reduced, and there was an increase in leukocytic infiltration and extravasation in group 6 compared with group 5.

At the ultrastructural level, a higher degree of leukocytic infiltration and extravasation, desquamation of surface epithelia, vascular stasis, features of contraction, and occasional morphological disintegration in vascular structure were evident in many Ru486 exposed endometrial samples (groups 2, 5 and 6). These features were absent in control, diclofenac, and

### Table I. Serum concentrations of oestradiol-17β and progesterone (nmol/l) in different treatment groups during cycle days (cd) 16–20

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Oestradiol-17β</th>
<th>Progesterone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cd16</td>
<td>cd18</td>
</tr>
<tr>
<td>Control</td>
<td>0.61</td>
<td>0.52</td>
</tr>
<tr>
<td>Mifepristone</td>
<td>±0.07</td>
<td>±0.05</td>
</tr>
<tr>
<td>(group 2)</td>
<td>0.63</td>
<td>0.60</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>±0.07</td>
<td>±0.07</td>
</tr>
<tr>
<td>(group 3)</td>
<td>0.53</td>
<td>0.52</td>
</tr>
<tr>
<td>Misoprostol</td>
<td>±0.07</td>
<td>±0.04</td>
</tr>
<tr>
<td>(group 4)</td>
<td>0.62</td>
<td>0.60</td>
</tr>
<tr>
<td>Mifepristone+</td>
<td>±0.08</td>
<td>±0.07</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>±0.06</td>
<td>±0.09</td>
</tr>
<tr>
<td>(group 5)</td>
<td>0.52</td>
<td>0.70</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>±0.09</td>
<td>±0.07</td>
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</table>

No significant changes among groups.
misoprostol treatment groups (groups 1, 3 and 4) and were relatively less frequent in endometrial samples following diclofenac plus RU486 treatment; however, no quantitative comparison was performed in this regard.

As shown in Table IV, morphometric assessment of immunostaining demonstrated highly significant increases in staining for oestrogen receptors (Figure 3) and progesterone receptors (Figure 4) in endometrial stromal cells following treatment with RU486 with or without diclofenac or misoprostol (groups 2, 5 and 6) compared with control (group 1), diclofenac (group 3), and misoprostol (group 4) treatments. No significant differences in immunopositive staining for oestrogen receptors and progesterone receptors in stroma were detected among treatment groups 1, 3, 5 and 6, respectively; corresponding uppercase letters denote the level of significance at 1% ($P < 0.01$).

The number of mitoses/1000 stromal cells (S3) was not detectable in any group.

The number of apoptotic ND B 29.2 cDe 7.9 Ef ND EF 53.6 Af 34.8 A bodies/1000 gland cells (G11) was detectable in control (group 1), diclofenac (group 3), and mifepristone (group 4) treatments. No significant differences in immunopositive staining for oestrogen receptors and progesterone receptors in endometrial glands were detected among treatment groups 1, 3, and 4, or treatment groups 2, 5, and 6.

Table II. Morphometric analysis of endometrial glands (mean ± SEM) on cycle day 20 in control (group 1), mifepristone (RU486, group 2), diclofenac (group 3), misoprostol (group 4), mifepristone plus diclofenac (group 5), and mifepristone plus misoprostol (group 6) treated monkeys

