Metalloproteinases and tissue inhibitor of metalloproteinase 1 (TIMP-1) in endometrial flushings from pre- and post-menopausal women and from women with endometrial adenocarcinoma


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The presence of metalloproteinase activity in endometrial flushings obtained from pre-menopausal women, during the proliferative and secretory phases of the menstrual cycle, control post-menopausal women and women with post-menopausal bleeding (PMB) with or without adenocarcinoma was analysed by zymography. In addition, quantitative measurements of matrix metalloproteinase 2 (MMP-2), MMP-3, MMP-9 and tissue inhibitor of metalloproteinase 1 (TIMP-1) in the flushings were obtained by ELISA. The zymography results showed eight bands of activity, with molecular weights ranging from 51 to 208 kDa in the flushings from pre-menopausal women and post-menopausal women, particularly those with adenocarcinoma. Both zymography and ELISA showed that MMP-2 and MMP-9 were the major metalloproteinases found in the flushings and only low concentrations of MMP-3 were found. Concentrations of MMP-2 in pre-menopausal women were higher in flushings obtained during the secretory phase of the menstrual cycle than those obtained in the proliferative phase (P < 0.05), suggesting that it may play a role in embryo implantation. Concentrations of MMP-2 (P < 0.001), MMP-9 (P < 0.05) and TIMP-1 (P < 0.001) in the flushings from post-menopausal control women were lower than those from pre-menopausal women. Concentrations of MMP-2 (P < 0.05) and TIMP-1 (P < 0.05) were higher in flushings from women with PMB without carcinoma compared with post-menopausal controls and concentrations of MMP-9 (P < 0.01) and TIMP-1 (P < 0.05) in flushings from women with adenocarcinoma were higher than in post-menopausal controls. Among subjects with PMB, concentrations of MMP-9 in women with adenocarcinoma were higher than those without carcinoma (P < 0.05). Our results show that concentrations of MMP-2, MMP-9 and TIMP-1, but not MMP-3, are associated with endometrial activity and, therefore, may have a role in the breakdown of endometrial tissue. In addition, the increased concentrations of MMP-9 in flushings of women with adenocarcinoma indicate that this particular proteinase is associated with the presence of endometrial neoplastic cells.

Introduction

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that possess proteolytic activities against several components of the extracellular matrix (Hulboy et al., 1997). They are secreted as inactive pro-enzymes which become activated by removal of an amino-terminal peptide. The function of the active enzyme is to digest the extracellular matrix. They are divided into collagenases, gelatinases or stromelysins according to their substrate specificity. Collagenases, such as MMP-1 and MMP-8, digest collagen type I, II, III, VII and X; gelatinases, such as MMP-2 and MMP-9, digest collagen type IV and denatured collagen (gelatin); stromelysins, including MMP-3, MMP-7 and MMP-10, degrade a variety of extracellular matrix components such as fibronectin, proteoglycans, laminin and collagen types IV, V and VII. The activity of these enzymes is inhibited by various tissue inhibitors of metalloproteinases (TIMPs). TIMP-1 forms stoichiometric complexes with active forms of all known MMPs and in addition binds pro-MMP-2. TIMP-2 shows similar inhibitory activity to TIMP-1 against MMPs but preferentially binds with MMP-2 (Zhang and Salamonsen, 1997).

There are several reports describing the presence of mRNA and protein of various MMPs and TIMPs in the human endometrium (Rodgers et al., 1994; Irwin et al., 1996; Jeziorska et al., 1996; Zhang and Salamonsen 1997). These
reports suggest that MMP-2, TIMP-1 and TIMP-2 are expressed in the endometrium relatively constantly throughout the entire menstrual cycle, although there may be a slight decrease in expression of TIMP-3 in the late proliferative phase of the menstrual cycle (Hulboy et al., 1997; Zhang and Salamonsen, 1997). Expression of other metalloproteinases appears to decrease during the early secretory phase of the menstrual cycle but concentrations then increase again at the end of the cycle, when they may have a role in the breakdown of endometrial tissue before and during menstruation (Rodgers et al., 1993; Marbaix et al., 1995). Endometrial MMPs, particularly MMP-2 and MMP-9, are also postulated to have a role in the breakdown of the subepithelial basement membrane in embryo implantation and further breakdown of extracellular matrix molecules in cytotrophoblast invasion (Martelli et al., 1993; Bischof et al., 1995).

