Up-regulation of matrix metalloproteinase-9 in human myometrium during labour: a cytokine-mediated process in uterine smooth muscle cells

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The pregnant uterus undergoes dramatic changes of tissue remodelling during the labour and post-partum period. We studied the production of matrix metalloproteinase 9 (MMP-9), as a major contributor of tissue remodelling, in human myometrium at parturition. The regulation of proMMP-9 by interleukin-1β (IL-1β) and tumour necrosis factor-α (TNF-α) was also investigated in human myometrial smooth muscle cells. MMP-9 was present in myometrial smooth muscle cells, interstitial fibroblasts and inflammatory cells. The gelatinolytic activities of proMMP-9 in myometrium increased dramatically during labour. IL-1β and TNF-α induced proMMP-9 in myometrial smooth muscle cells, but these effects did not seem to be mediated by protein kinase C. On the other hand, neither 17β-oestradiol nor progesterone itself affected proMMP-9 production in myometrial smooth muscle cells. Moreover, progesterone, which is known as a physiological suppressor of MMP-9 in other species, did not decrease the IL-1β- and TNF-α-induced production of proMMP-9. These results suggest that IL-1β and TNF-α are effective up-regulators of proMMP-9 in the tissue remodelling of human myometrium during labour.

Key words: interleukin-1β/matrix metalloproteinase-9/myometrium/smooth muscle cell/tumour necrosis factor-α

Introduction
During pregnancy, the uterus enlarges and transforms into a muscular organ sufficient to accommodate the fetus, placenta, and amniotic fluid. Uterine enlargement results from the stretching and hypertrophy of existing muscle cells and an accumulation of fibrous tissue, most of which is collagen, along with an increase in elastic tissue content (Cunningham et al., 1997). The enlarged uterus in pregnancy undergoes dramatic changes during labour and the post-partum period. The formation of the lower uterine segment with dilatation of the cervix is an essential part of parturition and the degradation of the deposited uterine collagen is an important process of uterine involution in the puerperium.

The matrix metalloproteinase (MMP)-9, also known as 92-kDa type IV collagenase / gelatinase B, degrades a variety of extracellular matrix components including collagens IV, V, and XI, elastin, proteoglycan, and gelatin (Hibbs et al., 1985; Murphy et al., 1991). MMP-9 is secreted from various inflammatory cells (Hibbs et al., 1985, 1987; Montgomery et al., 1993; Trocmé et al., 1998), tumour cells (Moll et al., 1990), and normal cells (Murphy et al., 1989) as a zymogen. From these points, MMP-9 is considered to be associated with cellular migration, invasion and tissue remodelling in reproductive processes (Riley et al., 1999; see also review by Hulboy et al., 1997). The 5′-flanking sequence of MMP-9 gene contains a NF-kB consensus sequence, a PEA3/ets motif, AP-1 sites, and a GT box (Sato and Seiki, 1993). The production of MMP-9 is regulated transcriptionally by many cytokines, growth factors and hormones acting through the promoter region of the MMP-9 gene (Dou et al., 1997; see also review by Nagase, 1997).

There is strong evidence to suggest that cytokines may be involved in the mechanisms of the normal labour. Cytokines that are known to be elevated in amniotic fluid and maternal and cord sera during labour include interleukin (IL)-1β (Romero et al., 1990), IL-6 (Romero et al., 1990), tumour necrosis factor (TNF)-α (Casey et al., 1989; Opsjon et al., 1993) and IL-8 (Romero et al., 1991). Recently, it was found that IL-1β and IL-8 concentration in the lower uterine segment increased during parturition, paralleling cervical dilation (Winkler et al., 1998). Moreover, previous studies have demonstrated that the secretion of MMP-9 from human cervical fibroblasts (Sato et al., 1996), trophoblasts (Shimonovitz et al., 1996) and endometrial stromal cells (Huang et al., 1998) is stimulated by cytokines and hormones. These findings suggest the modulation of MMP-9 by cytokines may take place in the human myometrium for tissue remodelling during parturition.

