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Matrix Metalloproteinase Expression in a Mouse Menstrual-Like Model by Pharmacologic Progesterone Withdrawal

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Abstract: MMPs are vital in menstruation. This study aimed to investigate their expressions in a mouse menstrual-like model by pharmacologic progesterone withdrawal. The model was prepared by administration of mifepristone after decidualization of ovariectomized mice. Immunohistochemistry was performed to detect the locations of Matrix Metalloproteinase (MMPs) proteins. MMP3 was present in the focal subluminal stromal zone, epithelium, decidual zone and interface between the basal zone and functional zone from 0-24 h and epithelium and subluminal epithelium stroma from 32-48 h. MMP7 was present in the basal zone, epithelium and mid decidual zone from 0-24 h and a similar location to MMP3 from 32-48 h. MMP9 was present in the basal zone and focal zone of the subluminal stroma from 0-24 h, epithelium and the edge of tissue breakdown at 32 h, basal zone and the subluminal stroma at 40 h and the subluminal stroma at 48 h. MMP13 was present in the mid decidual zone at 24 and 32 h and epithelium at 40 and 48 h. MMP2 was present in the basal zone and mid decidual zone from 0-24 h, the mid decidual zone at 32 h and no staining at 40 and 48 h. The locations of MMPs protein in the model displayed regional character and showed consistency with those in the mice model by physiological progesterone withdrawal and in primates.

Key words: Mouse, pharmacological withdrawal of progesterone, menstruation, matrix metalloproteinase, regional distribution

INTRODUCTION

Menstruation is the separation of the functional layer from the basal layer of the endometrium, accompanied by bleeding and occurs following progesterone withdrawal. The detailed mechanism of menstruation has not been fully elucidated, partly because of a lack of a suitable animal menstrual model.

The primitive animal model can be traced back to explanting from the human endometrium to rhesus monkey intraocular (Markee, 1940). Later, a model of menstruation in mice, a mammalian species which does not menstruate naturally was established in the 1980s (Finn and Pope, 1984). In the model, physiological progesterone withdrawal led to endometrial shedding and bleeding in ovariectomized mice prepared by sequential administration of estrogen and progesterone followed by oil infusion into the uterine lumen. The model was optimized by Salamonsen's group (Brasted *et al*., 2003) in which tissue breakdown was marked by 16 h, the decidual zone shed by 24 h with bleeding, then the endometrium regenerated completely within 48 h. The two models were defined as mouse menstrual-like models induced by physiological progesterone withdrawal. In the laboratory, a mouse menstrual-like model induced by pharmacological progesterone withdrawal was established (Xu *et al*., 2007) in which pharmacological progesterone withdrawal by mifepristone administration instead of physiological progesterone withdrawal was applied and shedding of the endometrium occurred with bleeding and subsequent regeneration as in the model induced by physiological progesterone withdrawal. The sequence of events was also consistent with the model induced by physiological progesterone withdrawal. The two mouse menstrual-like models are very useful for providing a platform to study menstruation mechanism.

Matrix Metalloproteinases (MMPs), a multigene family of enzymes that have the capacity to degrade components of the extracellular matrix are proved to play a role in the breakdown of the endometrium (Marbaix *et al*., 1996) and a considerable body of evidence supports the idea (Jabbour *et al*., 2006). In animal models, the locations of MMPs have been studied. In rhesus macaques, MMPs were confined to the upper functionalis

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zone and showed zone-specific tissue gradients during menstruation (Glasser *et al*., 2002; Rudolph-Owen *et al*., 1998). Expressions of MMPs were upregulated by progesterone withdrawal and decreased spontaneously after menstruation. Importantly, in a mouse menstrual-like model of menstruation induced by physiological withdrawal of progesterone, immunohistochemistry showed the location of MMPs during the whole breakdown and regeneration process and found that MMP9 was located in the basal zone of the decidualized endometrium at the edge of the decidua and co-located with some subsets of leukocytes. The staining of MMP13 was extracellular and MMP3 and MMP7 were abundant during re-epithelialization at levels similar to those in the newly forming epithelium (Brasted *et al*., 2003). However, no parallel data was reported in the mouse model by pharmacological progesterone withdrawal.

Here, researchers describe the study on the location of MMPs in the mouse menstrual-like model induced by pharmacological block of progesterone. Researchers observed a similar distribution as in the mouse menstrual-like model induced by physiological progesterone withdrawal. The similarities and differences in the two models were discussed.

