Regulation and restricted expression of interstitial collagenase suggest a pivotal role in the initiation of menstruation

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Collagenases are the only mammalian enzymes able to cleave, at neutral pH, the triple helical domain of fibrillar collagens, major constituents of the extracellular matrix of the endometrium. Interstitial collagenase is expressed, secreted and activated in human endometrium only just before and during menstruation. The expression of interstitial collagenase is restricted to the areas of the functional layer of the endometrium which are breaking down and to fragments which have been shed. In endometrial explants, combined sex steroids tightly control the expression, secretion and activation of interstitial collagenase, as well as the preservation of the extracellular matrix. These observations imply a pivotal role for this proteinase in the initiation of menstruation.

Key words: human endometrium/interstitial collagenase/matrix metalloproteinase-1/menstruation/sex steroids

Introduction

In Markee’s classic experiments with rhesus monkeys (Markee, 1940, 1948), all intra-ocular implants of endometrium constantly regressed during the few days preceding menstruation, whereas haemorrhages were limited to some implants, different from one menstrual cycle to the other. Small tissue fragments broke off from the implants, sedimented and vanished from the anterior chamber of the eyes. Although these events indicate that proteinases break down the tissue during menstruation, the nature of the involved enzymes is still a matter of conjecture. Recent evidence from our laboratory (Marbaix et al., 1992, 1995) and others (Hampton and Salamonsen, 1994; Rodgers et al., 1994) strongly support the hypothesis that interstitial collagenase [matrix metalloproteinase-1 (MMP-1)] plays a pivotal role in the initiation of menstruation (Eeckhout, 1990). This paper reviews the published arguments and adds new data supporting this hypothesis.

The extracellular matrix of the human endometrium contains argyrophilic fibres which constitute a well-developed network during the proliferative and secretory phases of the menstrual cycle, but which fragment and disappear at the end of the cycle and during menstruation (reviewed in Woessner, 1982). The argyrophilic fibres are identified as collagen fibres at the level of the electron microscope, and the endometrium contains various collagen types, most abundantly collagen III (Aplin et al., 1988). Collagen fibres have been observed in vacuoles inside stromal cells, in particular in the few days preceding menstruation (Sengel and Stoebner, 1970; Cornillie et al., 1985). These structures have been interpreted as heterophagosomes engaged in collagen digestion, thus involving lysosomal enzymes in menstruation.

The lysosomal concept (Henzl et al., 1972) of endometrial bleeding was supported further by the very high specific activities of several acid hydrolases in this tissue (Cornillie et al., 1990, 1991) and their reported increase during the secretory phase of the menstrual cycle (Wood et al., 1969). Furthermore, the concomitant increase in the
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non-sedimentable fraction of lysosomal enzymes (Rosado et al., 1977) suggested either an increased fragility of lysosomal membranes and subsequent leakage of enzymes, or the secretion of lysosomal hydrolases in the extracellular matrix. The secretion of a lysosomal enzyme was indeed observed during culture of human endometrium (Cornillie et al., 1990). Acid phosphatase, used as a marker enzyme of lysosomal acid hydrolases, has been detected by ultrastructural cytochemistry at the basolateral membranes of epithelial cells and between endothelial cells, as well as on the basement membranes of arterioles at the end of the late secretory phase and during the first day of menstruation (Henzl et al., 1972). It has also been proposed that the release of a lysosomal phospholipase could induce the production of arachidonic acid and subsequently prostaglandins, such as prostaglandins F<sub>2α</sub> and E<sub>2</sub> which are increased in the late secretory phase and during menstruation respectively (Downie et al., 1974). The vasoconstriction induced by prostaglandin F<sub>2α</sub> could, in turn, be responsible for endothelial lesions. Although these observations were compatible with a role for lysosomal hydrolytic enzymes in epithelial and endothelial disruption during menstruation, possibly by digesting components of the cell–cell junctions (Tabibzadeh et al., 1995), they provided no clue as to their possible involvement in the degradation of the endometrial extracellular matrix which precedes menstruation.

