Regulation of matrix metalloproteinase-9 in endometrium during the menstrual cycle and following administration of intrauterine levonorgestrel

Joanna L. Skinner, Simon C. Riley, Ailsa E. Gebbie, Anna F. Glasier and Hilary O. D. Critchley

Department of Obstetrics and Gynaecology, Centre for Reproductive Biology, The University of Edinburgh, 37 Chalmers Street, Edinburgh EH3 9EW, UK

1To whom correspondence should be addressed

Remodelling of endometrial tissues is fundamental to the cyclical changes that occur during the menstrual cycle, implantation and, in the absence of pregnancy, menstruation. The enzyme matrix metalloproteinase-9 (MMP-9) is recognized as important in these processes but its regulation is not well defined. These studies have demonstrated that MMP-9 activity is present in the endometrium and exhibits cyclical changes in its distribution in the glandular and stromal cells. MMP-9 protein is present throughout the cycle with highest expression, as determined by semi-quantitative analysis of specific MMP-9 immunoreactivity, in glandular cells during the mid secretory phase. A similar distribution was observed in first trimester decidua. In women with a levonorgestrel intrauterine system (LNG-IUS), which delivers high local concentrations of progesterone to the uterine cavity, MMP-9 is highly expressed in both endometrial glandular and stromal cells, and in the vasculature (endothelial and perivascular cells). It can be concluded that MMP-9 is stimulated directly or indirectly by progesterone. Furthermore, MMP-9 may play a role in the remodelling of the endometrium that occurs during the menstrual cycle and in the aetiology of the morphological changes and breakthrough bleeding associated with long-term progestagen administration via an LNG-IUS.

Key words: contraception–LNG/endometrium/matrix metalloproteinases/menstruation/tissue remodelling

Introduction

Tissue remodelling in the uterus is an important process during the normal menstrual cycle for the sequential destruction and reconstruction of the endometrial functionalis, for implantation processes and subsequent development of the placenta and fetal–maternal interface (Hulboy et al., 1997). The matrix metalloproteinases (MMP) are a family of zinc-dependent enzymes that are important for these tissue remodelling processes by degrading components of the extracellular matrix (ECM), basement membrane and interstitial matrix (Rawdanowicz et al., 1994). To date there have been 16 distinct MMP cloned and characterized in the human and the family member MMP-9 is a 92 kDa gelatinase enzyme that degrades components of the extracellular matrix and basement membrane components, specifically, collagens IV and V, elastin and gelatin (Hulboy et al., 1997). MMP-9 is expressed in the uterus and cellular sources include endometrial glandular and stromal cells, macrophages, neutrophils and first trimester cytotrophoblast cells (Jeziorska et al., 1996; Salamonsen and Woolley, 1996; Bischof et al., 1998). The gelatinase MMP-2 is also expressed and secreted by human endometrium (Irwin et al., 1994; Salamonsen et al., 1997). There is increasing evidence for a role for MMP in the tissue remodelling events of implantation and, in the absence of pregnancy, subsequent menstruation (Rodgers et al., 1994; Marbaix et al., 1995; Salamonsen and Woolley, 1996).

The regulation of MMP-9 is not well understood but it is controlled, at least in part, by progesterone. Maximal intraluminal secretion of MMP-9 by glandular epithelial cells occurs at a time when progesterone concentrations are highest during the ‘implantation window’ of early to mid secretory phase (Jeziorska et al., 1996) although its release from endometrial explants is inhibited by progesterone in vitro (Rodgers et al., 1994). This control of MMP-9 and indeed uterine function by progesterone is clearly complex, demonstrated by these differing responses found in vivo and in vitro. A compounding factor of this regulation is that the two isoforms of the progesterone receptor (PR), PRα and PRβ, are present in epithelial and stromal cells during the proliferative phase. In the secretory phase both receptor subtypes decline in the glandular compartment and PRβ decreases in the stroma (Wang et al., 1998).

The levonorgestrel-releasing intrauterine system (LNG-IUS; Leiras Oy, Finland) is associated with a 97% reduction in menstrual blood loss (Andersson and Rybo, 1990; Milson et al., 1991). However, the most common indication for discontinuation of use of this method and indeed other progestrone-only systems of contraception, with its associated dramatic reduction in menstrual bleeding, is the occurrence of the troublesome side-effect of unpredictable breakthrough bleeding (Findlay, 1996). The long-term administration of intrauterine levonorgestrel results in features of altered morphology and function of the endometrium (Critchley et al., 1998a) and down-regulation of receptors for oestrogen and both isoforms of the progesterone receptor (Critchley et al., 1998b). The paracrine mechanisms which account for the disturbance in bleeding patterns associated with intrauterine levonorgestrel delivery remain to be elucidated. Endometrium obtained from women using a LNG-IUS provides an opportunity to study the effect of high-dose local progestagen delivery upon endometrial development. The aims of this study were to investigate the
control of MMP-9 in the endometrium during the menstrual cycle and to elucidate further its regulation by local intrauterine LNG in vivo.