MATERIALS AND METHODS

Animals: Female virgin C57 mice 8-12 weeks old were obtained from the Animal Services of the National Research Institute for Family Planning. Mice were kept under controlled light (lights on from 0600-1800) and temperature (21±1°C) and allowed free access to food and water. Experimental and surgical procedures were approved by the Animal Ethics Committee of National Research Institute for Family Planning (Approval ID: 20100318).

Induction of the Mouse Menstruation Model: The manipulation of the animals followed the procedure described by Xu *et al*. (2007) as shown in Fig. 1. Animals were ovariectomized under anesthesia and allowed to recover for 1 week. On days 1, 2 and 3, all mice were subcutaneously (s.c.) injected daily with 100 ng of 17β-Estradiol (E2) (Alfa Aesar Inc., Heysham, UK) in arachis oil at 0930 h. After resting for 3 days, progesterone implants were inserted sc into the back of each mouse at 0930 h on day 7 and 50 ng of progesterone (Sigma-Aldrich Inc., St. Louis, MO, USA) and 5 ng of 17β-E2 in arachis oil were also injected sc. On days 8 and 9, 5 ng of 17β-E2 in arachis oil was injected sc at 0930 h. On day 9 at 1130 h, 20 μL of arachis oil was injected into the lumen of the left uterine horn of each mouse through a dorsal incision to induce decidualization. The right horn was not treated with arachis oil and served as a negative control. After 48 h, 120 mg kg^{-1} mifepristone (Beijing Zizhu Pharmaceutical Co., Ltd. Beijing, China) was given to the mouse by intragastric administration at 1130 h (regarded forthwith as 0 h). Mice were sacrificed by cervical dislocation at 0, 8, 16, 24, 32, 40 and 48 h (n = 10 for each time point) after mifepristone administration, uterine horns were harvested at each time interval and fixed in 4% paraformaldehyde, three sections each mice were for analysis of histomorphology and immunohistochemistry.

Immunohistochemical analysis of MMP3, MMP7, MMP9, MMP13 and MMP2: Immunohistochemistry was performed with PowerVision™ Two-Step Histostaining Reagent (Zhongshan GoldenBridge Biotechnology, Beijing, China). Briefly, uterine cross-sections were deparaffinized and rehydrated followed by an antigen retrieval procedure. After soaking in 3% H_2O_2 in methanol for 15 min, every other section was treated with rabbit anti-mouse primary antibody against MMP3 (diluted 1:50, Boster Biological Technology Co., Ltd. Wu han, China), MMP7 (diluted 1:100, Boster), MMP9 (diluted 1:50, Boster) and MMP13 (diluted 1:100, Boster), rabbit anti-human MMP2 (diluted 1:200, Biosynthesis Biotechnology Co., Ltd. Beijing, China) and incubated overnight at 4°C. Meanwhile, rabbit IgG2b antibodies matched to the MMPs IgG were applied to the adjacent sections as negative controls.

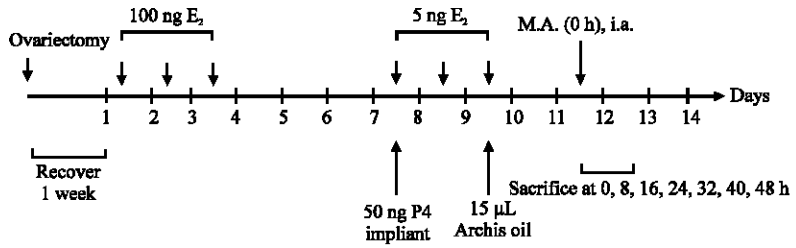

Fig. 1: Treatment schedule. Mifepristone administration was taken as 0 h. M.A. = Mifepristone Administration, E2 = Estradiol, P4 = Progesterone and i.a. = intragastric administration

The sections were then treated with biotinylated goat anti-rabbit secondary antibody (Zymed Laboratories, San Francisco, CA, USA) in blocking buffer and incubated for 30 min at 37°C. The antibody was visualized with 3, 3'-Diaminobenzidine tetrahydrochloride (DAB) solution counterstained with hematoxylin. Each treatment step mentioned above was followed by three 5 min washes in phosphate-buffered saline unless specifically described.