Other studies have suggested the importance of MMPs and their inhibitors in the invasive properties of cancer cells (Liotta et al., 1980; Ponton et al., 1991) and a recent study has indicated that high concentrations of MMP-2 and MMP-9 are expressed by endometrial carcinoma cells (Tamakoshi et al., 1995).

These previous studies have all been carried out using qualitative or semi-quantitative immunohistochemical, in situ hybridization or zymography techniques. Our previous studies have shown that the concentration of several proteins in uterine flushings correlates well with the amounts present in the endometrium in vivo (Li et al., 1993; Dalton et al., 1995; Hey et al., 1995). MMPs and TIMPs have been shown to be produced by a variety of endometrial cell types including epithelial cells, stromal cells and leucocytes. The advantage of measurement of proteins in endometrial flushings is that it has the potential to reflect the net amount of proteins in the endometrium derived from a number of cellular sources. In the present study amounts of MMPs and their inhibitors in uterine flushings obtained from normal fertile pre-menopausal women and three groups of post-menopausal women, (normal controls, those with post-menopausal bleeding and adenocarcinoma, and those with post-menopausal bleeding without adenocarcinoma) were therefore measured by zymography and a quantitative ELISA. Because of the size of the flushing sample obtained, measurements of only four different proteins could be performed and therefore the study was limited to that of MMP-2, MMP-3, MMP-9 and TIMP-1.

**Materials and Methods**

**Human subjects**

Uterine flushing samples were obtained from four groups of women for this study.

1. The first group were pre-menopausal normal fertile women volunteers. The ten women in this group were aged between 23 and 38 years and had regular menstrual cycles of between 25 and 35 days. None of the women had taken any steroid hormones for 2 months before the study. The mean number of children was 2.1 (range 1–4). For each subject a uterine flushing was obtained on day LH–4 and day LH+10 of the menstrual cycle. The samples were precisely timed according to the LH surge as described by Li et al. (1993).

2. The second group were control post-menopausal women; this group consisted of eleven post-menopausal volunteer subjects aged between 46 and 60 years who did not have any abnormal bleeding. None of these women was undergoing any form of hormone replacement therapy.

3. This group contained 11 post-menopausal women who were known to have endometrial adenocarcinoma. Their ages ranged from 57 to 84 years and they presented at the gynaecology clinic with post-menopausal bleeding. At the time of sampling none of these women was receiving any treatment.

4. The final group contained 11 post-menopausal women with post-menopausal bleeding, but no carcinoma. The women in this group were aged between 55 and 69 years. Again at the time of sampling none of these women was receiving any treatment.

Local ethical committee approval was obtained for this study and all samples were collected with the informed consent of the patients.

**Collection of uterine flushings**

A uterine flushing sample was obtained from each group of women using a modification of the technique described by Li et al. (1993). A bivalve speculum was inserted into the vagina and through it a Pipelle endometrial sampler, pre-loaded with 1 ml of sterile normal physiological (0.9% w/v NaCl) saline, was slowly introduced into the uterus. After about 30 s, the tip of the sampler was withdrawn by about 2 cm into the lower part of the uterine cavity; the saline solution was then aspirated slowly through the vacuum mechanism of the Pipelle sampler. The plunger was withdrawn by about 2 cm at a time, taking about 30 s in total. A volume of about 0.8 ml was consistently aspirated. The aspirate was immediately centrifuged at 220 g for 5 min and the supernatants were stored at −20°C for MMP and TIMP analysis.