Materials and methods

Patients and tissues

Normal endometrium was collected from women (n = 40) aged 38 years (median, range 25–47) undergoing minor gynaecological procedures (including laparoscopic sterilization, diagnostic laparoscopy for pelvic pain, endometrial sampling for regular but dysfunctional uterine bleeding) collected by Pipelle suction curette (Laboratoire CCD, Paris, France). All women described regular cycles (25–35 days). Endometrial samples were designated as menstrual, early proliferative, mid proliferative, late proliferative, ovulatory, early secretory, mid secretory and late secretory phase (n = 5 for each stage of cycle). Dating was based on the histological criteria of Noyes et al. (1950) and all biopsies were consistent with the date of the last menstrual period. No subject had received exogenous hormones or had used an intrauterine device in the 3 months prior to biopsy. A 10 ml sample of venous blood was collected at the time of each endometrial biopsy for estimation of serum oestradiol and progesterone concentrations by radioimmunoassay. Circulating concentrations of serum oestradiol and progesterone at the time of endometrial biopsy in women using a LNG-IUS have been previously reported (Critchley et al., 1998b) and reflect continued ovarian activity in subjects with a LNG-IUS in situ.

Endometrial tissue was collected from 14 women aged between 32 and 48 years (median 37) who provided informed consent for an endometrial biopsy prior to, and following, insertion of an LNG-IUS. All participants were fertile, described regular menstrual cycles (cycle length 25–35 days) and were not using 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, often also in association with heavy menses (n = 10), or heavy menstruation (n = 4). The study was longitudinal with each subject acting as her own control. Seven subjects underwent a pre-insertion endometrial biopsy in the proliferative phase and seven subjects in the secretory phase of the menstrual cycle prior to insertion of the LNG-IUS. The stage of the cycle prior to LNG-IUS insertion was defined according to the criteria of Noyes et al. (1950). All biopsies were performed in an outpatient setting with a Pipelle suction curette. Subsequent endometrial biopsies were collected 1, 3, 6 and 12 months following insertion of the LNG-IUS. Once the LNG-IUS was in situ, the histological appearance of the endometrium samples was indistinguishable whether collected in the follicular or luteal phase of the cycle, hence data at time periods of 1, 3, 6 and 12 months were pooled. The sex steroid receptor expression in this longitudinal series of biopsies has been previously reported (Critchley et al., 1998b).

A further five women (8–10 weeks amenorrhea) provided decidual tissue at the time of planned surgical termination of pregnancy. Decidual biopsies were collected following dilatation of the cervix and curettage of the uterine wall prior to vacuum aspiration of products of conception. Placental biopsies were also collected at this time. Decidual and placental villous biopsies were immunostained with a monoclonal antibody against cytokeratin (Dako MO821, High Wycombe, Bucks, UK) to confirm absence of trophoblast tissue (decidua parietalis).

Ethical approval for this study was granted by the Lothian Research Ethics Committee (reference: 170294/6/44 and 170293/6/73) and all tissue samples were obtained with informed written consent from all patients.

For zymography, endometrium was snap-frozen immediately after collection and stored at –70°C. For immunohistochemistry, all endometrium and decidua samples were fixed overnight in 10% neutral buffered formalin at 4°C, rinsed and stored in 70% ethanol and thereafter routinely waxed embedded. Sections (5 µm) were cut and mounted on 3-aminopropyltriethoxysilane-coated slides (Sigma, St Louis, MO, USA) for routine histopathology (haematoxylin and eosin staining) and immunolocalization of MMP-9.