RESULTS

Mouse menstrual-like model induced by pharmacological progesterone withdrawal: As previously described in the mouse menstrual-like model of menstruation induced by mifepristone treatment, the endometrium had developed full decidualization before mifepristone treatment by oil administration to the mouse uterine cavity for 48 h. (Fig. 2A1) With progesterone withdrawal, the decidual zone in the endometrium started focal cellular necrosis at 8 h was marked at 16 h (Fig. 2A2) and then the whole functional zone broke down and began to slough off into the uterine cavity from the basal zone at 24 h. At the same time, bleeding was visualized on vaginal smears. At 32 h, the endometrium was completely sloughed off into the uterine cavity while the epithelium began to repair by re-epithelialization (Fig. 2A3), the endometrium further repaired at 40 h (Fig. 2A4) and full repaired by 48 h (Fig. 2A5). The procedure of endometrial breakdown and repair occurred in the oil-induced decidualized uterus but the changes were not seen in the nondecidualized uterus.

Immunohistochemical analysis of MMP3, MMP7, MMP9, MMP13 and MMP2: The locations of protein MMPs in the endometrium were detected by immunohistochemistry over 48 h after mifepristone treatment in the Pharmacological mouse menstrual-like model in which endometrium went from breakdown and shedding to complete repair.

Immunohistochemical analysis of MMP3: In the decidualized uterus, positive staining for MMP3 emerged and was confined to the focal subluminal stroma and epithelial zones (including the glandular epithelium and luminal epithelium) at 0 and 8 h (Fig. 2B1). With progressive endometrial breakdown, immunoreactive staining was found in the decidual zone and was more marked in the interface between the basal zone and functional zone at 16 h (Fig. 2B2) then there was positive staining only in the epithelium at 24 h. With endometrial repair, weak staining was confined to the middle of the area of tissue breakdown while intense staining was present in the repaired luminal and glandular epithelial zone at 32 h. It was notable that intense staining appeared not only in the epithelium but also in the subluminal epithelial stroma at 40 and 48 h (Fig. 2B3). However, in the non-decidualized uterus, intense staining was only detected in the epithelial zone of the endometrium throughout the whole mifepristone treatment (Fig. 2B4). Negative control was also showed (Fig. 2B5).

Immunohistochemical analysis of MMP7: In the decidualized uterus, from 0-24 h, positive staining for MMP7 was located in the basal zone and epithelial zones (including the glandular epithelium and luminal epithelium) and with progesterone withdrawal, the intensity in the basal zone gradually increased. Meanwhile, positive stainings at 16 and 24 h were also visible in the middle of the decidual zone. With the endometrium repaired, weak staining was seen in areas of tissue breakdown and importantly, strong staining was visible in epithelium at 32 h (Fig. 2C1). Interestingly, there was also strong immunoreactive staining in the subluminal epithelial stroma at 40 h (Fig. 2C2) and 48 h (Fig. 2C3) with stronger intensity at 40 h than 48 h. However, staining in the non-decidualized uterus was only confined to the epithelium and the intensity in the epithelium at 32 and 40 h was weaker compared with that in the decidualized uterus and did not change during mifepristone treatment (Fig. 2C4). Negative control was also showed (Fig. 2C5).

Immunohistochemical analysis of MMP9: In the decidualized uterus, immunopositive MMP9 cells were located in the basal zone and the focal zone of the subluminal stroma at 0 h (Fig. 2D1). From 8-24 h, staining was confined to the basal zone and the intensity and frequency increased dramatically (Fig. 2D2). At 32 h, the functional layer sloughed off into the uterus, very weak staining was found in the reforming epithelium while intense staining was located within the area of tissue breakdown, especially at the edge of tissue breakdown. Apart from being present in the basal zone, the intense staining remained in the subluminal stroma at 40 h (Fig. 2D3) and till the complete repair at 48 h, the only visible staining was in the subluminal stroma. However, there was virtually no visible positive staining in the non-decidualized uterus during mifepristone treatment (Fig. 2D4). Negative control was also showed (Fig. 2D5).