More recently, MMP have been proposed to play a pivotal role in the initiation of menstruation (Eeckhout, 1990). MMP are a family of enzymes able to degrade most proteins of the extracellular matrix at neutral pH (for reviews see Woessner, 1991; Matrisian, 1992; Birkedal-Hansen et al., 1993). Three subfamilies can be distinguished among MMP, according to their substrate specificities. Collagenases (interstitial collagenase, MMP-1; neutrophil collagenase, MMP-8; rodent-type collagenase, MMP-13; and, as reported recently, inhibitor-free gelatinase A, MMP-2; Aimes and Quigley, 1995) are the only mammalian enzymes able to cleave the triple helical domain of fibrillar collagens at neutral pH. Thus they are good candidates to play an essential role in the degradation of the endometrial extracellular matrix. Gelatinases A and B (MMP-2 and MMP-9) can further degrade the denatured collagens and can also digest non-fibrillar collagens such as collagen type IV. Stromelysins-1 and -2 (MMP-3 and MMP-10) can degrade various components of the extracellular matrix, such as collagen type IV, laminin, fibronectin and proteoglycans.

MMP are synthesized as latent proenzymes and need the proteolytic cleavage of an ~10 kDa N-terminal propeptide to become active. All proMMP are constitutively secreted in the extracellular matrix, except MMP-8 (which is stored in the specific granules of neutrophils) and the recently described membrane-bound MMP, e.g. MT-MMP (which has been implicated in the activation of proMMP-2) (Sato et al., 1994). The physiological activators of procollagenases and of related proMMP are still unknown. Stromelysins-1 and -2, and matrilysin (MMP-7) can superactivate MMP-1, but these MMP need to be activated themselves, and the superactivation process is rather slow unless proMMP-1 is already partially activated. Kallikrein, cathepsin B and plasmin have been proposed as physiological activators of collagenase (Eeckhout and Vaes, 1977). The plasmin/plasminogen activator system is particularly active in the endometrium during the perimenstrual phase (Rybo, 1966), being involved in fibrinolysis of the menstrual thrombi. Furthermore, progesterone has been shown to regulate the production of plasminogen activators (Casslén et al., 1986) and of plasminogen activator inhibitor-1 (Casslén et al., 1992; Schatz et al., 1995) in endometrial tissue or stromal cells. Tissue inhibitors of metalloproteinases, as well as α<sub>2</sub>-macroglobulin, inhibit active MMP. Regulation of the expression of proMMP, their activation and their inhibition are thus probably involved in the control of the degradation of the extracellular matrix.

Materials and methods

Organ culture

Normal endometrial tissue was obtained at various periods during the menstrual cycle from biopsies performed for histological dating of the endometrium or from hysterectomies performed for pathologies unrelated to the endometrium. Proges-
terone and oestradiol serum concentrations were measured by a radioimmunoassay at the time of sampling in the majority of patients. Part of the tissue was fixed in freshly prepared 4% formaldehyde. The study was approved by the ethical committee of the University of Louvain, Bruxelles, Belgium.

Endometrial explants were cultured as described previously (Marbaix et al., 1992) in Dulbecco's modified Eagle medium (Gibco Europe, Merelbeke, Belgium), without serum and Phenol Red; culture medium was renewed every 6–24 h. Water-soluble complexes of 2-hydroxypropyl-β-cyclodextrin and progesterone or 17β-oestradiol (Sigma-Aldrich, Bornem, Belgium) were added to the culture medium at the indicated nominal concentrations, or were replaced by identical amounts of the vehicle alone in controls. In some cultures, 10 µM monensin (Calbiochem-Novabiochem, Bierges, Belgium) were added to block the secretion of proMMP-1 and thereby sensitize its immunodetection (Hembry et al., 1986). This ionophore abolishes proton gradients inside the cell, thus retaining the newly synthesized proteins in the Golgi vesicles (Tartakoff, 1983). Collected conditioned media were supplemented with 0.05 volumes of 1 M Tris-HCl buffer (pH 7.5), 1% (v/v) Triton X-100, 0.1 M CaCl₂, 60 mM NaN₃ and kept frozen at -80°C until use. At the end of the culture, explants were fixed for 6–12 h at 20°C in freshly prepared 4% formaldehyde and embedded in paraffin for morphological analysis.