**Protein and haemoglobin estimation**

Amounts of protein in each flushing sample were measured using the Bicinchoninic acid assay (Pierce, Rockford, IL) and BSA as a standard. Amounts of haemoglobin in the flushings were estimated using a Sigma diagnostic kit (Sigma, Poole, Dorset) with the haemoglobin provided as standards.

**Zymography**

All reagents were obtained from Sigma unless stated otherwise. Metalloproteinase activity in the flushings was measured qualitatively by gelatin zymography. Samples (5 μl or 20 μg total protein) were added to non-reducing buffer and run on a 6% polyacrylamide gel containing 0.1%
gelatin. A lane containing molecular weight markers (Sigma) was also included. Gels were run for approximately 2 h at 100 V. Gels were soaked in three changes of 2.5% (v/v) Triton X-100 for 30 min each and then incubated overnight at 37°C in 0.25 mol Tris buffer 1\(^{-1}\) containing 1 mol NaCl and 0.025 mol CaCl\(_2\) 1\(^{-1}\). The gels were then stained with 0.5% (w/v) Coomassie blue R-250, 10% (v/v) acetic acid, 30% (v/v) methanol and destained in 10% (v/v) acetic acid, 45% (v/v) methanol.

**MMP and TIMP ELISA**

The quantitative measurements of MMP-2, MMP-3, MMP-9 and TIMP-1 concentrations in endometrial flushings were obtained using BIOTRA ELISA kits obtained from Amersham Life Sciences and were performed according to the manufacturer’s instructions. Samples were diluted between 1:10 and 1:500 to ensure that they lay on the standard curve provided. The intra-assay variation range was 5–7%, while the inter-assay variation was 8–12%. The sensitivity of the assays were 0.37 ng ml\(^{-1}\) for MMP-2, 0.45 ng ml\(^{-1}\) for MMP-9, 2.35 ng ml\(^{-1}\) for MMP-3 and 1.25 ng ml\(^{-1}\) for TIMP-1.

**Statistical analysis**

The quantitative data obtained from the ELISA measurements were compared in the four groups of women using Student’s \(t\) test or the Mann–Whitney non-parametric analysis as appropriate.

**Results**

An example of the zymography results obtained from uterine flushing samples obtained during the proliferative and secretory phases of the menstrual cycle from pre-menopausal women is shown in Fig. 1. In this experiment equal volumes of each flushing were loaded onto each well of the gel. Similar results were obtained if the volumes were adjusted for protein content. Up to eight different activity bands ranging in molecular mass from 51 to 208 kDa were seen in the flushings. Only very small amounts of activity were seen when the gels were incubated in buffer containing EDTA (1 mmol l\(^{-1}\)) and the inhibition of activity in the presence of EDTA was the same for all bands. Two major bands with molecular masses of 98 and 73 kDa were present in all flushings. Concomitant zymography of flushings with cell culture supernatants of D3X cells (a human melanoma cell line known to produce MMP-2 and MMP-9) showed that the bands in wells from the D3X cell supernatants were in the same position as the 98 and 73 kDa bands seen in the flushings, indicating that these bands corresponded to MMP-9 (92 kDa) and MMP-2 (72 kDa), respectively. More minor bands of molecular masses 208, 143, 123, 90, 67 and 51 kDa were also present in some of the flushings. The results from the ten pre-menopausal women included in this study are summarized in Table 1. Similar proteinase activities, as determined by zymography, were seen in flushings obtained from the same women, but activities varied quite considerably between individuals. It was difficult to determine differences in the proteinase activity in flushings obtained during the proliferative and the secretory phases of the cycle using this method.

