Progestin receptor isoforms and prostaglandin dehydrogenase in the endometrium of women using a levonorgestrel-releasing intrauterine system

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This study has examined endometrial tissue in 14 normal women prior to insertion of a levonorgestrel-releasing intrauterine system (LNG-IUS) and thereafter longitudinally for up to 12 months post-insertion. The specific endpoints examined by immunohistochemistry were progesterone receptor (PR) subtypes A + B, oestrogen receptor (ER) and prostaglandin dehydrogenase (PGDH). Two antiprogestosterone receptor antibodies, one specific to PRB subtype and the other to PR subtype A + B, were employed to examine the localization of both PR isoforms. The activity of PGDH, a progestosterone dependent enzyme, was also measured. ER and PR A+B and PR subtype B were significantly down-regulated in glands and stroma in the presence of continuous intrauterine LNG delivery. There was an apparent increase in PR A immunoreactivity in endometrial glands between 6 and 12 months post-insertion. Consistent with down-regulation of both isoforms of PR was reduced glandular PGDH immunostaining following LNG-IUS insertion, and PGDH activity (as measured by metabolism of excess substrate in vitro). Furthermore, PGDH activity, known to be localized in the glands, significantly increased (P < 0.05) at 12 months post-insertion, coinciding with the observed increase in glandular PR A+B immunoreactivity at this time. Since the LNG-IUS suppresses the PRB so strongly, PR A is likely to be the subtype that mediates long term LNG action in the endometrium. PR A is the more suppressed of the two subtypes, and only PR A rises along with PGDH activity. Alterations to normal endometrial morphology and function, e.g. perturbation of normal sex steroid receptor expression, following exposure to high concentrations of local LNG, may play a role in the aetiology of bleeding disorders associated with the LNG-IUS. Further elucidation of local uterine mediators involved in the mechanism of bleeding problems is required. Key words: intrauterine levonorgestrel/PGDH/progestin receptor isoforms

Introduction

All progestogen-only contraceptives, including the levonorgestrel-releasing intrauterine system (LNG-IUS, Mirena, Leiras Oy, Finland) are associated with the problem of break-through bleeding. Even though the LNG-IUS is an extremely effective contraceptive which dramatically reduces menstrual blood loss (Andersson and Rybo, 1990; Milson et al., 1991) its use is associated with frequent and recurrent break-through bleeding in many women. Since the acceptability to women of modern approaches to fertility control, especially hormonal methods, is dependent upon the extent of menstrual bleeding disturbance associated with such methods (Odlind and Fraser, 1990), improvements in break-through bleeding rates with the LNG-IUS are highly desirable.

The aetiology of menstrual aberration associated with progestogen-only contraception is not understood. In fact, there are very limited data on the local effects of levonorgestrel on endometrial physiology. Well documented fluctuations in steroid receptor concentration take place during endometrial exposure to sex steroids in the normal cycle (Sullivan et al., 1988). Briefly, concentrations of glandular progesterone receptor (PR) increase in the follicular phase under the influence of oestradiol and decline following ovulation due to secretion of progesterone. There is no substantial change in endometrial stromal PR expression across the cycle (Snijders et al., 1992) and in first trimester decidua (Shi et al., 1993). Oestrogen receptor concentrations are also maximal in the proliferative phase and decline in both the glandular and stromal compartment in the secretory phase of the cycle (Garcia et al., 1988; Snijders et al., 1992). In an interesting exception, endometrium exposed to long-term systemic LNG delivered by sub-dermal implant (Norplant®) exhibits an increase in immunoreactivity of the stromal progesterone receptor when compared with control endometrium at all stages across the menstrual cycle (Critchley et al., 1993).