**Enzyme assays**

Collagenase activity was determined at 25°C with [³H]acetylated collagen in solution (Eeckhout et al., 1986). One unit of collagenase is defined as the amount of enzyme which degrades 1 µg of soluble collagen/min. Total collagenase activity was assayed after treating the conditioned medium for 2 h at 37°C with 2 mM 4-aminophenylmercuric acetate (Aldrich) to activate the proenzyme. This organomercurial is assumed to dissociate the cysteine of the propeptide from the Zn of the catalytic domain, thereby allowing auto-activation by intramolecular cleavage of the propeptide (Woessner, 1991). Spontaneously active collagenase was measured without pretreatment of the medium. Statistical comparisons were made using Wilcoxon's non-parametric two-samples test.

**Histological and immunohistological analysis**

Paraflin sections of non-cultured tissue and of explants were stained with haematoxylin and eosin or were impregnated with silver to stain the argentophilic fibre network (Gordon and Sweet, 1936). Immunohistochemical labelling of (pro)MMP-1 was performed with specific monoclonal antibodies (Zhang et al., 1993) kindly provided by Y. Okada (Kanazawa University, Kanagawa, Japan) and K. Iwata (Fuji Chemical Industries Ltd, Toyanna, Japan), using a biotin–streptavidin peroxidase procedure. Purified mouse monoclonal anti-Leu-1 antibodies (Becton-Dickinson Benelux, Erembodegem, Belgium), of the same immunoglobulin G isotype were used as controls. Briefly, after inactivation of the endogenous peroxidases with 0.3% H₂O₂ (v/v) for 30 min and the blocking of non-specific binding with phosphate-buffered saline containing 1% (w/v) bovine serum albumin for 1 h, both at room temperature, sections were incubated overnight at 4°C with 0.6 µg/ml anti-MMP-1 antibody. Immunolabelling was revealed by incubating the sections at room temperature with 2 µg/ml biotinylated sheep anti-mouse antibodies (Boehringer Mannheim Belgium, Bruxelles, Belgium) for 1 h and with 0.5 IU/ml peroxidase-conjugated streptavidin (Boehringer Mannheim) for 30 min. Peroxidase activity was detected by a 10 min incubation with 0.03% H₂O₂ and 0.6 mg/ml 3,3′-diaminobenzidine (Amersham Belgium, Gent, Belgium) in 50 mM Tris–HCl buffer, pH 7.4. Sections were counterstained with haematoxylin and microphotographs were taken using a Zeiss 450–490 run filter to better discriminate the brown signal from the blue background.

**In-situ hybridization**

A human MMP-1 cDNA (gift from H. Nagase, Kansas University, KS, USA) was inserted into a pBluescript® vector (KS²; Stratagene-Westburg, Leusden, The Netherlands) and both sense and antisense riboprobes were transcribed in vitro using [³⁵S]UTP (Amersham kit) by the T3 RNA polymerase (Boehringer Mannheim). The sense riboprobe was transcribed from position 204 to 662
Regulation and restricted expression of interstitial collagenase after the ATG start codon of the human MMP-1 cDNA, i.e. from a region coding for the cysteine switch and for a part of the catalytic domain. The antisense probe was transcribed from position 1879 to 1394 after the ATG start codon, i.e. from a region coding for a small part of the haemopexin-like domain and for the 3' untranslated region. The specificity of the cDNA was checked in Northern blots by hybridization with sense riboprobes transcribed from MMP-2, MMP-3 and MMP-8 cDNAs [gifts from G.I. Goldberg (Washington University, St Louis, MO, USA), H. Nagase and H. Tschesche (University of Bielefeld, Germany) respectively].

After paraffin removal, tissue sections were treated with 1 μg/ml proteinase K (Boehringer Mannheim) at 37°C for 20 min, acetylated, dehydrated and air dried. Sections were then hybridized overnight at 50°C with antisense and sense riboprobes (30 000–50 000 c.p.m/μl) which had been denatured for 2 min at 80°C just before starting the hybridization. The hybridization mixture contained 100 mM dithiothreitol, 50% (v/v) formamide, 0.3 M NaCl, 20 mM sodium acetate, 5 mM EDTA, 0.2% bovine serum albumin, 0.2% Ficoll, 0.2% polyvinylpyrrolidone, 10% dextran sulphate (all w/v) and 700 μg/ml yeast tRNA. After hybridization, sections were washed three times for 15 min and once for 45 min at room temperature in 4× SSC (1× SSC is 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0), rinsed in 2× SSC and incubated for 25 min at 55°C in a stringent wash with 50% (v/v) formamide, 0.15 M NaCl, 1 mM EDTA, 10 mM dithiothreitol and 20 mM Tris-HCl, pH 8.0. Slides were then treated for 30 min at 37°C with 30 μg/ml RNase A and 1 μg/ml RNase T1 (Boehringer Mannheim), washed, dehydrated, air dried and dipped into K5 Ilford emulsion diluted with the same volume of 300 mM ammonium acetate and 1% (v/v) glycerol. Slides were exposed for 1 week at 4°C, developed with G150 Agfa for 75 s and fixed with G350 Agfa for 15 min. Sections were lightly counterstained with haematoxylin.