Up to now, little is known about the production and regulation of MMP-9 in human myometrium in relation to parturition. In this study, we demonstrated the localization of MMP-9 and changes of its expression in the lower uterine segment before and during labour at term pregnancy. We are the first to report that cytokines are related to the production of MMP-9 in the human myometrium using human myometrial smooth muscle cells in vitro. Furthermore, we have also analysed the protein kinase C (PKC)-mediated pathway of cytokines for the production of MMP-9 in myometrial smooth muscle cells, which is related to tissue remodelling.
**Materials and methods**

**Reagents**

The following reagents were obtained commercially: Dulbecco’s modified Eagle’s medium (DMEM) and fetal bovine serum (FBS) from Whittaker Bioproducts (Walkersville, MD, USA); mouse monoclonal antibodies to MMP-9 (Ab-3, catalogue number IM37L) from Oncogene Research Products (Cambridge, MA, USA); antibody against α-smooth muscle actin, anti-human MMP-9 antibody (catalogue number MAB936), recombinant human IL-1β and tumour necrosis factor-α from R&D Systems Inc. (Minneapolis, MN, USA); enhanced chemiluminescence (ECL) from Amersham Pharmacia Biotech (Amersham Korea Ltd.); DAB chromogen and autohaematoxylin from Research Genetics (Huntsville, AL, USA); Bio-Rad DC Protein Assay from Bio-Rad Laboratories (Hercules, CA, USA); and other reagents were purchased from Sigma Chemical Co. (St Louis, MO, USA).

**Tissue samples**

Myometrial tissue was obtained during caesarean section from 24 term pregnant women with and without labour (n = 12, each group). This study was approved by the Institutional Review Board for Clinical Research at Samsung Medical Center and informed consent for the study was obtained from all participating patients. Myometrial tissues were excised from the upper portion of a low segment uterine incision. In women who had had previous caesarean sections, care was taken to avoid scar tissue and to exclude the serosa. Myometrial tissue was dissected free of the decidua, snap-frozen in liquid nitrogen, and stored at -70°C.

Gestational periods were confirmed by use of the first trimester crown–rump length and/or second trimester biparietal diameter. In all patients with labour, the labour began spontaneously and its progress was assessed.

**Immunostaining**

The immunostaining of MMP-9 was performed on paraffin-embedded sections using antibody to MMP-9 (Oncogene Research products, Ab-3). Myometrial tissues were fixed in 10% formalin and embedded in paraffin. Tissue sections were mounted on slides coated with 0.1% poly-L-lysine. After deparaffinization and rehydration, tissue sections were blocked with normal serum and then incubated for 1 h with a monoclonal antibody to MMP-9 at a dilution of 1:50. Tissue sections were treated in 0.1% Triton X-100, and they were incubated with a 1:1000 diluted biotinylated secondary antibody. After incubation in a streptavidin buffer, the antibody complexes were visualized by incubation with stable DAB chromogen for 5 min. Sections were counterstained with autohaematoxylin, dehydrated and mounted.

**Cell culture**

Myometrial tissue was excised from term pregnant women before labour and washed in Hank’s balanced salt solution. After being minced into 3 mm sizes, tissue fragments were digested with 2 mg/ml collagenase XI and 200 IU/ml DNAse I in DMEM for 20 min at 37°C with gentle agitation. We sieved the digested tissue solution and centrifuged the filtrate for 5 min at 300 g. The cell pellets were washed three times in DMEM with 10% FBS and plated in a culture flask (Kornyei et al., 1993). The purity of myometrial smooth muscle cells was determined by cytochemical staining of the cells with antibody against α-smooth muscle actin. This antibody showed positive staining in >90% of cells.