Zymography

Gelatinase activity was detected by zymography using methods described previously with minor modifications (Rawdanowicz et al., 1994). Briefly, tissues were homogenized [extraction buffer 0.2% sodium dodecyl sulphate (SDS) in H2O; 100 µg tissue wet weight/ml buffer] and protein concentrations measured. Samples were separated (50 µg protein loaded) by SDS–polyacrylamide gel electrophoresis (PAGE) using gels (7.5%) containing gelatin (1 mg/ml) using non-reducing conditions. Gels were washed (2.5% Triton X-100) and incubated in zymography digestion buffer (50 mM Tris, 0.2 M NaCl, 5 mM CaCl2, 1 µM ZnCl2, 0.02% Brij-35, pH 7.6) at 37°C for 18 h. Gels were then stained (0.5% Coomassie blue R250 in 30% methanol/10% glacial acetic acid) at 23°C for 3 h, destained (staining solution omitting the Coomassie blue) to reveal the discrete areas where gelatinase activity had hydrolysed the substrate.

Immunolocalization of MMP-9

Immunoreactive MMP-9 was localized using standard techniques. In brief, sections were dewaxed, rehydrated and endogenous peroxidase activity blocked by incubation with H2O2 (3% v/v for 20 min; Sigma). Sections were washed extensively and a further blocking step of normal horse serum (5% v/v for 20 min) applied. Tissue sections were then incubated (17 h at 4°C) with the primary mouse monoclonal antibody (2 µg/ml final concentration) raised against a peptide sequence corresponding to amino acids 624 to 644 in the carboxy-terminal domain of MMP-9 (Insight Biotechnology Ltd, Wembley, Middlesex, UK). The primary antibody was detected using a biotinylated horse anti-mouse antibody and an avidin–peroxidase complex (Vector Laboratories, Burlingame, CA, USA), then visualized with 3,3’-diaminobenzidine as chromagen (Vector) according to the manufacturer’s instructions. Sections were counterstained, dehydrated and mounted.

The specificity of the MMP-9 antibody used for immunohistochemistry was confirmed using immunoblotting techniques. In brief, conditioned medium samples collected from endometrial explants were dialysed and concentrated by lyophilization. Secreted proteins were separated by PAGE (7.5% gels) and transferred to a nitrocellulose membrane (0.45 µm pore size; Biorad, Hemel Hempstead, Herts, UK) by wet blotting. The membrane was blocked with 5% bovine serum albumin, before application of the MMP-9 antibody, which was detected using the same methods used for immunohistochemistry.

Negative controls were performed by replacing the primary antibody with non-immune mouse immunoglobulin at a concentration of 1:500 in diluted horse serum (purified MMP-9 antigen was not available). Sections of placenta collected at term were used as a positive control, which demonstrated strongly positive and consistent immunolocalization of MMP-9 in cytotrophoblast and extravillous trophoblast cells throughout all the experiments performed, as described previously (Riley et al., 1997).

Scoring and analysis of immunoreactivity

Immunostaining intensity and proportion of positive immunoreactivity of epithile in all tissue sections was assessed semi-quantitatively, on a 4-point scale. A score of 3 indicated intense immunostaining of
MMP-9 in endometrium

Figure 1. Gelatin zymogram demonstrating matrix metalloproteinase (MMP)-9 (92 kDa gelatinase B) and MMP-2 (72 kDa gelatinase A) in homogenates of endometrium from the proliferative (Pr), ovulatory (Ov) and secretory (S) phases of the menstrual cycle and decidua collected from the first trimester of pregnancy (D). Amniotic fluid (AF) collected at term after spontaneous delivery was used as a positive control.

>95% for the cell types, that is, glandular or stromal cells examined. Scores of 1 and 2 indicated weak or moderate immunostaining, respectively. A score of zero indicated an absence of immunoreactivity. The scoring was performed blind by a single observer, without prior knowledge of phase of the menstrual cycle. The mean ± SEM was calculated. The data were analysed by one-way analysis of variance (ANOVA) using Fisher’s PLSD (protected least significant difference) coefficient to assign significance.

Results

MMP-9 activity in endometrium and decidua

Gelatin zymography demonstrated that MMP-9 activity, corresponding to 92 kDa, was detectable in endometrium tissue samples from throughout the menstrual cycle and also from decidua collected during the first trimester of pregnancy (Figure 1). In addition, MMP-2 enzyme activity (corresponding to 72 kDa) was evident in endometrium and decidual samples (see Figure 1). The specificity of this activity as being derived from MMP was confirmed by incubation with EDTA (5 mM) or o-phenanthroline (2.5 mM), with both treatments inhibiting activity of all bands (data not shown).

Immunolocalization of MMP-9 during the menstrual cycle

The specificity of the MMP-9 antibody was confirmed by immunoblot (Figure 2). A single band at the expected 92 kDa molecular weight was identified as a secreted product in culture medium conditioned by tissue explants of endometrium. This aligned with the strong single band in the amniotic fluid positive control.