<table>
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<tr>
<th>Index</th>
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<th>Group 4</th>
<th>Group 5</th>
<th>Group 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of vacuolated glands/mm$^2$, G1</td>
<td>35.2 ± 3.2</td>
<td>30.2 ± 2.6</td>
<td>33.1 ± 2.8</td>
<td>31.0 ± 2.0</td>
<td>35.4 ± 2.8</td>
<td>35.6 ± 1.4</td>
</tr>
<tr>
<td>Volume fraction of endometrium occupied by glands (%; G2)</td>
<td>25.7$^B$ ± 19.0$^B$</td>
<td>26.3$^E$ ± 24.2$^E$</td>
<td>18.4$^AF$ ± 18.4$^AF$</td>
<td>25.8$^B$ ± 25.8$^B$</td>
<td></td>
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<tr>
<td>Average diameter of glands (μm; G3)</td>
<td>95.9$^B$ ± 85.1$^CD$</td>
<td>101.3$^EF$ ± 101.3$^EF$</td>
<td>79.9$^AF$ ± 79.9$^AF$</td>
<td>94.9$^B$ ± 94.9$^B$</td>
<td></td>
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<tr>
<td>Number of supranuclear bodies/1000 gland cells (G4)</td>
<td>61.6$^B$ ± 69.7$^CD$</td>
<td>60.5$^E$ ± 63.1$^E$</td>
<td>68.4$^AF$ ± 68.4$^AF$</td>
<td>62.7$^B$ ± 62.7$^B$</td>
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<tr>
<td>Number of vacuolated gland cells/1000 gland cells (G5)</td>
<td>361.2$^B$CD ± 151.7</td>
<td>158.7$^EF$ ± 158.7$^EF$</td>
<td>137.7$^AF$ ± 137.7$^AF$</td>
<td>66.6$^B$ ± 66.6$^B$</td>
<td></td>
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<tr>
<td>Number of mitoses/1000 gland cells (G6)</td>
<td>65.2 ± 26.3</td>
<td>29.1 ± 13.2</td>
<td>18.1 ± 18.1</td>
<td>22.3 ± 5.6</td>
<td>20.3 ± 7.2</td>
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<tr>
<td>Degree of pseudo-stratification (score, G9)</td>
<td>0.7$^b$ ± 1.4$^d$</td>
<td>0.8$^c$ ± 0.8$^c$</td>
<td>1.1$^a$ ± 1.1$^a$</td>
<td>0.9$^b$ ± 0.9$^b$</td>
<td></td>
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<tr>
<td>Volume fraction of endometrium occupied by lumen (%; G10)</td>
<td>0.7$^d$ ± 0.1</td>
<td>0.1 ± 0.1</td>
<td>0.8$^a$ ± 0.8$^a$</td>
<td>0.5 ± 0.5</td>
<td>0.6 ± 0.6</td>
<td></td>
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<tr>
<td>Amount of secretion (score, G11)</td>
<td>1.0$^bc$ ± 0.6$^b$</td>
<td>0.7$^d$ ± 0.7$^d$</td>
<td>1.1$^EF$ ± 1.1$^EF$</td>
<td>0.6$^A$ ± 0.6$^A$</td>
<td>0.8$^a$ ± 0.8$^a$</td>
<td></td>
</tr>
<tr>
<td>Number of apoptotic bodies/1000 gland cells (G12)</td>
<td>ND$^B$</td>
<td>ND$^B$</td>
<td>29.2$^DE$</td>
<td>7.6$^F$</td>
<td>ND$^EF$</td>
<td>53.6$^AF$</td>
</tr>
</tbody>
</table>

ND, non-detectable. a, b, c, d, e and f denote significant ($P < 0.05$) differences from group 1, 2, 3, 4 and 6, respectively; corresponding uppercase letters denote the level of significance at 1% ($P < 0.01$).

The number of mitoses/1000 gland cells (G6) was not detectable in any group. G1–G12 identify the different indices evaluated; how they were measured is described in Materials and methods.

Table III. Morphometric analysis of endometrial stroma (mean ± SEM) on cycle day 20 in control (group 1), mifepristone (RU486, group 2), diclofenac (group 3), misoprostol (group 4), mifepristone plus diclofenac (group 5), and mifepristone plus misoprostol (group 6) treated monkeys