Results

**Secretion and activation of collagenase is limited to the perimenstrual phase.**

When endometrial explants are cultured for 1 day without added sex steroid, tissues presumably mimic to a large extent their in-vivo situation at the time of sampling, especially with regard to their sex steroid content. Although the exact concentration of sex steroids within the endometrial tissue is not known, it can be assumed that tissue concentrations correlate with those assayed in the serum of the patients at the time of sampling. Only endometria sampled during the perimenstrual period released high levels of total collagenase activity during the first day of culture (Marbaix et al., 1995). Furthermore, spontaneously activated

![Figure 1. Collagenase secretion and activation are restricted to the perimenstrual period. Twenty-one endometria sampled throughout the menstrual cycle were cultured for 1 day in the absence of sex steroid. Endometria are distributed according to their histological appearance in a menstrual cycle starting at ovulation. Oestradiol (A) and progesterone (C) concentrations were measured in the serum of patients at the time of sampling (a). Collagenase activity was assayed in the conditioned media (b), either without treatment to measure the spontaneously activated collagenase during culture (○) or after treatment with aminophenylmercuric acetate to activate the proenzyme and thus measure the total collagenase activity secreted (○). Values are means ± SD or measurements in pools. Total activities of 13 out of the 21 endometria are reproduced from Marbaix et al. (1995) (with kind permission of the Biochemical Society and Portland Press, London, UK).
Collagenase was only detected in media conditioned by menstrual endometria. The inverse relationship between plasma steroid concentration and collagenase secretion and activation is evident in Figure 1.

**Sex steroids regulate the secretion of collagenase**

Menstrual endometria released high levels of total collagenase activity throughout the 4 days of culture without sex steroid (Figure 2a). In contrast, total collagenase activity in media conditioned by non-menstrual endometria increased during days 2 and 3 of culture without sex steroid, reaching levels similar to those observed with menstrual endometria. Progesterone added at physiological concentrations (50–100 nM) significantly reduced the level of total collagenase activity released by non-menstrual explants but failed to inhibit when used with menstrual explants.

Oestradiol added at the physiological concentration of 1 nM together with progesterone significantly enhanced the inhibitory effect of progesterone (Figure 2a). The inhibitory effect of progesterone and of combined oestradiol and progesterone was still observed after 1 or 2 days of culture without sex steroid (Figure 2b and c), demonstrating that the tissue remained hormone responsive for at least 2 days of culture without added sex steroid. Indeed, oestradiol and progesterone receptors remained detectable by immunohistochemistry in explants cultured for 2 days in the same conditions (data not shown). Moreover, when both sex steroids were added to the culture medium after 2 days, a significant inhibition could be maintained during days 4 and 5 of culture (Figure 2b). Progesterone