There are two distinct subtypes of the human progesterone receptor, PR A and PR B (Clark et al., 1987). PR A subtype is the shorter form, lacking 164 amino acids from the N-terminal fragment (Tung et al., 1993). Our studies of progesterone receptor localization have involved two antibodies: a rabbit polyclonal against PR B isoform and a monoclonal antibody that detects both A + B subtypes. Thus we refer to PR A+B as the receptor detected by antibody that recognizes both subtypes of the PR and PR B as the receptor detected by antibody specific to the B-isoform. It is not possible to raise an antibody specific to the PR A isoform. We assume that PR A is the subtype responsible for positive immunoreactivity when the PR B cannot be detected. Our group has recently described the presence of PR B immunoreactivity in the nuclei of endometrial glandular and stromal cells throughout the normal menstrual cycle and in decidua of early pregnancy. PR subtype B is preferentially expressed in the mid-proliferative phase, and
declines dramatically in the secretory phase. By inference, the PR$_A$ subtype is the subtype responsible for all immunostaining during the secretory phase, premenstrually and immediately post-menstruation. Further, preferential expression of PR subtype A was observed in first trimester decidua (Wang et al., 1998). It is not known whether this differential expression of the two forms of progesterone receptor in endometrium results in different responses to steroid exposure. There are no data on progesterone receptor subtype expression in levonorgestrel-exposed endometrium.

The local release of vasoactive substances such as prostaglandins has been implicated in the contraceptive actions of intrauterine contraceptive devices. The presence of an intrauterine device (IUD) increases the production of prostaglandin E$_2$ (PGE$_2$) in secretory endometrium (Hillier and Kasonde, 1976). In studies of decidua exposed to antigestogens, blockade of the action of progesterone results in a decrease in prostaglandin metabolism and an increase in prostaglandin E, which in turn may facilitate leukocyte traffic in the endometrium. The interaction between progesterone, prostaglandins and leukocyte traffic are of relevance to the contraceptive effects of the IUD. The main prostaglandin metabolizing enzyme, prostaglandin dehydrogenase (PGDH), has been shown to be progesterone dependent (Casey et al., 1980).

This study has therefore examined endometrial tissue in normal women prior to insertion of a levonorgestrel-releasing intrauterine system (LNG-IUS) and thereafter longitudinally for up to 12 months post-insertion in order to ascertain changes in endometrial sex steroid receptor localization and activity of PGDH, a progesterone dependent enzyme, during local delivery of levonorgestrel to the uterine cavity.

Materials and methods

Ethical approval for the study was obtained from Lothian Research Ethics Committee (reference: 1702/94/6/44). Informed consent was obtained from 14 women aged between 32 and 48 years (median age 37 years). All subjects were fertile, had reported regular menstrual cycles (cycle length 25–35 days) and had not used hormonal or intrauterine contraception in the 6 months prior to inclusion in the study. The indication for insertion of the LNG-IUS was either for contraception (n = 10) or heavy menstruation (n = 4). The study was longitudinal, with each subject acting as her own control. All subjects underwent a pre-insertion endometrial biopsy either in the proliferative (n = 7) or secretory (n = 7) phase of the cycle, immediately after which the LNG-IUS was inserted. The stage of the cycle prior to insertion of the LNG-IUS was defined with reference to serum sex steroid concentrations and histological dating, according to the criteria of Noyes et al. (1950). Biopsies were performed in an outpatient setting with a Pipelle suction curette (Laboratoire CCD, Paris, France). Further endometrial samples were collected 1, 3, 6 and 12 months following insertion of the LNG-IUS. Since histological appearances were indistinguishable in the follicular or luteal phase, once the LNG-IUS was in situ, data at each time period (i.e. 1, 3, 6 or 12 months) post-IUS insertion were pooled regardless of cycle stage. All endometrial tissue samples were fixed overnight in 10% neutral buffered formalin at 4°C, rinsed and stored in 70% ethanol and thereafter routinely wax embedded. Sections 5 µm thick were cut for routine histopathology (haematoxylin and eosin staining) and immunohistochemistry (progesterone receptor, subtypes A + B, progestin receptor subtype B, oestrogen receptor and PGDH immunolocalization). Tissue was also transported to the laboratory in ice-cold RPMI 1640 medium for measurement of PGDH activity in the endometrial cytosol. A venous blood sample was collected at the time of biopsy. Serum was separated and frozen at −20°C for subsequent radioimmunoassay of oestradiol and progesterone (Yong et al., 1992). The inter-assay coefficients of variation (CV) for these assays were 11.0 and 10.0% respectively; intra-assay CV were 8.0 and 8.0% respectively.