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</thead>
<tbody>
<tr>
<td>Diameter of venular capillary (μm, S1)</td>
<td>18.5 ± 2.0</td>
<td>17.2 ± 0.4</td>
<td>17.9$^F$ ± 0.3</td>
<td>18.6$^EF$ ± 0.5</td>
<td>16.8$^df$ ± 0.3</td>
<td>15.6$^AB$</td>
</tr>
<tr>
<td>Amount of oedema (score, S2)</td>
<td>2.0$^b$ ± 1.1$^d$</td>
<td>1.6$^P$ ± 2.0$^F$</td>
<td>1.2$^af$ ± 0.7$^ab$</td>
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</tr>
<tr>
<td>Amount of leukocytes (score, S3)</td>
<td>0.7$^B$ ± 0.1</td>
<td>1.9$^CD$ ± 0.9$^F$</td>
<td>0.5$^EEF$ ± 1.1$^af$</td>
<td>2.1$^A$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infiltration (score, S4)</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.1</td>
<td>0.06 ± 0.1</td>
<td>0.1 ± 0.1</td>
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<tr>
<td>Amount of extravasation (score, S5)</td>
<td>ND$^B$ ± 0.8$^DE$</td>
<td>ND$^EF$ ± 0.4$^d$</td>
<td>0.9$^A$ ± 0.9$^A$</td>
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</table>

ND, non-detectable. a, b, c, d, e and f denote significant ($P < 0.05$) differences from group 1, 2, 3, 4 and 6, respectively; corresponding uppercase letters denote the level of significance at 1% ($P < 0.01$).

The number of mitoses/1000 stromal cells (S3) was not detectable in any group.
RU486 and PG modulators on endometrium

Glandular cells showed higher oestrogen receptor staining (Figure 3D) following misoprostol treatment (group 4) compared with control (group 1), diclofenac (group 3) and RU486 exposed endometria (groups 2, 5, and 6). However, progesterone receptor immunostaining in glands was significantly higher in group 2 (Figure 4B), group 5 (Figure 4E), and group 6 (Figure 4F) when compared with control group (Figure 4A). The percentage of immunostained area for progesterone receptors in the glandular epithelium of treatment groups 2 (Figure 4B) and 6 (Figure 4F) was also significantly higher than that of group 3 (Figure 4C). Small arteries present in the endometrium functionalis also showed positive immunostaining for oestrogen receptors and progesterone receptors, although a high degree of heterogeneity was observed (Figure 5). Occasionally, perivascular cells of capillaries showed distinct nuclear labelling for oestrogen receptors and progesterone receptors. Contrary to the findings in stroma and glands, morphometric analysis (Table IV) revealed a significant decrease in the percentage of stained area for both oestrogen receptors and progesterone receptors in arteries after treatment with RU486 (groups 2, 5, and 6) compared with control, diclofenac, and misoprostol treatment groups. However, no significant differences in immunostaining for oestrogen receptors and progesterone receptors were detected among treatment groups 1, 3, and 4, or treatment groups 2, 5, and 6.

Discussion

A single dose of mifepristone (RU486) on day LH+2 causes repression of secretory transformation in the endometrium functionalis (Swahn et al., 1990; Danielsson et al., 1994, 1997; Ghosh et al., 1996), with resultant pregnancy protection in women and in rhesus monkeys (Danielsson et al., 1993; Ghosh and Sengupta, 1993). Subsequently, it has been reported that the window for the application of mifepristone can be wider; a single dose of the antiprogestin (2 mg/kg body weight) given either on cycle day 16 or on cycle day 20 to rhesus monkeys was effective in preventing pregnancy (Ghosh et al., 1994). However, at a lower dose (1 mg/kg body weight) RU486 was found to be only partially effective (Ghosh et al., 1993a). By contrast, mifepristone administered over a period of 3 days in divided doses (i.e., 2 mg/day) on cycle days 16–18 provided 100% protection against pregnancy (Nayak et al., 1997). Using this dose schedule in the present study, we report discernible changes in the glandular and stromal compartments of the endometrium on cycle day 20 of preimplantation stage of gestation in the rhesus monkey.

Following mifepristone treatment, endometrial glands exhibited an inhibition of secretory differentiation characterized by a reduction in secretion, diameter and vacuolation. It is likely that retardation of secretory differentiation of glands in mifepristone treated groups resulted in overcrowding of cells.