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**Figure 2. Regulation of collagenase secretion in culture by sex steroids.** (a) Explants from a menstrual endometrium (∆ and ▲) and from a proliferative endometrium (○, ● and ■) were cultured for 4 days. Culture medium was devoid of sex steroid (—H; △ and ○; six and seven groups of explants respectively) or supplemented with either 100 nM progesterone (+P; ▲ and ●; six and four groups of explants respectively) or both 1 nM oestradiol and 100 nM progesterone (+E+P; ■; five groups of explants). Total collagenase activity was measured in all conditioned media. Medians are presented and statistical comparisons were made within each culture (proliferative or menstrual), with either explants cultured without added sex steroid (∗∗P < 0.05; ∗∗∗P < 0.01) or explants cultured with progesterone alone (+P < 0.05). Figure reproduced from Marbaix et al. (1995), with kind permission of Portland Press. (b) Twelve groups of explants from a proliferative endometrium were cultured for 5 days in the presence of 1.5 μM insulin. No sex steroid was added during the first 2 days of culture (□; —H). In six groups of explants, culture was pursued without sex steroid (□; —H), whereas in six other groups of explants, 1 nM oestradiol and 50 nM progesterone were added to the culture medium during the last 3 days of culture (■; +E+P). Medians of the total collagenase activity secreted are presented. Statistical significance is as in (a). (c) Explants from an early secretory endometrium were cultured for 4 days in the presence of 1.5 μM insulin. Thirteen groups of explants were cultured during day 1 (d 1) without sex steroid (—H) and during the next 3 days (d 2–4) either without sex steroid (□) or with 50 nM progesterone (●; +P). Seventeen other groups of explants were cultured during day 1 with 1 nM oestradiol (+E) and during the next days without sex steroid (△), with 1 nM oestradiol (△) or with 50 nM progesterone (■). Medians of the total collagenase activity secreted are presented. Statistical comparisons were made by reference to □ or △ (∗P < 0.05, ∗∗P < 0.01), to ▲ (∗∗P < 0.05) or to ● (∗∗∗P < 0.05; ∗∗∗P < 0.01).
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alone ceased to inhibit after 3 days of culture (Figure 2c).

Tissue priming with oestradiol during the first day of culture amplified the inhibitory effect of progesterone added during the following days of culture (Figure 2c). Indeed, explants primed with oestradiol during 1 day and cultured with 50 nM progesterone thereafter secreted significantly less total collagenase activity than explants not primed during the first day of culture, as well as significantly less total collagenase activity than explants cultured with oestradiol alone throughout the culture period. Although oestradiol had a moderate but significant inhibitory effect by itself on the secretion of collagenase throughout culture, oestradiol priming had no effect on its own because no difference with non-primed tissue was observed when pursuing the culture without sex steroid.

**Sex steroids regulate the activation of collagenase**

When endometrial explants were cultured without sex steroid, a significant proportion of the total collagenase activity secreted during day 2 of culture was found in a spontaneously active form (Figure 3). In contrast, when explants from the same endometria were cultured in the presence of physiological concentrations of oestradiol and progesterone, not only was the secretion of total collagenase activity decreased, but the proportion of spontaneously active collagenase was also reduced significantly ($P < 0.005$, using Wilcoxon’s paired rank-sum test).

**Expression of interstitial collagenase is limited to areas of tissue breakdown**

MMP-1 mRNA was detected by in-situ hybridization only in endometria sampled in the very last days of the late secretory phase and during menstruation ($n = 7$), but was totally absent during all other periods of the menstrual cycle ($n = 10$; Kokorine et al., 1996). When present, MMP-1 mRNA was restricted to foci of stromal cells of the functional layer, where the latter showed morphological signs of menstrual breakdown such as tissue fragmentation and disappearance of the argyrophilic fibrillar network (Figure 4). Furthermore, when shedded menstrual tissue was examined, MMP-1 mRNA was found in numerous stromal cells preferentially localized at the periphery of the fragments, realizing a characteristic ‘tear along the dotted line’ pattern (Kokorine et al., 1996).

**Sex steroids preserve the extracellular matrix of endometrial explants**

The well-developed network of argyrophilic fibres present in non-menstrual endometria was perfectly preserved in explants from these endometria cultured for 2 days in the presence of physiological concentrations of oestradiol and progesterone (Figure 5a). In such conditions, the explants secreted low levels of total collagenase. In striking contrast, explants from the same endometria cultured for 2 days without added sex steroid released high levels of total and spontaneously active colla-
Figure 4. Expression of matrix metalloproteinase-1 (MMP-1) in menstrual endometrium is limited to areas of matrix breakdown. A hysterectomy was performed for myomas in this 37 year old woman during a menstrual period. Serial histological sections of the endometrium show fragmentation of the superficial portion of the functional layer (a, haematoxylin–eosin), where the MMP-1 mRNA is detected in stromal cells by in-situ hybridization (b). The network of argyrophilic fibres is interrupted in the functional layer and has disappeared in the upper layer of the mucosa, where MMP-1 is expressed (c, silver impregnation).