In-vitro measurement of PGDH activity

Tissue was washed in ice-cold saline, weighed, chopped with scissors and homogenized for 20 s in 0.5 ml of ice cold metabolism buffer [20% glycerol, 2 mM diithiothreitol (Sigma, Dorset, UK) in 0.1 M phosphate buffer, pH 8.4]. Homogenate was centrifuged at 2000 g in a refrigerated centrifuge at 4°C and supernatant was stored at −40°C until assessed. PGDH activity in all samples was determined at the same time.

Frozen supernatant was thawed on ice and diluted in metabolism buffer. Samples of 100 µl of enzyme were incubated in 1 ml metabolism buffer containing 1 mM NAD (Sigma) and 5 µg PGE$_2$. After 30 min, the reaction was stopped by adding 2 ml of methoxyamine hydrochloride (10 mg/ml) in acetate buffer pH 5 to derivatize keto groups to their methyl oxime form. 15-Keto PGE was measured as its methyl oxime by ELISA, using a specific antibody and 15-keto PGE conjugated to horse radish peroxidase.

Immunohistochemistry procedures

All immunohistochemical protocols were optimized to determine the correct conditions for maximal specific staining, and all negative controls displayed absent immunostaining.

Progesterone receptor (subtypes A + B) immunoreactivity with NCL-PGR antibody

Tissue sections were dewaxed and rehydrated in descending grades of alcohol. Sections were washed in distilled water and 0.1 M Tris buffered saline (TBS, pH 7.4–7.6, for 10 min). Non-specific endogenous peroxidase activity was blocked with 3% hydrogen peroxide in distilled water for 5–10 min at room temperature. An antigen retrieval step was performed. Tissue sections were microwaved at high power in 0.01 M sodium citrate buffer (pH 6.0) for 10 min and then allowed to stand in the microwave oven for a further 20 min. After a further wash in buffer, tissue sections were exposed to a 20 min non-immune block using diluted normal horse serum. The primary antibody raised in mouse against human progesterone receptor was used at a dilution of 1:40 (NCL-PGR, recognizing A + B subtypes, Novocastra Laboratories, Newcastle, UK). Following primary antibody binding, the sections were incubated for 30 min at room temperature with biotinylated horse anti-mouse IgG (Vecta stain, PK-4002; Vector Laboratories, Peterborough, UK). Sections were thereafter incubated for 30 min at room temperature with a mixture of avidin and biotin complex coupled to a horseradish peroxidase enzyme (Vecta stain elite, PK 6101; Vector Laboratories). The site of bound enzyme was identified by application of 3,3-diaminobenzidine in H$_2$O$_2$ (DAB Kit, SK-4100; Vector Laboratories). Thereafter tissue sections were washed in distilled water and counterstained with Harris’ haematoxylin, a non-specific nuclear stain. Sections were rinsed with tap water, dehydrated and cleared in xylene before mounting in Pertex (Cellpath, Hemel Hempstead, UK). Negative controls were included by replacing the primary antibody with mouse immunoglobulin (at a matching concentration) at a dilution of 1:6000 in PBS.

Similar protocols were used for localization of progesterone receptor

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semi-quantitative scores of sex steroid receptor immunoreactivity data supports the subsequent statistical analyses performed on the subjective semi-quantitative scoring of immunostaining patterns. Such tissue sections a high correlation (0.963) of objectively measured intense immunoreactivity. We have previously reported in endometrial Progesterone receptor subtype B (PRB) immunohistochemistry features in each immunostaining protocol were as follows.

5 faint immunoreactivity; 2 intensity and localization of immunoreactivity in entire tissue sections.

A semi-quantitative scoring system was employed for assessment of Scoring and immunohistochemistry analysis

PRETREATMENT

Pretreatment of the sections was necessary for localization of the estrogen receptor. Tissue sections were microwaved at high power in 0.01 M sodium citrate buffer (pH 6.0) for 10 min then allowed to rest in the microwave for 20 min. The protocol was thereafter conducted as described above for the progesterone receptor. Primary antibody ER1D5 (DAKO Laboratories, High Wycombe, UK) was used at a dilution of 1:25. Negative controls were included by replacing primary antibody by non-immune mouse immunoglobulin at the equivalent concentrations.