Figure 1. Morphology of endometrial gland in control (A), mifepristone (B), diclofenac (C), misoprostol (D), mifepristone plus diclofenac (E) and mifepristone plus misoprostol (F) treated monkeys. All endometrial samples were collected on day 20 of ovulated, mated cycles. Bars, 20 (A), 15 (B, C) and 10 (D, E, F) μm.

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Figure 2. Morphology of endometrial stroma in control (A), mifepristone (B), diclofenac (C), misoprostol (D), mifepristone plus diclofenac (E) and mifepristone plus misoprostol (F) treated monkeys. Microhaematoma and leukocytic egression are evident in mifepristone exposed endometrial stroma (B, E, F). All endometrial samples were collected on day 20 of ovulated, mated cycles. Bars, 10 (A, B, C, F), 5 (D) and 15 (E) µm.

Table IV. Morphometric analyses (mean ± SEM) of immunohistochemical staining of oestrogen receptors and progesterone receptors in endometrium on cycle day 20 following different treatments*

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<th>Group 6</th>
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<tbody>
<tr>
<td>Immunopositive oestrogen receptors (nuclear area, %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Gland cells</td>
<td>46.1±D</td>
<td>53.0±d</td>
<td>49.4±d</td>
<td>69.5±e</td>
<td>58.1±</td>
<td>47.3±</td>
</tr>
<tr>
<td>±5.8</td>
<td>±5.8</td>
<td>±6.5</td>
<td>±3.6</td>
<td>±3.7</td>
<td>±7.1</td>
<td></td>
</tr>
<tr>
<td>Stromal cells</td>
<td>50.1±B</td>
<td>72.5±c</td>
<td>54.7±e</td>
<td>49.1±F</td>
<td>67.9±</td>
<td>63.7±A</td>
</tr>
<tr>
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<td>±4.6</td>
<td>±3.9</td>
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<td>±2.8</td>
<td>±1.8</td>
<td></td>
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<tr>
<td>Vascular cells</td>
<td>41.4±b</td>
<td>31.8±C</td>
<td>50.7±EF</td>
<td>41.3±EF</td>
<td>28.7±A</td>
<td>25.3±A</td>
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<tr>
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<td>±4.2</td>
<td>±1.4</td>
<td>±3.4</td>
<td>±4.3</td>
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<tr>
<td>Immunopositive progesterone receptors (nuclear area, %)</td>
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<tr>
<td>Gland cells</td>
<td>35.3±B</td>
<td>58.1±C</td>
<td>42.5±f</td>
<td>48.8±</td>
<td>52.3±A</td>
<td>53.7±A</td>
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<td>±6.7</td>
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<tr>
<td>Stromal cells</td>
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<td>79.5±CD</td>
<td>66.7±EF</td>
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<td>74.3±</td>
<td>80.0±A</td>
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<td>±1.9</td>
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<tr>
<td>Vascular cells</td>
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</table>

*See Table II for group definitions. a, b, c, d, e and f denote significant (P < 0.05) difference from group 1, group 2, group 3, group 4, group 5 and group 6, respectively; corresponding upper case letters denote the level of significance at 1% (P < 0.01).

giving rise to observed increases in the volume fraction occupied by gland cells and pseudostratification in these groups (Li et al., 1988a). Also, a subset of glands showed apoptosis with focalized epithelial desquamization. Similar changes have been reported earlier in women and in rhesus monkeys (Li et al., 1988a; Swahn et al., 1990; Danielsson et al., 1994; Ghosh et al., 1996).

Since uterine hormone receptors are suggested to be primarily regulated by oestrogen and progesterone, endometrial oestrogen receptor and progesterone receptor measurements...
Figure 3. Immunohistochemical localization of oestrogen receptors in endometrial samples from control (A, bar, 50 µm), mifepristone (B, bar, 25 µm), diclofenac (C, bar, 50 µm), misoprostol (D, bar, 25 µm), mifeprifione plus diclofenac (E, bar, 50 µm) and mifepristone plus misoprostol (F, bar, 50 µm) treated animals. A higher degree of nuclear staining in glandular epithelium is evident in misoprostol (D) exposed endometrium, while stromal staining is higher in mifepristone treated endometrial samples (B, E, F).