Figure 5. Sex steroids preserve the argyrophilic fibres during organ culture. Explants from a mid-secretory endometrium were cultured for 2 days in the presence of 1 nM oestradiol and 100 nM progesterone (a) or without sex steroid (b). Histological sections were silver stained. In this experiment, explants cultured with oestradiol and progesterone (a) released 0.37 U/ml of total collagenase activity during the second day of culture, 16% of which was spontaneously activated during culture. In contrast, explants cultured without sex hormone (b) secreted 2.48 U/ml of total collagenase activity, 73% of which was spontaneously activated during culture.

Interstitial collagenase is focally expressed at arterioles

MMP-1 was immunolocalized in numerous stromal cells in explants cultured without sex steroid and in the presence of 10 μM monensin, which efficiently blocked the secretion of the protein. Some of these stromal cells were particularly localized in or around the wall of small vessels (Figure 6). A similar localization in the wall of arterioles was observed in non-cultured menstrual endometria by in-situ hybridization of MMP-1 mRNA (Kokorine et al., 1996).
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Discussion

The expression of interstitial collagenase is clearly restricted to menstruation. By Northern blot analysis, MMP-1 mRNA was only detected in endometrial tissues sampled around menstruation (Hampton and Salamonsen, 1994; Marbaix et al., 1995). These results were confirmed by in-situ hybridization studies (Rodgers et al., 1994; Kokorine et al., 1996). Furthermore, explants cultured in conditions as close as possible to the in-vivo situation secrete high levels of collagenase activity only when sampled around menstruation (Marbaix et al., 1995; Figure 1), and a significant proportion of the secreted collagenase is spontaneously activated only in menstrual endometria (Figure 1).

Regulation of the expression of MMP-1 is remarkably reproduced in organ culture by added sex steroids. Indeed, physiological concentrations of progesterone, especially when combined with oestradiol, decrease the steady state level of MMP-1 mRNA (Marbaix et al., 1995) and inhibit the secretion and activation of collagenase by endometrial explants (Marbaix et al., 1992; Figures 2 and 3). Oestradiol is found to moderately inhibit by itself the secretion of MMP-1. However, the increased inhibition observed when oestradiol is combined with progesterone, or when oestradiol has primed the tissue, suggests that oestradiol also acts by increasing the expression of progesterone receptors. Similarly, the level of preservation of the progesterone receptors could account for the lower responsiveness of explants to progesterone alone after 1 day of culture without sex steroid, than to combined oestradiol and progesterone, even after 2 days of culture without sex steroid.

Evidence from in-vitro regulation by oestradiol and progesterone provides a reasonable explanation for the absence of expression, secretion and activation of MMP-1 during all non-menstrual periods of the cycle, i.e. when the endometrial mucosa should be protected against breakdown. Indeed, the high concentrations of progesterone during the secretory phases of the cycle can completely block the expression, secretion and activation of MMP-1. During the proliferative phase, oestradiol could also inhibit MMP-1 expression by itself and/or could make the tissue responsive to the low concentration of progesterone by increasing the amount of progesterone receptors. At the end of the cycle, the fall in the concentrations of both sex steroids and receptors would relax the inhibition and allow menstruation to proceed.

Localization of the expression of MMP-1 further supports a role for this proteinase in menstruation. Indeed, MMP-1 mRNA is found exclusively in the functional layer of the endometrium during menstruation, and in particular at foci undergoing fragmentation and lysis of the extracellular matrix, as assessed by the disappearance of the network of argyrophilic fibres (Figure 4). The presence of numerous stromal cells showing a strong in-situ hybridization signal for MMP-1 mRNA at the periphery of shedded menstrual fragments also suggests that the proteinase is involved in tissue breakdown. Modifications of the basement membrane components of endometrial vessels have been observed at menstruation (Kelly et al., 1995) and, despite the better preservation of the argyrophilic fibres in the wall of some arterioles, the finding of MMP-1 mRNA and of MMP-1 at or around arterioles and small vessels (Figure 6) could be relevant in the genesis of the focal haemorrhages accompanying menstrual shedding of the endometrium.

Figure 6. Matrix metalloproteinase-1 (MMP-1) is expressed in stromal cells in the wall of small endometrial vessels. This explant from a menstrual endometrium was cultured for 1 day without sex steroid and with 10 μM monensin (to inhibit the secretion of MMP-1). In this histological section, MMP-1 immunolabelling is preferentially localized at or immediately around small arteries (arrowheads). Notice also sparse labelling of the stromal cells.
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