Progesterone receptor subtype B (PRB) immunohistochemistry

No antigen retrieval step was required to expose the epitope. A non-immune block was conducted with normal goat serum. Tissue sections were incubated with a 1:200 dilution of anti progesterone receptor B antibody (rabbit anti-human polyclonal antibody, raised against a 19 amino acid sequence from the N-terminal end, unique to PRB subtype–DQQSLSDVGEYSGRAEATR– and coupled through a C-terminal cysteine to keyhole limpet haemocyanin) in TBS for 60 min at 37°C. Western blotting of endometrial tissue samples collected during the periovulatory phase revealed a strong band at expected molecular mass of 120 kDa (Wang et al., 1998). Primary antibody binding was detected with an avidin–biotin peroxidase detection system. Sections were incubated with a biotinylated goat anti-rabbit IgG antibody made up in dilute normal goat serum for 60 min at room temperature. Thereafter the protocol was as described above for localization of the progesterone receptor. Negative controls were performed by replacing the primary antibody with non-immune serum of the equivalent concentration.

Oestrogen receptor (ER) immunohistochemistry

Pretreatment of the sections was necessary for localization of the estrogen receptor. Tissue sections were microwaved at high power in 0.01 M sodium citrate buffer (pH 6.0) for 10 min then allowed to rest in the microwave for 20 min. The protocol was thereafter conducted as described above for the progesterone receptor. Primary antibody ER1D5 (DAKO Laboratories, High Wycombe, UK) was used at a dilution of 1:25. Negative controls were included by replacing primary antibody by non-immune mouse immunoglobulin at the equivalent concentrations.

Prostaglandin dehydrogenase (PGDH) immunohistochemistry

Detection of PGDH immunoreactivity employed a rabbit polyclonal antibody (Cascade Biochem Ltd, Reading, UK) with an avidin–biotin peroxidase detection system. Endogenous peroxidase activity was quenched with 3% hydrogen peroxide in distilled water for 10 min at room temperature. A non-immune block was conducted using 10% normal swine serum for 20 min at room temperature. Sections were incubated overnight with the primary antibody (dilution 1:500) at 4°C. Biotinylated secondary antibody (biotinylated swine anti-rabbit) and ABC complex (Vecta stain, ABC Kit, PK 4002: Vector Laboratories) were then applied for 30 min each at room temperature. The peroxidase substrate diaminobenzidine (DAB) was used to visualize the reaction (SK4100; Vector Laboratories). Slides were lightly counter stained with Harris haematoxylin. Rabbit immunoglobulin at a dilution of 1:500, i.e. the equivalent concentration as primary antibody, was substituted for negative control sections.

Scoring and immunohistochemistry analysis

A semi-quantitative scoring system was employed for assessment of intensity and localization of immunoreactivity in entire tissue sections. The score of zero indicated an absence of immunoreactivity; 1 = faint immunoreactivity; 2 = strong immunoreactivity and 3 = very intense immunoreactivity. We have previously reported in endometrial tissue sections a high correlation (0.963) of objectively measured immunoreactivity (measured by computerized image analysis) and subjective semi-quantitative scoring of immunostaining patterns. Such data supports the subsequent statistical analyses performed on the semi-quantitative scores of sex steroid receptor immunoreactivity (Wang et al., 1998).

A one-way analysis of variance (ANOVA) with Fisher’s PLSD coefficient was used to evaluate whether or not there were significant differences in the expression of epitopes. The results with a P value of <0.05 or ≤0.01 were considered significant differences.

Results

Prior to insertion of the LNG-IUS, endometrial biopsies conducted in the follicular and luteal phases of the ovarian cycle displayed normal proliferative and secretory features respectively. All subjects in whom biopsies were performed in the follicular phase (proliferative histology) had circulating progesterone concentrations of ≤10 nmol/l (range 0–10, mean 3.4, SEM 1.9). Women in whom biopsies were performed in the luteal phase (secretory histology) had serum progesterone concentrations ranging between 6 and 28.6 nmol/l (mean 17.7, SEM 2.9) (see Table I).