The quantitative measurements of MMP-2, MMP-3, MMP-9 and TIMP-1 in the uterine flushings from the same ten pre-menopausal women during the proliferative and secretory phase of the cycle showed similar results to those seen with zymography. Concentrations of MMP-2 (median 320 ng ml\(^{-1}\); range 21–975), MMP-9 (median 59 ng ml\(^{-1}\); range 4–2760) and TIMP-1 (median 816 ng ml\(^{-1}\); range 90–8345) in all flushings were considerably greater than that for MMP-3 (median 32 ng ml\(^{-1}\); range 0–107) and concentrations of MMP-2 and MMP-9 in the flushings obtained from the same women were very similar, but varied considerably between women. Concentrations of MMP-2 were significantly higher in flushings obtained in the secretory phase of the cycle (median 394 ng ml\(^{-1}\); range 91–975) compared with those obtained during the proliferative phase (median 170 ng ml\(^{-1}\); range 21–438) (\(P < 0.05\)). There was no significant difference between the concentrations of the other proteins in flushings obtained during the proliferative and secretory phases of the cycle.

A zymography gel of uterine flushing samples from pre- and control post-menopausal women (Fig. 2a) and from women with post-menopausal bleeding (PMB) with and without adenocarcinoma (Fig. 2b) was obtained by adding equal volumes of flushing to the gel and again similar results were obtained if the volumes were adjusted for protein concentration. Very few bands of activity were seen in the flushings obtained from post-menopausal women. However bands of activity were seen in flushings from women with PMB and these bands were of a similar molecular weight to those seen in the flushings obtained from pre-menopausal women. The proteinase activity seen in flushings from both groups of post-menopausal women with PMB (Table 2) indicates that some women with adenocarcinoma had extremely high concentrations of MMP-2 and MMP-9 in their uterine flushings.
Table 1. Summary of zymography results from pre-menopausal women

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Pro, proliferative and sec, secretory phases of the menstrual cycle.

The quantitative amounts of MMP-2, MMP-3, MMP-9 and TIMP-1 in the uterine flushings from post-menopausal women are shown in Fig. 3. For the pre-menopausal women, values shown are those in flushings obtained on both days LH-4 and LH+10. For some samples, the volume of flushing obtained was not large enough to allow analysis of all four proteins and therefore values are present for only nine or ten flushings in some cases. Concentrations of MMP-2, MMP-9 and TIMP-1 in flushings from post-menopausal control subjects without bleeding were significantly lower than those of pre-menopausal women (P < 0.001, P < 0.05 and P < 0.001, respectively). Concentrations of MMP-2 and TIMP-1 were significantly higher in flushings from women with PMB without carcinoma compared with those of post-menopausal controls (P < 0.05 in both cases) and concentrations of MMP-9 and TIMP-1 in flushings from women with adenocarcinoma were significantly higher than those in postmenopausal control women (P < 0.01 or P < 0.05 for MMP-9 and TIMP-1, respectively). In women presenting with PMB, concentrations of MMP-9 in women with adenocarcinoma (median 385 ng ml⁻¹; range 7–1395) were significantly higher than those without adenocarcinoma (median 10 ng ml⁻¹; range 6–710) (P < 0.05). There were no significant differences in concentrations of MMP-3 in pre- and post-menopausal women, and between post-menopausal women with or without bleeding and/or adenocarcinoma.

There was no difference in the amounts of protein in flushings obtained from pre-menopausal women during the secretory and proliferative phases of the cycle (Table 3). Concentrations of protein in flushings from post-menopausal control women were significantly lower than those in flushings from pre-menopausal women (P < 0.05). Concentrations of protein in flushings from women with

Fig. 2. Zymography gels showing proteinase activity in flushings from (a) pre- and post-menopausal women and (b) women with post-menopausal bleeding with and without carcinoma. 5 μl of flushing was run in each lane. (a) Flushings from pre-menopausal women were run in lanes 4 and 5, and from post-menopausal women in lanes 1–3. Lane 7 contains molecular weight markers. (b) Flushings from women with adenocarcinoma were run in lanes 3–5 (subjects 1–3, respectively), and from women with post-menopausal bleeding (PMB) and no carcinoma in lanes 6–8 (subjects 1–3, respectively). Lane 1 contains molecular weight markers.
considerably, post-menopausal women were also noted to have lower concentrations of haemoglobin, presumably due to the absence of endometrial bleeding.