Positive immunoreactivity for MMP-9 was predominantly visualized in the cytoplasm of the majority of glandular epithelium in both the proliferative, secretory and menstrual phases of the cycle (Figure 3a–c). In endometrial stromal cells, a heterogeneous distribution of lower intensity MMP-9 immunoreactivity was observed, with immunostaining more marked in the secretory phase (Figure 3b), especially in the late secretory stage. The intensity of MMP-9 immunostaining expressed by semi-quantitative analysis during the proliferative and secretory phases of the cycle is demonstrated in Figure 4. There was no significant difference in the intensity or distribution of MMP-9 immunoreactivity between these phases of the menstrual cycle. In the endometrial vasculature, MMP-9 immunoreactivity was localized in endothelial cells and also in perivascular cells in some vessels throughout the menstrual cycle (Figures 3a–c). All negative control sections consistently demonstrated no positive staining (representative section, Figure 3d). All biopsies collected during the normal menstrual cycle were from the functionalis layer, since a Pipelle sampling device (suction curette) was employed.

Immunolocalization of MMP-9 in decidua and placenta during early pregnancy

MMP-9 immunoreactivity was localized in decidual tissue in a similar pattern of distribution and intensity to that seen in the mid and late secretory phases of the cycle (Figure 3f). Maximal MMP-9 immunostaining was present in glandular epithelial cells, whereas in stromal cells staining was heterogeneous from strong to less intense and absent. The localization of MMP-9 in the vasculature was similar to that found during the menstrual cycle, evident in endothelial and some perivascular cells (Figure 3f). In placenta, MMP-9 was localized in villous tissue predominantly in syncytiotrophoblast, but not in cytotrophoblast cells (Figure 3g).

Immunolocalization of MMP-9 in endometrium exposed to intrauterine levonorgestrel

Endometrial biopsies collected prior to insertion of the LNG-IUS demonstrated normal morphology consistent with features of the proliferative or secretory phase described, and localization of MMP-9 immunoreactivity was comparable to normal tissues collected from the same stage of the cycle as reported above.

All subjects in whom biopsies were collected in the follicular phase (proliferative histology) had circulating progesterone concentrations of ≤10 nmol/l (range 0–10, 3.4 ± 1.9 nmol/l, mean ± SEM). Subjects in whom biopsies were collected in the luteal phase (secretory histology) had serum progesterone concentrations between 6 and 29 nmol/l (17.7 ± 2.9). Post
insertion of the LNG-IUS serum progesterone and oestradiol concentrations were within normal follicular and luteal concentrations as expected, and have been previously published (Critchley et al., 1998b).

Following insertion of the LNG-IUS, endometrial morphology was modified with widespread pseudo-decidualization evident in all biopsies. MMP-9 immunoreactivity remained marked in the glandular compartments, although it became less evident with increased atrophy of these glands (Figure 3e). In contrast to the normal cycle, following insertion of the LNG-IUS regardless of the time period at which the biopsy was taken, intense MMP-9 immunoreactivity was present in the stromal cells of all biopsies (Figure 3e). There was a significant ($P < 0.05$) increase in MMP-9 immunoreactivity in the endometrium collected following insertion of the LNG-IUS, compared with biopsies collected in both the proliferative and secretory phases of the cycle (Figure 5).

**Discussion**

In normal endometrium MMP-9 enzyme activity is present throughout the menstrual cycle. MMP-9 immunoreactivity was localized predominantly in glandular epithelial cells with the highest quantities observed in the mid to late secretory and

**Figure 3.** Localization of matrix metalloproteinase-9 immunoreactivity in endometrium collected from the menstrual cycle in the (A) proliferative phase, predominantly in glandular epithelium, (B) early secretory phase, predominantly in glandular epithelium, in the vasculature and in some stromal cells and (C) during the menstrual phase in glandular epithelium and vasculature (note stromal breakdown); (D) a representative negative control section demonstrating no positive non-specific staining; (E) from a woman with a levonorgestrel intrauterine system in situ strongly in decidualized stromal cells with less immunostaining in atrophic glands (F) in decidua collected from the first trimester of pregnancy in glandular epithelium, stromal cells and vasculature, and (G) in placenta in villous tissue in syncytiotrophoblast. $v =$ vasculature; $s =$ syncytiotrophoblast; $c =$ cytotrophoblast; $g =$ glandular epithelium; $ag =$ atrophic gland; $st =$ stromal cells. All scale bars $= 50 \mu m$. 