After confluency, the media were replaced with Phenol Red-free and serum-free DMEM for 2 days. The level of MMP-9 production by myometrial smooth muscle cells was determined by measuring gelatinolytic activity of culture media after treatment of cells with 1 µmol/l 17β-oestradiol, 1 µmol/l progesterone, 100 nmol/l phorbol 12-myristate 13-acetate (PMA), 1 ng/ml IL-1β, and 20 ng/ml TNF-α. Control treatments received equal volumes of vehicles. At the end of the incubation, which lasted for 18 h, the media were removed and frozen at -70°C for further analysis within 1 week. To examine whether IL-1β and TNF-α act through new protein synthesis in the production of proMMP-9, myometrial smooth muscle cells were treated with cycloheximide and cytokines. In all experiments, myometrial smooth muscle cells from the 5th up to the 10th passage were used. Each experiment was carried out more than three times for statistical analysis.

**Gelatin zymography**

The pulverized tissue samples and cells were lysed in RIPA buffer [50 mmol/l Tris–Cl, 150 mmol/l NaCl, 1% NP-40, 0.5 or 1% sodium deoxycholate and 0.1% sodium dodecyl sulphate (SDS), pH 7.5] containing 1 mmol/l phenylmethylsulphonyl fluoride for 1 h on ice. Samples were centrifuged and protein concentrations for each sample were determined for supernatants using the Bio-Rad DC Protein Assay (Bio-Rad Laboratories, USA). Proteins in culture media were concentrated in trichloroacetic acid (final concentration 10% v/v) for 30 min, and their concentrations were determined. Protein (20 µg) was separated by 8% SDS–polyacrylamide gel electrophoresis (PAGE) in reducing conditions and electrotransferred to a polyvinylidene-fluoride membrane. Blots were blocked with 5% non-fat dry milk in TTBS, then incubated with mouse monoclonal antibody to MMP-9 (R&D Systems Inc., catalogue number MAB936). After washings, blots were incubated with anti-mouse horseradish peroxidase-conjugated secondary antibodies for 1 h, washed again, and then incubated with ECL for detecting the proteins as recommended by the manufacturer.

**Statistical analysis**

All results are given as mean ± SEM. The data were tested by the Wilcoxon rank sum test and the Kruskal–Wallis test. P < 0.05 was considered to be significant.

**Results**

Immunostaining in human myometrium at term pregnancy showed immunoreactivities for MMP-9 in myometrial smooth muscle cells and interstitial fibroblasts in addition to inflam-
Figure 1. Immunostaining for matrix metalloproteinase-9 (MMP-9) in human myometrium at term pregnancy. In myometria, immunoreactivity for MMP-9 was detected mostly in myometrial smooth muscle cells (arrow) and inflammatory cells (open arrow). The intensity of immunoreactivity for MMP-9 in myometrial smooth muscle cells was stronger during labour (B) than before labour (A). Original magnification (A) ×400 and (B) ×200, bar = 15 µm.

Immunoreactivity for MMP-9 was detected mostly in myometrial smooth muscle cells (arrow) and inflammatory cells (open arrow). The intensity of immunoreactivity for MMP-9 in myometrial smooth muscle cells was stronger during labour (B) than before labour (A). Original magnification (A) ×400 and (B) ×200, bar = 15 µm.

The intensity of immunostaining for MMP-9 in myometrial smooth muscle cells was stronger during labour than before labour (Figure 1). As shown in Figure 2A, the amount of proMMP-9 in human myometrium increased during labour compared to that before labour. In gelatin zymography, multiple clear bands with molecular weights of 120, 88 and 64 kDa were observed in myometrial tissue extracts in addition to the 92 kDa and 72 kDa bands that are proMMP-9 and proMMP-2 respectively (Figure 2B). The 120 kDa band corresponds to the MMP-9 and tissue inhibitor of metalloproteinase (TIMP)-1 complex, while the 88 and 64 kDa bands correspond to active MMP-9 and MMP-2. The gelatinolytic activities of active MMP-9 correlated positively with those of proMMP-9. The gelatinolytic activity of the proMMP-9 on gelatin zymogram showed significantly higher activities (2.5-fold) in the myometria during labour than in those before labour (Figure 2C). In Figure 2, the two left and right lanes of the labour group were from women with cervical dilatations of ~3–4 cm and ~7–8 cm respectively. From the clinical viewpoint, the activities of MMP-9 in myometrium increased parallel to the progression of labour.