Immunohistochemical analysis of MMP13: No positive staining was detected in the endometrium of the decidualized uterus from 0-16 h. Positive staining was visible till the endometrium breakdown occurred evidently, namely at 24 h when staining was widespread

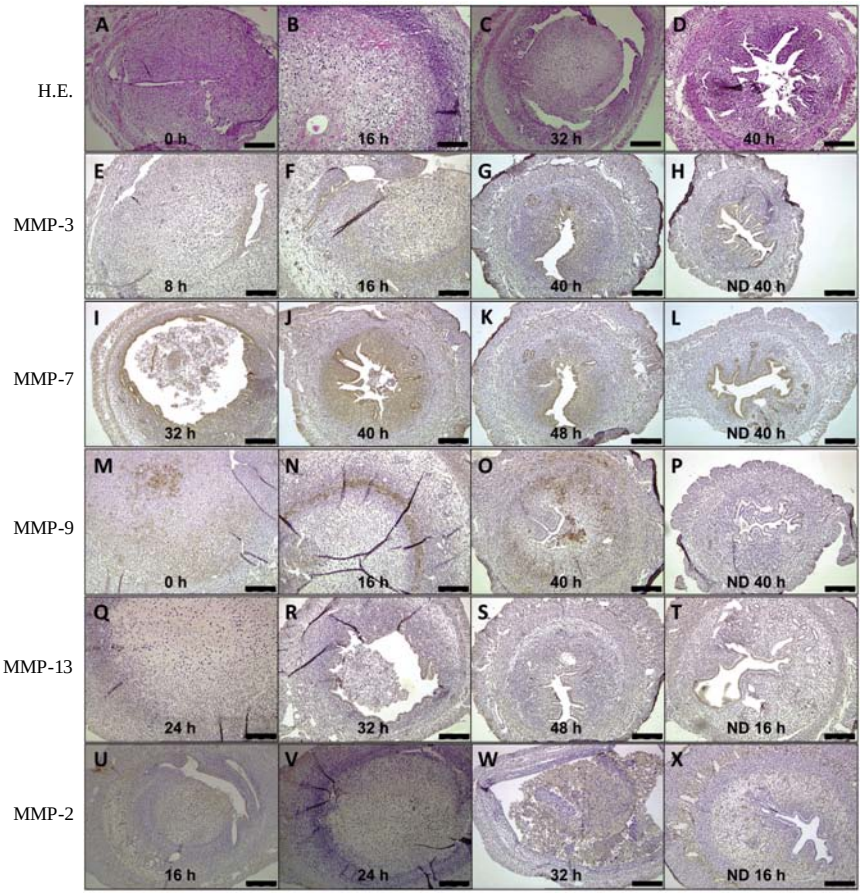

Fig. 2: A1-A5) Representative morphologic changes in endometrium shown with hematoxylin and eosin staining and (B1-F5) photographs MMPs (MMP3, MMP7, MMP9, MMP13 and MMP2) immunostaining at different timepoints after mifepristone administration. A1) The murine endometrium had undergone extensive decidualization at 0 h. A2) Tissue necroses were most drastic by 16 h. A3) The whole functional zone was sloughed into the uterine lumen by 32 h, A4) regenerated from the basal zone of the tissue by 40 h, A5) repaired well at 48 h. B1) Positive staining for MMP3 confined to focal subluminal stroma zone and epithelial zones 8 h, B2) the decidual zone and stronger in the interface between the basal zone and functional zone at 16 h, B3) epithelium and subluminal epithelium stroma at 48 h in decidualized uterus. B4) Intense stainings were only detected in the epithelium zone of the endometrium throughout the whole mifepristone treatment in nondecidualized uterus. C1) Strong staining for MMP7 was in the reforming epithelium at 32 h and C2) reached the peak at 40 h, C3) then reduced at 48 h and C2) also visible in the subluminal epithelium stroma at 40 h and C3) 48 h in decidualized uterus. C4) The staining was only confined to the epithelium during the process of mifepristone treatment in the nondecidualized uterus. D1) Immunopositive MMP9 were in the basal zone and the focal zone of subluminal stroma at 0 h, D2) only in the basal zone and their intensity increased dramatically at 16 h, D3) in basal zone and the subluminal stroma at 40 h in decidualized uterus. D4) There was nearly no visible positive stainings during the whole mifepristone treatment in the nondecidualized uterus. E1) Positive staining for MMP13 was widespread in the whole decidual zone at 24 h E2) throughout the middle of breaking-down endometrium at 32 h and E3) only mainly in the zone of re-epithelialization at 48 h in the decidualized uterus. E4) Positive staining was in epithelium during the process of mifepristone treatment in the non-decidualized uterus. F1) MMP2 staining was in the middle of decidual zone at 16 h and F2) at 24 h and F3) only in the endometrium breakdown at 32 h in decidualized uterus. F4) Intense staining was seen in the whole stromal zone at 16 h in non-decidualized uterus. Negative controls were also showed (Fig. 2B5, 2C5, 2D5, 2E5, 2F5). Bars = 200 μm (A-T, W-X), 310 μm (U-V). ND = Non-Decidualization. Insert images were whole cross-section fields of uterine tissues