Insertion of a LNG-IUS produced widespread morphological changes in endometrial histology: a pseudo-decidualized stroma with atrophic glands was observed in all biopsies collected following insertion of the LNG-IUS.

\[
PR_{A+B} \text{ immunoreactivity}
\]

Prior to LNG-IUS insertion, all proliferative phase biopsies displayed positive \(PR_{A+B}\) immunoreactivity in both glands and stroma. Secretory phase endometrial biopsies collected prior to IUS insertion showed reduced \(PR_{A+B}\) immunostaining in glands (\(P \leq 0.001\)) but no reduction in stromal \(PR_{A+B}\) immunoreactivity. However, a significant decrease in \(PR_{A+B}\) immunoreactivity was observed in the stromal and glandular compartments of endometrium (see Figure 1a-c) following insertion of a LNG-IUS. Figure 2 illustrates the change in glandular and stromal \(PR_{A+B}\) immunoreactivity in endometrium following insertion of a levonorgestrel IUS.

There were significantly lower (\(P \leq 0.001\)) \(PR_{A+B}\) immunostaining levels in the glandular epithelium 1, 3 and 6 months after insertion of a LNG-IUS compared to progesterone receptor immunoreactivity in biopsies prior to insertion of the

<table>
<thead>
<tr>
<th>Biopsy time</th>
<th>n</th>
<th>Serum oestradiol range mean (SE)</th>
<th>Serum progesterone range mean (SE)</th>
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<tr>
<td>Pre-insertion: proliferative phase</td>
<td>5*</td>
<td>62–343</td>
<td>0–10</td>
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<tr>
<td></td>
<td></td>
<td>145 (53)</td>
<td>3.4 (1.9)</td>
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<tr>
<td>Pre-insertion: secretory phase</td>
<td>7</td>
<td>166–614</td>
<td>6–28.6</td>
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<td></td>
<td></td>
<td>300 (52.7)</td>
<td>17.7 (2.9)</td>
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<tr>
<td>Post-insertion 1 month</td>
<td>11</td>
<td>217–2281</td>
<td>0–23.2</td>
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<tr>
<td></td>
<td></td>
<td>637 (201)</td>
<td>3.7 (1.1)</td>
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<td></td>
<td>13</td>
<td>49–813</td>
<td>0–19.9</td>
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<td>380.7 (105.8)</td>
<td>3.8 (1.1)</td>
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<td>14</td>
<td>69–1929</td>
<td>0–16.3</td>
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<td></td>
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<td>446.2 (120.6)</td>
<td>3.7 (1.0)</td>
</tr>
<tr>
<td>12 months</td>
<td>14</td>
<td>67–1288</td>
<td>0–56.6</td>
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<td>460.4 (124.4)</td>
<td>11.6 (3.1)</td>
</tr>
</tbody>
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* Serum oestradiol and progesterone concentrations were unavailable from two patients whose biopsies prior to IUS insertion were collected in the proliferative phase (hence \(n = 5\)).
Progestin receptor isoforms with intrauterine levonorgestrel

**Figure 1.** Progesterone receptor (PR<sub>A</sub> + PR<sub>B</sub>) and progesterone receptor subtype B stromal and glandular immunoreactivity in endometrium exposed to intrauterine LNG. (a) Strong positive PR (A + B) immunostaining in proliferative endometrium prior to insertion of a LNG-IUS. (b) Reduced endometrial PR (A + B) immunostaining 1 month post-insertion of a LNG-IUS. (c) PR (A + B) immunostaining 12 months post-LNG-IUS insertion. Note increased immunostaining in glands and stroma. (d) Strong positive PR<sub>B</sub> immunoreactivity in proliferative endometrium prior to LNG-IUS insertion. (e) Low intensity PR<sub>B</sub> immunostaining 1 month post-insertion of a LNG-IUS. (f) Persistent low PR<sub>B</sub> immunoreactivity 6 months following insertion of a LNG-IUS. Scale bar for a, b, d, e (shown in d) = 50 µm. Scale bar for c and f (shown in f) = 25 µm.