In cell culture, myometrial smooth muscle cells prepared from term pregnant women before the onset of labour produced only a very little amount of proMMP-9 (Figure 3). Treatment of these cells with 1 µmol/l 17β-oestradiol and 1 µmol/l progesterone also failed to increase the production of proMMP-9.
Up-regulation of MMP-9 in human myometrium during labour

Figure 2. Expression of promatrix metalloproteinase-9 (proMMP-9) in human myometrium before and during labour at term pregnancy. (A) Immunoblot for proMMP-9 in myometria before and during labour. (B) Gelatin zymography. ProMMP-9 and active MMPs were more strikingly expressed in myometria during labour than those before labour. (C) Quantification of gelatin zymography. The relative activities of proMMP-9 were quantified by densitometry, taking one of the myometria before labour as 1 arbitrary unit. This is a representative view of three experiments with a different set of samples. All three zymograms showed similar results. Data are presented as mean ± SEM. *Significantly different from value for pregnant myometria before labour (P < 0.05, Wilcoxon rank sum test).

9, even though progesterone receptors, but not oestrogen receptors were detected by immunoblotting (data not shown). When these cells were treated with 1 ng/ml IL-1β, 20 ng/ml TNF-α, or 100 nmol/l PMA, a gelatinolytic enzyme of 92 kDa, that was further characterized as proMMP-9 by immunoblotting, was induced. Furthermore, both IL-1β and TNF-α up-regulated the production of proMMP-9 in a dose-dependent manner (Figure 4). On the other hand, the 120 kDa bands corresponding to the MMP-9 and TIMP-1 complex were observed but were not prominent on the zymogram. The expression of proMMP-2 was constitutive in culture media. The activities of proMMP-2, however, were refractory to IL-1β, TNF-α, and PMA treatment in these cells.

Since PMA induced proMMP-9 in myometrial smooth muscle cells, we investigated whether PKC mediated the production of proMMP-9 by IL-1β and TNF-α. Staurosporine, an inhibitor of PKC at 100 nmol/l, did not influence the production of proMMP-9 in these cells (data not shown). Nor did 100 nmol/l staurosporine block the IL-1β- and TNF-α-induced production of proMMP-9 to any significant degree (Figures 5, 6). Similarly, the depletion of intracellular PKC activity by pretreatment of these cells with 500 nmol/l PMA did not obviously affect the induction of MMP-9 by IL-1β and TNF-α (data not shown).

Figure 3. Up-regulation of promatrix metalloproteinase-9 (proMMP-9) by phorbol 12-myristate 13-acetate (PMA), interleukin-1β (IL-1β) and tumour necrosis factor-α (TNF-α) in human myometrial smooth muscle cells. (A) Immunoblot for proMMP-9. Confluent myometrial smooth muscle cells from 5th to 10th passage in 100 mm diameter dishes were treated with 1 µmol/l 17β-oestradiol (E2), 1 µmol/l progesterone (P4), 100 nM PMA, 1 ng/ml IL-1β, and 20 ng/ml TNF-α in Dulbecco’s modified Eagle’s medium (DMEM) for 18 h. The proteins in harvested culture media from triplicate dishes were concentrated and subjected to immunoblotting for proMMP-9. (B) Gelatin zymography. Confluent cells in 6 multi-well plates were treated with 17β-oestradiol, progesterone, PMA, IL-1β, and TNF-α for 18 h. An aliquot of the culture media from triplicate wells was subjected to gelatin zymography.