Table 1: The number and percentage of mice showing the same staining patterns described at different time points after mifepristone treatment in the mouse menstrual-like model

	The number and percentage of mice with the same staining pattern n (%)						
MMPs	0 h	8 h	16 h	24 h	32 h	40 h	48 h
MMP2	7 (100)	7 (88)	5 (83)	5 (71)	5 (71)	8 (100)	5 (83)
MMP3	5 (71)	5 (63)	6 (100)	7 (100)	6 (86)	8 (100)	6 (100)
MMP7	6 (86)	6 (75)	5 (83)	7 (100)	5 (71)	7 (88)	5 (83)
MMP9	6 (86)	8 (100)	6 (100)	5 (71)	6 (86)	6 (75)	5 (83)
MMP13	7 (100)	8 (100)	4 (67)	6 (86)	7 (100)	6 (75)	6 (75)

in the whole decidual zone (Fig. 2E1). Positive staining was located throughout the middle of the breaking-down endometrium at 32 h (Fig. 2E2) and there was also positive staining in the reforming luminal epithelium and glandular epithelium. Intense staining was mainly in the zone of re-epithelialization at 40 and 48 h (Fig. 2E3). However, in the nondecidualized uterus, positive staining was restricted to the luminal epithelium and glandular epithelium as in the decidualized uterus and the intensity showed consistency between the two groups during the process of mifepristone treatment (Fig. 2E4). Negative control was also showed (Fig. 2E5).

Immunohistochemical analysis of MMP2: In the decidualized uterus, immunoreactive MMP2 was restricted to the basal zone at 0 and 8 h. Apart from the basal zone, positive MMP2 staining was also located in the middle of the decidual zone at 16 h (Fig. 2F1). With breakdown of the endometrium, weaker staining was confined to the middle of whole decidual zone at 24 h (Fig. 2F2). As the endometrium repaired, strong staining was present only in the area of endometrial breakdown at 32 h (Fig. 2F3) and was not present at 40 and 48 h. However, in the non-decidualized uterus, intense staining was seen in the whole stromal zone from 8-24 h (Fig. 2F4) and was absent at other time points during mifepristone treatment. Negative control was also showed (Fig. 2F5 and Table 1). The number and percentage of mice with the same staining patterns as described: the number mice of successfully being built the menstrual-like model at 0, 8, 16, 24, 32, 40 and 48 h was respective 7, 8, 6, 7, 7, 8, 6 and the percentage of these mice showed staining patterns as above described ranged from 67-100%.

DISCUSSION

In this study, sequential expressions of MMP3, MMP7, MMP9, MMP13 and MMP2 were assessed by immunohistochemistry in the mouse menstrual-like model induced by pharmacologic progesterone withdrawal. The sublocations of MMPs during endometrial breakdown and repair closely mimicked that in humans during perimenstrual and menstrual phases of the menstrual cycle and importantly were nearly similar to that in the mouse menstrual-like model induced by physiological progesterone withdrawal.

In primates, menstruation occurs naturally and strong correlative evidence exists supporting the role of MMPs in menstruation (Curry and Osteen, 2003; Salamonsen and Woolley, 1999). Studies using *in vitro* culture systems of isolated stromal and epithelial cells and explants have shown the presence of a number of MMPs including MMP1, MMP2, MMP3, MMP7, MMP9, MMP10 and MMP11 in premenstrual and menstrual endometrium (Curry and Osteen, 2003; Irwin *et al*., 1996; Jeziorska *et al*., 1996, 1995; Marbaix *et al*., 1996; Salamonsen and Woolley, 1999; Zhang and Salamonsen, 2002; Zhang *et al*., 2000) and more, localization of MMP mRNA and protein in the endometrium has demonstrated the regional distribution in menstruation in primates (Glasser *et al*., 2002; Rudolph-Owen *et al*., 1998). Importantly, to the known, locations of MMPs protein in the mouse menstrual-like model induced by physiological progesterone withdrawal was the only detail report at molecular level until now, so the identical evaluation indexes were also studied in the mouse menstrual-like model to provide a comparison with the mouse menstrual-like model induced by physiological progesterone withdrawal at molecular level (Kaitu'u *et al*., 2005) and to confirm the suitability of using these mouse menstrual-like models to study the mechanism of primates menstruation at a molecular level.