Intrauterine system collected in the proliferative phase. These levels were equivalent to those seen in the luteal phase. However, between 6 and 12 months following insertion, there was an increase in PR<sub>A+B</sub> immunostaining in endometrial glands (P < 0.05). In the endometrial stroma, PR<sub>A+B</sub> immunostaining was also significantly decreased 1, 3, and 6 months following insertion of the LNG-IUS when compared to pre-insertion stromal immunoreactivity (P < 0.05), but, unlike the glands, there was no increased staining evident in the 12 month samples.

**PR<sub>B</sub> immunoreactivity**

PR subtype B immunostaining was evident in glands and stroma in proliferative phase biopsies prior to IUS insertion, although immunostaining was much less intense than when an antibody recognizing A + B subtypes was employed. Glandular PR subtype B immunoreactivity was significantly reduced in the secretory phase (P < 0.01) but persisted in the stromal compartment of secretory phase biopsies collected pre-IUS insertion. Figure 1d–f and Figure 3 illustrate a decrease or absence of progesterone receptor subtype B immunoreactivity in both glandular and stromal (P < 0.01) compartments of endometrium 1, 3, 6 and 12 months following insertion of a LNG-IUS, with no indication of an increase in immunostaining in the 12 month samples.

**Oestrogen receptor (ER) immunoreactivity**

ER immunoreactivity was also confined to the nuclei of endometrial glands and stromal tissue. Positive strong immunostaining was displayed in glands and stroma of proliferative phase biopsies prior to LNG-IUS insertion. Pre-insertion biopsies collected in the secretory phase displayed reduced glandular (P < 0.05) and stromal immunoreactivity (not significant). There was a significant fall (P < 0.001) in oestrogen receptor
immunoreactivity in the glandular compartment of endometrial biopsies collected at 1, 3, 6 and 12 months post-insertion, when compared with endometrial tissue collected pre-insertion in the proliferative phase (Figure 4). Oestrogen receptor immunoreactivity in stromal endometrial tissue was significantly reduced ($P < 0.01$) 1, 3, 6 and 12 months following insertion of the IUS when compared with stromal endometrium collected from subjects prior to insertion in the proliferative phase (Figure 4). These levels were equivalent to those seen in the luteal phase, and showed no increase in the 12 month samples.

**PGDH metabolism**

PGDH activity, as measured by metabolite (µg/ml per mg tissue) was greater in endometrial samples collected in the secretory phase. Low levels of PGDH activity were observed following insertion of a LNG-IUS both 1 and 3 months post-insertion. Metabolism significantly increased ($P < 0.05$) at 12 months post-IUS insertion (Figure 5).

Positive immunoreactivity for PGDH was observed in the glandular cytoplasm from all endometrial biopsies collected prior to insertion of the LNG-IUS during the secretory phase of the cycle. Reduced PGDH immunostaining was observed in endometrial tissue collected following insertion of the LNG-IUS (data not shown).

**Serum oestradiol and progesterone concentrations**

Circulating serum oestradiol and progesterone concentrations at the time of endometrial biopsy are documented in Table I.

**Discussion**

This study describes a significant decline in immunostaining of ER and PR (subtype A + B) in glands and stroma in endometrium exposed to intrauterine levonorgestrel. Furthermore, the availability of an antibody directed against a sequence at the N terminal end, unique to PRB subtype, has demonstrated a more substantial and sustained down-regulation of the
PRB subtype in the glands and stroma of the LNG-exposed endometrium, whereas a distinct increase in staining with the PR A + B antibody was evident in the glands at 12 months. Consequently, the data herein show by inference that the PR A subtype is likely to be the isoform mediating LNG action in the endometrium.

The observation of down-regulation of PRB (see Figure 3) in endometrium exposed to intrauterine levonorgestrel is consistent with our own observations of progesterone receptor subtype expression in the normal cycle and in early decidua (Wang et al., 1998). It would appear that regardless of whether the exposure of the endometrium to high concentrations of progesterin was endogenous (as in luteal phase or early pregnancy) or exogenous (LNG-IUS) PRB is suppressed to a greater extent than the PR A subtype.