Endometrial flushing samples were obtained from pre-menopausal and post-menopausal women. In the pre-menopausal group, samples were collected on Day LH-4 and Day LH+10. In the post-menopausal group, samples were obtained from women with PMB (no carcinoma) and PMB (carcinoma). The protein concentrations of each group are shown in Table 3.

Table 3. Concentrations of protein (median and range) in uterine flushings obtained from pre- and post-menopausal women

<table>
<thead>
<tr>
<th>Group</th>
<th>Protein concentration (mg ml⁻¹)</th>
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<td>Pre-menopausal women</td>
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<tr>
<td>Day LH-4</td>
<td>7.4 (1.2-30)</td>
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<tr>
<td>Day LH+10</td>
<td>7.2 (4.8-20)</td>
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<td>Post-menopausal women</td>
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<tr>
<td>Control</td>
<td>1.4 (0.48-5.98)</td>
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<tr>
<td>PMB (no carcinoma)</td>
<td>21.6 (1.1-50)</td>
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<tr>
<td>PMB (carcinoma)</td>
<td>31.5 (4.9-90)</td>
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PMB both with and without adenocarcinoma varied considerably, but were significantly higher than those in flushings from post-menopausal control women (P < 0.001 and P < 0.05, respectively). There was no correlation between concentrations of specific MMPs in the flushing and total protein concentration.

Haemoglobin measurements were carried out to assess the contamination of the uterine flushing with blood. No haemoglobin was detected in any of the flushings obtained from pre-menopausal women or post-menopausal control women. Haemoglobin was present in 2 of 11 (range 1.02-1.35 g dl⁻¹) flushings from women with PMB without adenocarcinoma and 5 of 11 (range 0.62-5.47 g dl⁻¹) flushings from women with adenocarcinoma. There was no correlation between concentrations of specific MMPs in the flushings and the presence or concentration of haemoglobin.

Discussion

There have been several reports using qualitative or semi-quantitative methods showing the expression of various metalloproteinases and their inhibitors in endometrial flushings. However, we have previously shown that concentrations of endometrial proteins in flushings are a good indicator of endometrial secretory activity (Li et al., 1993; McKenna et al., 1993; Dalton et al., 1995; Hey et al., 1995), and would therefore expect concentrations of MMPs in the flushing to reflect endometrial production. However, in contrast to the proteins measured in previous studies, blood cells may be a significant source of the MMPs and TIMP-1 measured in this study. In comparison to early studies (Mathiis and Aitkin, 1978), in this study no haemoglobin was detected in the flushing samples from pre-menopausal and post-menopausal control women. This lack of haemoglobin suggests that the MMPs and TIMP-1 measured in these flushings are not due to contamination with blood cells. The presence of haemoglobin in a proportion of the flushings from post-menopausal women suggests contamination of these samples with blood. However, the fact that concentrations of MMPs and TIMP-1 showed no correlation with the presence or absence of, or the concentration of, haemoglobin suggests that the concentrations in the flushings are unlikely to be due to contamination with blood.
Concentrations of (a) matrix metalloproteinase 2 (MMP-2), (b) MMP-9, (c) tissue inhibitor of metalloproteinase 1 (TIMP-1) and (d) MMP-3 in uterine flushings of pre- and post-menopausal women. Post-Con, post-menopausal control women; PMB, women with post-menopausal bleeding without adenocarcinoma; PMB+Car, women with post-menopausal bleeding and adenocarcinoma; Pre, pre-menopausal women. The horizontal bars represent the median values.