Figure 4. Immunostaining scores assessed by semi-quantitative analysis of matrix metalloproteinase-9 immunoreactivity in glands and stromal cells of endometrium throughout the early (E) mid (M) and late (L) stages of the proliferative (P), ovulatory (Ov), secretory (S) and menstrual (M) phases of the menstrual cycle D = decidua from early pregnancy (n = 5 tissue samples collected from different women at each time point). Open bars = glands; solid bars = stromal cells.

Figure 5. Immunostaining scores assessed by semi-quantitative analysis of matrix metalloproteinase-9 immunoreactivity in glands and stromal cells of endometrium collected from women in the proliferative (prolif) and secretory (secret) phases of the menstrual cycle D

peri-menstrual phases of the cycle. In decidua in early pregnancy, MMP-9 was present in glands and also in decidualized stromal cells. These findings indicate a role for MMP-9 in the remodelling processes that occur during menstruation and at implantation (Marbaix et al., 1992; Salamonsen and Woolley, 1996) Following intrauterine delivery of levonorgestrel by a LNG-IUS there was a significant increase in endometrial MMP-9 immunoreactivity, indicating that progesterone may be involved, at least indirectly, in the control of MMP-9 expression. In addition, MMP-2 enzyme activity is present in endometrium obtained throughout the menstrual cycle and during early pregnancy, as demonstrated by zymography.

The findings in this study demonstrate that in the normal menstrual cycle MMP-9 protein was immunolocalized to glandular epithelial cells throughout the menstrual cycle with maximal intensity in the glandular epithelium. A previous study which examined MMP-9 localization in human endometrium (Jeziorska et al., 1996) showed similar findings in the proliferative phase. As in this present study, intraluminal secretion was evident with peak concentrations in glandular secretion and uterine fluid at the peri-implantation period. However, Jeziorska et al. (Jeziorska et al., 1996) observed a disappearance of MMP-9 immunoreactivity in the late secretory phase and perimenstrually, contrary to our findings. Furthermore, Rodgers et al. (Rodgers et al., 1994) reported that expression of MMP-9 mRNA was most evident in late secretory and menstrual endometrium. Such observations support a role for MMP-9 in remodelling of the extracellular matrix and in the production of glandular secretions which may be associated with blastocyst recognition and implantation. In stromal cells, MMP-9 immunoreactivity was generally low throughout the cycle with only an increase in the late secretory phase, where it may have a role in the profound changes in morphology during decidualization as well as at menstruation. By dual immunolocalization, Jeziorska et al. (Jeziorska et al., 1996) noted that MMP-9 immunoreactivity was present in polymorphonuclear leukocytes and macrophages, with little MMP immunoreactivity in stromal cells and resident leukocytes, including mast cells. The observation of MMP-9 expression in subpopulations of leukocytes within the endometrium at critical times, for example pre- and peri-menstrually, is consistent with a role for these cell types as a source of endometrial MMP-9 expression (Jeziorska et al., 1996).

Immunoreactive MMP-9 was localized in the endometrial vasculature in endothelial and perivascular cells throughout the menstrual cycle and may play a role in the angiogenesis and development of the vasculature, as well as the breakdown of vessels during menstruation. Roberts et al. (Roberts et al., 1992) in an electron microscopy study demonstrated extensive breakdown of the vascular basal lamina prior to menstruation. Specifically in the pre-menstrual period structural interactions between endothelial and neighbouring pericytic cells are impaired. This endothelial cell hypertrophy evident in the luteal phase of the cycle has been attributed to rising concentrations of progesterone in combination with oestrogen. In late secretory endometrium the necrosis evident in stromal tissues is distinct from that in capillary endothelial cells (Roberts et al., 1992).

In women using an intrauterine device (IUD), overall secretion of gelatinase activity increases throughout the cycle, with increased MMP-9 secretion during the proliferative phase (Martelli et al., 1993). This activity was attributed to bone marrow-derived cells, in agreement with the findings of Jeziorska et al. (Jeziorska et al., 1996). This may be involved in the rise in blood loss at menstruation associated with IUD use.

The role of progesterone in the regulation of endometrial MMP-9 expression is unclear. These and previous studies (Jeziorska et al., 1996) have demonstrated that glandular MMP-9 production increases at a time when progesterone also increases, but it is maintained when progesterone concentrations fall in the perimenstrual period. The LNG-IUS releases 20 µg of levonorgestrel daily directly to the uterine cavity and this results in the typical morphological changes in the endometrium associated with long-term use of a progestagen.
suppressed of the two subtypes and thus PR A is likely to be given the name of a method.

vasculature) which is the major problem with this contraceptive method.