We previously reported that PR A is the predominant subtype in secretory endometrium and decidua of early pregnancy, that is, in a progesterone dominated steroid environment (Wang et al., 1998). PR A protein is also the dominant isoform, as determined by Western blot analysis in human myometrium (Viville et al., 1997). The significance of the predominance of PR A is not known. Morphologically, the endometrium in the presence of a LNG-IUS shows features of decidualized stroma (Nilsson et al., 1978; Silverberg et al., 1986). Hence in the presence of ‘pseudo-decidualization’, the predominance of PR A is consistent with observations of PR subtype expression in early decidua (Wang et al., 1998). Differential expression of PR subtypes is likely to determine response to progestins, although the action of ligand occupied PR B receptors may work through an alternative mechanism (Tung et al., 1993). In endometrium exposed to a local exogenous LNG (LNG-IUS), sufficient PR A isoform is maintained to mediate the long term morphological and functional characteristics of progesterin action, for example decidualization.

There was an interesting apparent increase in PR A immunoreactivity in endometrial glands between 6 and 12 months post-insertion. Further, this apparent rise in PR A was related to increased PGDH activity between 6 and 12 months following insertion of the intrauterine system. However, there was an apparent discrepancy between PGDH activity (Figure 5) and PR concentration (Figure 2) during the secretory phase. This may have been due to the chronic high exposure of the endometrium to local progestins. Nevertheless, the observations described here concerning prostaglandin metabolism (reduced PGDH immunostaining and reduced in-vitro PGDH activity post-LNG-IUS insertion) support the hypothesis that with a down-regulation of PR, the endometrium is less responsive to progestin-mediated events.

The observations of elevated PGDH immunoreactivity in endometrial biopsies collected in the secretory phase prior to insertion of a LNG-IUS are consistent with reported data concerning PGDH enzyme activity in the secretory phase and premenstrually (Casey et al., 1980). The decline in PGDH immunostaining following insertion of a LNG-IUS is consistent with our observation of reduced PR A + B and PR B immunoreactivity post-insertion of a LNG-IUS. From a functional viewpoint, if reduced steroid receptor is available to bind the ligand then only a limited functional response can be initiated (that is, reduced induction of PGDH activity). This study has thus demonstrated a consistent functional response of the endometrium to the reduced number of progesterone receptors. The continued suppression of PRB by exogenous LNG is by inference, at least in part, mediated by the PR A subtype.

The in-vitro data for PGDH activity are in keeping with these observations. Prostaglandin metabolism is elevated in tissue collected in the secretory phase, prior to insertion of a LNG-IUS and reduced thereafter. Small sample numbers (n = 3) precluded estimation of a significant difference when compared with secretory phase biopsies. The observation is, however, entirely consistent with the literature (Casey et al., 1980). Following insertion of the LNG-IUS, metabolism was low at 1 month and 3 months. This is consistent with the down-regulation of PR in the glands of LNG-exposed endometrium. However, there was a significant increase in metabolism (PGDH activity) 12 months post-LNG-IUS insertion. This coincided with the observed significant increase in glandular PR immunoreactivity (which by inference was presumably PR A subtype) 12 months following insertion of the LNG-IUS.

In the present study, a significant reduction in ER immunoreactivity has been reported up to 12 months following insertion of the LNG-IUS. In the normal cycle there is a significant decline in the ER of the glands and stroma in the functional layer of endometrium, with the transition from proliferative to secretory phase of the cycle. However, there is no significant fall in ER (or PR) immunoreactivity in the stromal compartment of the basal endometrium (Snijders et al., 1992). The endometrium of users of a LNG-IUS is atrophic, but is morphologically distinct from normal basal endometrium (own observations). At any time, the steroid environment within the endometrium will reflect the local progestogen (LNG) levels and the circulating serum oestradiol and progesterone concentrations. Mean serum oestradiol concentrations remained in the mid follicular range (Critchley et al., 1990) during the 12 months following LNG-IUS insertion. Mean serum progesterone levels were indicative of the fact that biopsies were collected during either the follicular or luteal phases (see Table 1).