Figure 4. Dose-dependent up-regulation of promatrix metalloproteinase-9 (proMMP-9) by interleukin-1β (IL-1β) and tumour necrosis factor-α (TNF-α) in human myometrial smooth muscle cells. (A) Gelatin zymography. Confluent cells in 6 multi-well plates were treated with IL-1β at 0.1, 0.2, 1, and 2 ng/ml respectively for 18 h. An aliquot of the culture media from triplicate wells was subjected to gelatin zymography. ProMMP-9 was up-regulated by IL-1β up to concentration of 2 ng/ml. (B) Gelatin zymography. Confluent cells in 6 multi-well plates were treated with TNF-α at 5, 10, 20, and 40 ng/ml for 18 h. ProMMP-9 was up-regulated by TNF-α up to concentration of 40 ng/ml.
Figure 5. Interleukin-1β (IL-1β) induces the production of promatrix metalloproteinase-9 (proMMP-9) in human myometrial smooth muscle cells. Confluent cells were treated with 1 ng/ml IL-1β plus 2.5 μg/ml cycloheximide (CHX), 100 nmol/l staurosporine (STA) and 1 μmol/l progesterone (P₄), respectively. (A) Immunoblot for proMMP-9. The proteins in harvested culture media from triplicate 100 mm diameter dishes were concentrated and subjected to immunoblotting for proMMP-9. (B) Gelatin zymography. An aliquot of the culture media from triplicate wells of 6-multi-well plates was subjected to gelatin zymography. (C) Densitometric analysis of gelatin zymography. Three separate sets of experiments were conducted and the relative amounts of proMMP-9 were quantified by densitometer, taking the proMMP-9 value without treatment as 1 arbitrary unit for each set of experiments. Statistical analyses were conducted with relative values of each set of experiments. Data are the means ± SEM from three independent experiments using cells from different origins at different passages. *Significantly different from values for control (CTL) untreated cells (P < 0.05, Kruskal–Wallis test).

Cycloheximide at 2.5 μg/ml suppressed basal production of proMMP-9 in myometrial smooth muscle cells. It also profoundly down-regulated the production of proMMP-9 by IL-1β and TNF-α. In addition, progesterone has been reported to suppress the IL-1- and/or PMA-mediated production of MMP-9 in rabbit uterus cervical fibroblasts (Imada et al., 1997). To investigate the suppressive action of progesterone on the production of proMMP-9 by IL-1β and TNF-α in human myometrial smooth muscle cells, we added progesterone to culture media containing IL-1β and TNF-α. As shown in Figures 5 and 6, progesterone at 1 μmol/l did not suppress the IL-1β- and TNF-α-induced production of proMMP-9. Though, in comparison with the control, the production of proMMP-9 by IL-1β with staurosporine or progesterone in Figure 5 (lane 5 and 6) was increased, the difference was not statistically significant despite the trials being carried out four times. However, the secretion and activity of proMMP-9 was significantly increased compared with the control.

Discussion

The tensile strength of the growing uterus in pregnancy is principally determined by the structural protein collagen. The collagen in the uterus during pregnancy increases in amount ~10-fold in various mammalian species (Morrione and Seifter, 1962; Woessner and Brewer, 1963). After delivery, the collagen content of the uterus rapidly decreases, which is a conspicuous feature of post-partum involution. Most of the collagen bundles are degraded extracellularly. Activated collagenases cleave collagen bundles into fragments, which then denature at body temperature to gelatin. Gelatinase action then cleaves these fragments to small peptides that are rapidly removed by the bloodstream (Shimizu et al., 1983). Increased activity of MMP-9 in human myometrium, therefore, would be required for the uterine tissue remodelling during labour and the post-partum period. In this study, we observed increased activities of proMMP-9 in myometrial tissues during labour, compared with before labour at term pregnancy. Furthermore, the values of proMMP-9 in human myometrium increased as labour progressed as assessed by cervical dilatation. It was reported that interstitial collagenase, which degrades fibrillar collagen types I, II, and III and catalyses the initial cleavage of collagen, increased 13-fold in the lower uterine segment during active labour with a cervical dilation of 4–8 cm (Rajabi et al., 1988).
This finding is consistent with our results in that activities of MMPs increased as labour progressed in human myometrium.