Figure 4. Immunohistochemical localization of progesterone receptor (progesterone receptors) in endometrial samples from control (A, bar, 50 µm), mifepristone (B, bar, 25 µm), diclofenac (C, bar, 50 µm), misoprostol (D, bar, 50 µm), mifepristone plus diclofenac (E, bar, 50 µm) and mifepristone plus misoprostol (F, bar, 50 µm) treated animals. A higher degree of nuclear staining in glandular epithelium and stromal cells evident in mifepristone treated endometrial samples (B, E, F).

Figure 5. Immunohistochemical localization of oestrogen receptors (A–C) and progesterone receptors (D, E) in blood vessels of endometrial samples collected from control (A, D), mifepristone (B, E) and diclofenac (C) treated animals. Vascular muscle cells, pericytes and cuff decidual cells and occasionally endothelial cells are immunopositive. The degree of immunopositive precipitation is less in mifepristone (B, E) treated endometrium. Bar, 20 µm.
may help to define the state of endometrial maturation (Bouchard et al., 1990). The disappearance of both oestrogen receptors and progesterone receptors in the mid- to late-luteal phases represents a progesterone mediated response and may therefore be used as an index of cumulative activity of progesterone during the luteal phase (Garcia et al., 1988). Post-ovulatory administration of anti-progesterin has been shown to inhibit progesterone-dependent down-regulation of steroid receptors in both glandular and stromal cells (Berthois et al., 1991; Maentausta et al., 1993; Danielsson et al., 1994; Cameron et al., 1996). In the present study we report higher progesterone receptors in glandular and stromal cells and higher oestrogen receptors in stromal cells and an apparent increase of oestrogen receptor immunopositive staining in the glandular epithelium following RU486 treatment. Diclofenac treatment alone did not influence oestrogen receptor and progesterone receptor expression in glandular and stromal cells. The PGE analogue, misoprostol, in contrast, enhanced oestrogen receptor immunopositive staining only in glandular epithelium with a higher tendency for progesterone receptor expression. Interestingly, secretory activity in glands following misoprostol treatment was also stimulated and we speculate that it is mediated through an up-regulation of glandular cell oestrogen receptor and progesterone receptors. Co-administration of mifepristone with a PG synthesis inhibitor (diclofenac) or a PGE analogue (misoprostol) failed to alter the expression of steroid receptors (oestrogen receptors and progesterone receptors) in glandular, stromal and vascular cell types of the endometrium. Generally, a higher degree of oestrogen receptor immunopositivity was detected in glands compared with progesterone receptor staining in all groups. Taken together, it appears that oestrogen receptors and progesterone receptors are differentially regulated in epithelial and stromal compartments during the luteal phase (Ferenczy, 1994).

Mifepristone is known to induce vascular changes in progesterone dominated primate endometrium. Administration of this drug to women and monkeys in the post-ovulatory phase of the cycle has been shown to cause endometrial haemorrhage associated with vascular stasis, vasoconstriction, vasoregression and enhanced leukocytic infiltration (Li et al., 1988a; Johannisson et al., 1989; Ghosh et al., 1996). In the present study, a decrease in diameter in a small population of venular capillaries following mifepristone treatment was observed. These changes consequent to mifepristone treatment might affect blood flow in the upper functionalis of endometrium. Consistent with such previous reports, in spite of a very low dose of mifepristone exposure, we found a significant decrease in the degree of oedema, and an increase in the amount of extravasation and leukocytic infiltration.