Although the flushing samples used in this and our previous studies appear to show no tissue contamination, to minimise the possibility of the contribution of endometrial tissue to the proteins measured, the flushings were centrifuged on collection and the supernatants frozen immediately. Previous studies have suggested that uterine fluids contain plasma proteins such as albumin (Shirai et al., 1972). However, in these studies it was acknowledged that the flushings were contaminated with blood, which may have been the source of albumin found. Although for the majority of our flushings there was no such contamination, it is possible that the MMPs and TIMP-1 measured in the flushings may have resulted from transfer from the blood through the stromal and epithelial cell compartments into the luminal cavity.

Protein estimations carried out on the flushing samples obtained from the various groups of women showed that concentrations in post-menopausal control women were lower than those in pre-menopausal women, which probably reflect the decrease in endometrial secretory activity in these women. Concentrations of protein in the flushings from pre-menopausal women in this study were ten times higher than those seen in a previous study (Dawood and Fazleabas, 1986). In the earlier study the protein concentration was measured in a 10 ml flushing whereas in this study a 1 ml flushing was obtained. The 10-fold difference in results therefore suggests that both studies are showing similar endometrial protein production. In contrast to this earlier study, no increase in protein concentration was seen in secretory phase flushings in the study reported here. Protein concentrations were higher in women with post-menopausal bleeding (PMB) than in both pre-menopausal women and control post-menopausal women. However, the fact that no correlation was seen between concentrations of MMPs and TIMP-1 in flushings and protein concentrations in any group of women indicates that the specific concentrations of these proteins in the flushings is independent of total protein concentration.

The zymography results obtained in this study showed eight distinct bands of proteinase activity in the flushings from pre-menopausal women corresponding to molecular masses of approximately 208, 143, 123, 98, 90, 73, 67 and 51 kDa. A similar pattern of bands has been reported in the supernatants from cultured stromal and epithelial cells (Martelli et al., 1993). The molecular masses suggest that the bands at 73 and 67 kDa correspond to the inactive and active forms of MMP-2, and that at 51 kDa is MMP-3 or stromelysin. It is also likely that the bands at 98 and 90 kDa correspond to the inactive and active forms of MMP-9, although the true molecular masses of these molecules are
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reported slightly lower at 92 and 83 kDa, respectively. A possible reason for this discrepancy is that the molecular mass standards are run in reducing buffer, whereas the flushing samples are run in non-reducing buffer or that addition of gelatin to the gel alters the electrophoretic mobility of the protein. The zymography and ELISA results suggest that considerably more MMP-2 and MMP-9 are produced by the human endometrium than MMP-3, which was only present in the flushing in very small amounts.

In general, the proteinase activity shown by zymography correlated well with activities found by ELISA, both with respect to activities of proteases in each group of women and the identity of individual proteases present in the flushings. In addition, concentrations of MMP-9 in individual flushings detected by ELISA showed an excellent correlation with the 98K/90K activity bands seen by zymography. MMP concentrations and particularly those of MMP-2 and MMP-9, as assessed by both methods, appeared to vary greatly between individual pre-menopausal subjects, and were more similar in the same subjects taken at different times of the menstrual cycle. The reason for this is unknown.

The ELISA results obtained in this study suggest that concentrations of MMP-9, MMP-3 and TIMP-1 are similar during the proliferative and secretory phases of the menstrual cycle. Other reports have suggested that concentrations of MMP-9 and MMP-3 decrease during the early secretory phase of the menstrual cycle, but then increase very late on in the cycle at the onset of menstruation (Rogers et al., 1994; Jeziorska et al., 1996). In the present study flushing samples were obtained on days LH-4 and LH+10 only, so any subtle changes may have been missed. Concentrations of MMP-2 measured by ELISA were clearly higher in flushings obtained in the secretory phase compared with those obtained in the proliferative phase. Previous studies have suggested that, unlike other proteinases, concentrations of MMP-2 do not fall in the secretory phase of the menstrual cycle (Irwin et al., 1996; Hulboy et al., 1997). Other workers have postulated that MMP-2 and MMP-9 may play a role in embryo implantation (Bischof et al., 1995). The increase in MMP-2 in flushings obtained during the secretory phase of the cycle is consistent with a biological role for MMP-2 in embryo implantation. The results presented here agree with other studies suggesting that TIMP-1 concentrations do not vary during the menstrual cycle (Zhang and Salamonsen, 1997) and therefore, that proteinase activity changes during the menstrual cycle are controlled by changes in concentrations of MMP and not of their inhibitors. However, it should be noted that both zymography and ELISA measure proMMPs and activated MMPs irrespective of whether they are complexed with TIMPs and, therefore, these measurements may not reflect their true biological activities in vivo.