MMP3 positive staining was confined to the focal areas of endometrial decidualization at 0 and 8 h in the mouse menstrual-like model which was consistent with expression patterns in the mouse menstrual-like model induced by physiological progesterone withdrawal (Kaitu'u *et al*., 2005). However, in the menstrual-like model, in contrast to the other mouse menstrual-like model, the staining increased at the interface between the basal zone and functional zone at 16 h, along with that of MMP9, suggesting that MMP3 may activate MMP9 or may be activated in concert with MMP9 to degrade the extracellular matrix as reported in humans (Hampton and Salamonsen, 1994; Jeziorska *et al*., 1996). The main role of MMP3 is in repairing tissue and functional studies have also revealed a significant impairment of wound-healing in MMP3 knockout mice (Bullard *et al*., 1999). In the study, during the repair process MMP3 was located in the epithelium and subluminal epithelial stroma in the decidualized uterus at 40 and 48 h but it was also present in the epithelial zone of the endometrium in the non-decidualized uterus, thus MMP3 is most likely to be involved in endometrial stroma repair. In the mouse menstrual-like model induced by physiological

progesterone withdrawal, MMP3 was also identified in and around the area of re-epithelialization in the decidualized uterus, though there was no corresponding data on the non-decidualized uterus as in the mouse menstrual-like model. MMP3 protein significantly increased only in the stroma cells surrounding the sites of re-epithelialization also supporting the results in the model (Kaitu'u *et al*., 2005). In rhesus monkeys, MMP3 was upregulated after menstruation and expression was very restricted to a group of stroma cells beneath the luminal epithelium (Glasser *et al*., 2002; Rudolph-Owen *et al*., 1998), a very similar expression pattern to that in the two mouse menstrual-like models.

In the mouse menstrual-like model, MMP7 was detected in the basal zone as in the model induced by physiological progesterone withdrawal (Kaitu'u *et al*., 2005). More importantly, MMP7 showed a strong relationship with tissue repair as did MMP3 and a significant impairment of wound-healing was also found in MMP7 knockout mice (Parks, 1999). In the mouse menstrual-like model, though rather weak staining was also observed in the epithelium of the non-decidualized uterus, the same location in the decidualized uterus showed strong staining at 32 and 40 h and furthermore, MMP7 has been previously shown to have a functional role in re-epithelialization following wounding of the trachea (Parks *et al*., 2001). It is likely that MMP7 was important in epithelium repair in the study as the other mouse menstrual-like model (Kaitu'u *et al*., 2005). In humans and rhesus monkeys, MMP7 was also confined to the epithelial cells (Glasser *et al*., 2002; Rodgers *et al*., 1993, 1994; Rudolph-Owen *et al*., 1998). More importantly, in rhesus monkeys, there were lower levels of MMP7 in the shedding upper functionalis and the strongest signal was in the epithelium and residual functionalis which showed consistency with the two mouse menstrual-like models (Kaitu'u *et al*., 2005). Thus MMP7 appears to participate in postmenstrual extracellular matrix remodeling.

MMP9 immunostaining was mainly located in the basal zone and increased from 0-24 h in the mouse menstrual-like model which showed consistency with the mouse menstrual-like model induced by physiological progesterone withdrawal in which MMP9 was located in the basal zone and increased dramatically after progesterone withdrawal (Kaitu'u *et al*., 2005). MMP9 immunostaining appeared before 24 h, earlier than MMP13 (at 24 h) and was located in the basal zone where breakdown first occurred. MMP13 appeared in the decidualized zone where breakdown was followed by that of the basal zone (result not published), so the sequences and spatial expression patterns of MMP9 and MMP13 indicated that the effect of MMP9 in tissue breakdown may occur before that of MMP13 as the conclusion from the other mouse menstrual-like model (Kaitu'u *et al*., 2005). In humans, MMP9 expression was only detected in late secretory and menstrual phases (Rodgers *et al*., 1994), being equal to the period from 0-24 h after progesterone withdrawal in mice, so the expression patterns in humans and mouse menstrual-like models showed temporal similarities (Kaitu'u *et al*., 2005). MMP9 was also found most abundantly in a band of tissue at the interphase between the endometrium destined to be lost during menstruation and the basalis endometrium. The location of MMP9 in humans was very similar to that in the two mouse menstrual-like models (Kaitu'u *et al*., 2005) and further suggested that MMP9 was vital in endometrial breakdown, especially in the process of predecidualized endometrium shedding from the basal zone. In addition, MMP9 has previously been co-localized with neutrophils in the region of tissue degradation (Vincent *et al*., 1999). Although, neutrophils were not distinguished by immunohistochemistry in the present study, histomorphology showed that MMP9 seemed to be co-localized with neutrophils. It is well recognized that leukocytes including neutrophils are capable of producing regulatory molecules including cytokines, chemokines and a range of enzymes that are important either directly in matrix degradation or indirectly by activation of other proteases. MMP9 was in the subluminal stroma and displayed a similar location in the repair period in the two mouse menstrual-like models at 40 and 48 h (Kaitu'u *et al*., 2005) indicating that MMP9 was also involved in endometrial repair, especially stroma repair. In the neutrophil knockout mouse menstrual-like model, endometrial repair was blocked (Kaitu'u-Lino *et al*., 2007), suggesting that MMP9 from neutrophils may play a role in endometrial repair.