Post-ovulatory luteal phase endometrium is marked by arteriolar growth mainly in the sub-epithelial zone (Shephard and Bonnar, 1980; Johannisson, 1990) and this is considered to be a progesterone mediated event (King and d’Arcangues, 1992); and oestrogen receptors and progesterone receptors are expressed in media and adventitia of arterial walls (Horwitz and Horwitz, 1982; Perrot-Appalan et al., 1988). In this study, RU486 exposure led to significantly reduced oestrogen receptor and progesterone receptor expression in the endometrial vascular bed. Thus, the endometrial vasculature in progesterone exposed endometrium is one of the primary targets of RU486 action.

Evidence from various studies suggests that PG play a cardinal role in endometrial vascularity, implantation and decidualization, and that timed application of PG synthesis inhibitor inhibits or delays these processes (Kennedy, 1994). Despite extensive literature on primate endometrial PG synthesis and its in-vivo and in-vitro regulation, precise understanding about the involvement of PG in the process of implantation in primates still remains rudimentary. Indirect evidence indicates that PGE may be involved through a complex network of other cellular signals in the endometrial implantation reaction in primates, including humans (Smith, 1991; Edwards and Brody, 1995; Psychoyos et al., 1995; Ghosh and Sengupta, 1996). In our study conducted in conception cycles of rhesus monkeys a stimulatory action of a PGE analogue on the glandular epithelium was observed as evidenced by a very high degree of supranuclear vacuolation and secretion. Furthermore, inhibition of PG synthesis by diclofenac treatment induced a mild degree of apoptosis and repressed secretory activity in endometrial glands. However, diclofenac or misoprostol treatments alone did not affect the stromal compartment. To our knowledge, similar observation in any primate model has not been reported. Keys and Kennedy (1990) have reported that indomethacin treatment induced degenerative changes in the rat endometrium. We have earlier reported that diclofenac treatment during early- to mid-luteal period results in partial inhibition or delay of implantation (Nayak et al., 1997).

PG may be involved in mediating the anti-implantation action of mifepristone in the luteal phase (Smith, 1990). In the present study, inhibiting PG synthesis by diclofenac treatment along with mifepristone administration further accentuated glandular degeneration with a substantial increase in apoptotic bodies, while treatment with a PGE analogue (misoprostol) along with mifepristone reversed most of the glandular degenerative features following treatment with mifepristone alone. Mifepristone is known to stimulate primarily PGE production by stromal cells and to inhibit PG catabolism in vitro by human secretory phase endometrial cells (Kelly et al., 1986). It has also been reported that mifepristone treatment led to a decline in the PGE/PGF ratio in rat uteri (Peplov, 1993). Thus, inhibition of PG production by diclofenac treatment may attenuate the vasoregressive effect of mifepristone in the endometrium. On the other hand, misoprostol, along with mifepristone, induced menstruation-like changes in the endometrium with constriction of venular capillaries, decreased stromal oedema and increased leukocytic infiltration and extravasation. In fact, there was significant reduction in tissue yield following mifepristone plus misoprostol treatments, and two monkeys in this group exhibited endometrial bleeding on day 20. It has been reported that in women, RU486 along with misoprostol treatment in late luteal phase is potentially useful in inducing menstruation (World Health Organization Task Force on Post-ovulatory Methods of Fertility Regulation, 1995).

In conclusion, the anti-gestatory action of low-dose mifepri-
stone in the early luteal phase of mated cycle in the rhesus monkey involves endometrial desynchronization with discernible changes in glandular, vascular and stromal compartments of the implantation stage endometrium. Inhibition of PG synthesis by diclofenac when co-administered with mifepristone, accentuated such glandular degeneration, while co-administration of a PGE analogue (misoprostol) and mifepristone induced menstruation-like changes in the stroma. However, the endometrial responses to mifepristone were not mimicked either by PGE or diclofenac alone. We suggest that the anti-nidatory activity of mifepristone is most likely mediated through a multifactorial mechanism and the PG milieu in the endometrium could be a single module in this complex mechanism. The preimplantation stage endometrial prostaglandin milieu following luteal phase mifepristone administration with and without diclofenac or misoprostol treatments remains to be explored.

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