The concentrations of MMP-2, MMP-9 and TIMP-1 in pre-menopausal women were significantly higher than those of post-menopausal control subjects; however, there was no difference in the concentrations of MMP-3 between the two groups. The zymography results also suggested that the concentrations of all MMPs are lower in the endometrium of post-menopausal women. This probably reflects the atrophic nature of the endometrium and an associated decrease in secretory activity in these women.

The zymography results showed the same eight bands of activity in the flushings from pre-menopausal women and women with PMB both with and without adenocarcinoma, and this pattern of results agreed with those reported by Tamakoshi et al. (1994). Both the zymography and ELISA results show that in women with PMB, regardless of whether there is underlying carcinoma, the proteinase activity, and in particular that of MMP-2, MMP-9 and TIMP-1, was higher than those of control post-menopausal women. The reason for the increase in these proteins in flushings from women with PMB, but without carcinoma, is not clear, but may reflect a surge in ovarian steroid production leading to bleeding and stimulation of endometrial secretory activity. The ELISA results also showed that among women with PMB, those with adenocarcinoma appeared in addition to have higher concentrations of MMP-9 than did women without adenocarcinoma. Increased MMP production is a recognized characteristic of many different carcinoma cells (Davies et al., 1993; Tamakoshi et al., 1995) and high concentrations of MMP-2 and MMP-9 have been shown to be produced by endometrial carcinoma tissue (Tamakoshi et al., 1994; Tamakoshi et al., 1995). Previous studies have suggested that MMP concentrations correlate with the invasive nature of the tumour (Liotta et al., 1980; Davies et al., 1993). In the present study, no correlation was seen with the stage of tumour, but as only 3 of the 11 tumours were either grade II or grade III, the numbers are too small to be significant.

The results in the present study give no indication of the cellular source of the various proteinases measured. Previous studies have suggested that the stromal cells are the major source of the MMPs and TIMP-1 measured in this study (Hulboy et al., 1997). However, other reports have suggested that endometrial leukocytes may be an important source of these proteinases (Martelli et al., 1993) and that epithelial cells may produce the lower molecular weight MMPs (Martelli et al., 1993; Rodgers et al., 1994). Our previous studies using endometrial flushings have been on epithelial cell products, which would be expected to be secreted into the lumen of the endometrium. It is possible that stromal cell products may not be secreted into the uterine cavity and that MMPs may be specifically retained by binding to extracellular matrix components, and therefore concentrations in endometrial flushings may reflect the secretion of these proteins by the endometrium less accurately than has previously been shown for epithelial cell products (Li et al., 1993; Dalton et al., 1995; Hey et al., 1995). This may be another reason for some of the differences in our results and those of others.

In summary, eight bands of MMP activity were detected in endometrial flushings from pre- and post-menopausal women. In addition, quantitative measurements of MMP-2, MMP-3, MMP-9 and TIMP-1 were obtained. Both types of result have shown that MMP-2 and MMP-9 are the major gelatinase enzymes produced by the endometrium and that concentrations of MMP-2 are increased in the secretory phase of the menstrual cycle, indicating that it may have a role in embryo implantation. In post-menopausal women
concentrations of MMP-2, MMP-9 and TIMP-1 proteins, but not of MMP-3, are associated with endometrial activity and therefore may have a role in the breakdown of endometrial tissue. In addition, MMP-9 is increased in women with PMB and adenocarcinoma, indicating that it may be produced by endometrial neoplastic cells.

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