Short-term administration of synthetic progestogens decreases the PR content of both endometrial epithelium and stroma in pre- and post-menopausal women (Lane et al., 1988). Continuous delivery of levonorgestrel exerts part of its contraceptive action via PR in endometrium and thus subsequent biological activity is presumed to be dependent upon the concentrations and availability of functional PR (Lau et al., 1996). The observations reported here on PR expression in endometrium exposed to intrauterine levonorgestrel are completely opposite to observations in endometrium from Norplant® users (sub-dermal LNG). In the presence of elevated circulating progestogen concentrations, PR immunoreactivity was persistently raised in Norplant® exposed endometrium. The mechanism by which this observation may be explained and whether the observed increase in stromal PR immunoreactivity is associated with an increased number and concentration of functional PR is unknown (Critchley et al., 1993). According to Lau et al.
(1996), Norplant results in a decrease in PR mRNA levels as measured by in-situ hybridization, which implied a change in turnover rate of PR mRNA and protein. Similar data on PR mRNA levels in endometrium exposed to intrauterine delivery of levonorgestrel are not yet available.

Pekonen et al. (1992) have also observed functional differences between continuous local intrauterine and subdermal levonorgestrel delivery. Intrauterine LNG was a potent stimulator of stromal cell IGFBP-1 production. IGFBP-1 is a product of decidua from vellent gonadal stromal cells (Bell, 1991). Subdermal delivery of LNG produced no such effect. This observed difference is likely to reflect dose-dependency effects of LNG. Intrauterine LNG produced endometrial LNG levels 1000 times greater than serum concentrations. The latter are of the same order of magnitude with subdermal implants (Nilsson et al., 1982; Pekonen et al., 1992). In the presence of such high local progestin concentrations, endogenous, systemic progesterone would be masked, despite circulating progesterone concentrations remaining in the normal follicular–luteal phase range (Table I). Women using a LNG-IUS usually have normal oestrogen-varying cycles (Luukkainen, 1991) and there is no reduction in oestradiol concentrations (Luukkainen et al., 1990). LNG, however, is not progesterone and the ligand (LNG)–receptor complexes may not necessarily reproduce all the classic 'progestin' actions.

Insertion of a LNG-IUS produced widespread morphological changes in endometrial histology. In this study, a pseudo-decidualized stroma, with atrophic glands, was evident in all biopsies collected post-insertion of the IUS. These observations are consistent with earlier reports (Nilsson et al., 1978; Silverberg et al., 1986). A more detailed description of the histological features in biopsies in this series will be reported elsewhere.

The dramatic reduction in menstrual blood loss (Andersson and Rybo, 1990; Milson et al., 1991) described in users of a LNG-IUS has been considered to be due to epithelial and glandular endometrial atrophy (Nilsson et al., 1978; Silverberg et al., 1986) associated with the LNG-IUS. However, disturbances in endometrial bleeding associated with the intrauterine system remain a major problem for users. It is likely that subtle endometrial mechanisms responsible for the control of normal uterine bleeding have been disturbed. The present data provide novel information on the perturbation of local sex steroid receptor expression (down-regulation) in the presence of a LNG-IUS and evidence to support interference with normal function (PGDH activity). Thus disturbance of sex steroid receptor expression, as described in this study, may play a role in the control of mechanisms responsible for aberrations in normal bleeding patterns. Interestingly, bleeding patterns are described to improve (Luukkainen, 1991) 6 months or so following insertion of a LNG-IUS, at a time when at least PR (by inference subtype A) had somewhat increased. Further work is necessary to elucidate the mechanisms responsible for this major problem of menstrual aberration with progestin-only contraceptive methods. Examination of more local mechanisms in endometrium exposed to an intrauterine levonorgestrel system should help explain some of the potential mechanisms regulating endometrial bleeding.

Acknowledgements

Thanks are due to Teresa Drudy, Gail Carr and Vivien Grant for technical assistance, to Dr Sharon Cameron for help with endometrial biopsy collection, to Linda Harkness and Nancy Evans for assistance with the steroid radioimmunoassays, and to Mrs Vicky Watters for secretarial support. We acknowledge the assistance of Mr Tom McFetters and Mr Ted Pinner for the illustrations. We are grateful to Professor David T. Baird and Professor Robert M. Brenner for their helpful comments on the manuscript. The levonorgestrel-releasing intrauterine systems were supplied by Leiras-Oy, Finland. The study was supported by Wellcome Trust project grant No: 044744/Z/95/Z.

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Received on October 24, 1997; accepted on February 4, 1998