MMP-9 may be involved in the tissue remodelling that occurs during the functionalis layer of the endometrium with long-term treatment with progestagen contraceptives (Marsh et al., 1995). MMP-9 may also have a role in the aetiology of breakthrough bleeding (since MMP-9 is localized in the vasculature) which is the major problem with this contraceptive method.

In women using a LNG-IUS, both isoforms of the progesterone receptor (PR\(A\) and PR\(B\)) are down-regulated and there is a reduction in some progesterone-dependent endometrial markers (Crichley et al., 1998b) including glycofelin (Mandellin et al., 1997). Interestingly PR\(B\) isoform is the more suppressed of the two subtypes and thus PR\(A\) is likely to be the subtype that mediates long term levonorgestrel action in the endometrium (Crichley et al., 1998b; Wang et al., 1998). Similarly, studies in vitro demonstrate that after several days in culture, more MMP-9 is secreted by stromal than epithelial cells in culture (Salamonsen et al., 1997) and progesterone withdrawal is the optimal stimulus for inducing MMP-9 expression in these cells. Nevertheless, this study and others (Jeziorska et al., 1996) indicate that the stromal cells are not the principal source of MMP-9 during the normal menstrual cycle and that these alterations in stromal cell MMP-9 expression do not occur in vivo during the normal cycle but are a response to decidualization during early pregnancy or exogenous progesterone treatment, or to in-vitro culture (Salamonsen et al., 1997).

There is recent evidence for the cell specificity of progesterone receptor regulation (Tseng and Zhu, 1997) with progestin stimulating progesterone receptor mRNA in cultured human endometrial stromal cells. This stromal–decidual cell system is novel in that progestin induces numerous differential pathways are involved in the regulation of PR isoforms in endometrial stromal cells.

MMP are also controlled after secretion by inhibition of enzyme activity by endogenous tissue inhibitors of metalloproteinases (TIMP). TIMP-1, -2 and -3 are localized in endometrium and are secreted by decidualized stromal cells in culture, but it is proposed that they are not regulated during the menstrual cycle and their role would appear to be maintenance of tissue integrity (Hulbey et al., 1997; Zhang and Salamonsen, 1997).

In placenta during the first trimester, MMP-9 is localized in syncytiotrophoblast, but not cytotrophoblast, cells. However, cytotrophoblast cells collected at this time and maintained in culture secrete MMP-9, although this may be due to alterations in phenotype in vitro (Librach et al., 1994). At this stage, interactions between trophoblast and decidua are important for the establishment of the fetal–maternal interface. An as yet unidentified factor in medium conditioned by decidualized stromal cells stimulates gelatinase activity and MMP-9 release by first trimester trophoblast cells (Bischof et al., 1998). This may be an important component of the control of trophoblast invasion at this time.

The antibody used in this study does not distinguish between the latent and active forms of MMP-9, so care must be taken in the interpretation of these data, although the major form of MMP-9 detected by zymography was the latent 92 kDa form. The concentrations of total protein as assessed here by semi-quantitative analysis of immunohistochemistry does not establish enzyme activity within tissues.

In summary, this study confirms that MMP-9 activity is present in normal endometrium and decidua with an increased MMP-9 expression in levonorgestrel-exposed endometrium. Maximal MMP-9 immunoreactivity was evident at times when progesterone exposure was highest during the early and mid secretory phases, that is, around the time of anticipated implantation. MMP-9 may also have a role in menstruation with its expression maintained in the late secretory phase in association with progesterone withdrawal. Regulation of MMP-9 is clearly complex and it is likely to involve progesterone either directly or indirectly via other local mediators. High quantities of MMP-9 immunoreactivity were present in endometrium exposed to intrauterine progestagen delivered by the LNG-IUS, and MMP-9 may have a role in the aetiology of breakthrough bleeding associated with the use of this intrauterine contraceptive system.

Acknowledgements

We wish to acknowledge the technical assistance of Miss Teresa Drudy and Miss Rose Leask and we are grateful to Mrs Vicky Watters for secretarial help and Mr Tom McFetters and Mr Ted Pinner for assistance with the illustrations. The levonorgestrel intrauterine systems were provided by Leiras Oy, Finland. Support was provided by the Wellcome Trust project grant No. 004474/ZZ/95/G to HODC and The Scottish Hospital Endowment’s Research Trust to SCR.

References


Received on June 10, 1998; accepted on November 30, 1998