MMP13 was expected to a play significant role in tissue destruction in the mouse menstrual-like model with immunohistochemistry staining was only detected once breakdown was evident (at 24 h after mifepristone treatment), becoming widespread in the whole decidual zone which showed consistency with the mouse menstrual-like model induced by physiological progesterone withdrawal (Kaitu'u *et al*., 2005). In humans and rhesus monkeys, MMP1, equivalent to the mouse homologue MMP13 was significantly upregulated only at the time of menstruation and not during other phases of the menstrual cycle (Hampton and Salamonsen, 1994; Marbaix *et al*., 1995).

In the mouse menstrual-like model, at 24 h after progesterone withdrawal, the decidual endometrium shed into the uterine cavity and bleeding was obvious at 24 h,

corresponding to the menstruation phase in primatess, so the temporal expression pattern of MMP13 in mice were in agreement with that in primatess. Furthermore, in humans and rhesus monkeys, MMP1 was confined to groups of stromal cells in the functional layer and correlated with the foci of overt tissue lysis. In the two mouse menstrual-like models, the whole decidual zone, especially the predecidual zone was equivalent to the functional layer, so regional patterns of MMP13 in the two mouse menstrual-like models were also consistent with those in primatess and further supported that MMP13 was of major importance in endometrial breakdown. In addition, although MMP13 immunostaining was detected in the luminal epithelium and glandular epithelium in the decidualized uterus from 32h-48 h and also in the nondecidualized uterus, it seemed that MMP13 was not functional in epithelial repair in the mouse menstrual-like model.

There was no comparable result the in the other mouse menstrual-like model but in human obvious changes in MMP1 only occurred in menstruation (Hampton and Salamonsen, 1994; Marbaix *et al*., 1995), supporting the result that MMP13 has a role in breakdown but not in repair.

There are no data on MMP2 immunohistochemistry in the mouse menstrual-like model induced by physiological progesterone withdrawal. However, in the human tissue explant model, progesterone inhibited MMP2 secretion and this effect was antagonized by mefipristone (Marbaix *et al*., 1992).

In the mouse menstrual-like model, although MMP2 was located in the decidual zone in the decidualized uterus at 16 and 24 h, there was also a positive signal in the nondecidualized uterus, so it could not be concluded that MMP2 was functional only in the decidual zone.

However, there was a similar localization in the decidualized uterus at 32 h but not in the nondecidualized uterus and at the same time, the decidual zone had already sloughed off from the basal zone which was in agreement with the result from rhesus monkeys that the staining was confined to the shedding stroma in menstruation (Glasser *et al*., 2002; Rodgers *et al*., 1994; Rudolph-Owen *et al*., 1998; Vincent *et al*., 1999).

CONCLUSION

Although, there was a small difference between the two mouse menstrual-like models, mmp protein expression patterns showed near consistency indicating that although, the means of progesterone withdrawal was different in the two mouse menstrual-like models, the complicated downstream molecular mechanisms that progesterone withdrawal triggered may be identical. Furthermore, the expression patterns of MMPs in the mouse menstrual-like models also showed consistency with those in primates, increasing our confidence in the use of mouse menstrual-like models which are a very important platform to study molecular mechanisms of menstruation, a unique physiological phenomenon in primates.

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