MMP-9 is known to be produced by many inflammatory cells such as macrophage (Hibbs et al., 1987), polymorphonuclear leukocytes (Hibbs et al., 1985), T-lymphocytes (Montgomery et al., 1993), and B-lymphocytes (Trocmé et al., 1998). The increased concentration of MMP-9 in the lower uterine segment during labour, thus, has been regarded as a result of the release of zymogens by inflammatory cells recruited by IL-8 (Osmers et al., 1995). However, the results of our in-vivo immunostaining and in-vitro experiments indicate that human myometrial smooth muscle cells produce MMP-9 when stimulated by IL-1β and TNF-α. Indeed, it was reported that human myometrial smooth muscle cells and human fibroblasts produce collagenases that are indistinguishable immunologically and display essentially identical kinetic behaviour (Roswit et al., 1988).

Experiments with cycloheximide in vitro indicate that de novo protein synthesis is required for the IL-1β- and TNF-α-induced production of MMP-9. It is reported that the expression of MMP-9 is regulated by the PKC pathway in tumour cells (Mackay et al., 1992) and T-lymphocytes (Zhou et al., 1993). The actions of IL-1β and TNF-α on the production of proMMP-9 in myometrial smooth muscle cells, however, did not seem to be dependent on PKC activation. High doses of staurosporine (100 nmol/l) decreased neither the IL-1β- nor the TNF-α-induced production of proMMP-9 in this study to any significant degree. This result was further confirmed by the down-regulation of PKC activity in myometrial smooth muscle cells which were pretreated with PMA (500 nmol/l) for 48 h prior to treatment with IL-1β and TNF-α. In human cervical fibroblasts, it was reported that TNF-α-induced production of proMMP-9 was not mediated by PKC, whereas the effects of IL-1β was through PKC (Sato et al., 1996). The slight decrease in the production of proMMP-9 by IL-1β plus staurosporine in our study (Figure 4) might reflect some coexistence of uterine fibroblasts in primary culture of myometrial smooth muscle cells.

It should be noted that several previous investigations have suggested a role for sex steroid hormones and hormone analogues in the regulation of MMPs in uterine tissue. Addition of either progesterone (1–5 µmol/l) or the potent synthetic progestational steroid medroxyprogesterone acetate (MPA, 0.1–0.5 µmol/l) into cultured rat uterine smooth muscle cells resulted in a cessation of collagenase production by the cells (Jeffrey et al., 1990). Progesterone, also, at a physiological concentration has been shown to increase the values of TIMP-2 (Imada et al., 1994) and to suppress the IL-1- and/or PMA-mediated production of MMP-9 in rabbit uterine cervical fibroblasts (Imada et al., 1997). It also has been reported that MMP-9 was induced in rat amnion immediately prior to parturition (Lei et al., 1995). These studies suggest that the production of MMP-9 is suppressed by progesterone during pregnancy, but is increased at the end of pregnancy by progesterone withdrawal in those species. In humans and other primates, however, there is no decrease in the concentration of plasma and myometrial progesterone before the onset of labour. In our study, progesterone did not significantly inhibit the IL-1β- and TNF-α-induced production of MMP-9 in human myometrial smooth muscle cells. It is unclear, however, whether this result reflects differences between species or in-vitro change of myometrial smooth muscle cells because the oestrogen receptor was lost and only the progesterone receptor was present in these cells.

It has been shown that interstitial collagenase is produced by rat myometrial smooth muscle cells during the post-partum period, and not during pregnancy (Blair et al., 1986). In that study, it was proposed that serotonin may activate the gene for IL-1β, after which the induced IL-1β acts via either an autocrine or paracrine mechanism to maintain a continuous source of IL-1β and IL-1α, which then goes on to induce interstitial collagenase (Dumin et al., 1998). Furthermore, it was reported that the MMP-3, MMP-2, and MMP-9, but not interstitial collagenase, convert the inactive 33 kDa precursor IL-1β into the biologically active 18 kDa mature IL-1β in a caspase-1-independent pathway (Schonbeck et al., 1998). On the other hand, MMP-3 degrades mature IL-1β, producing a loss of biological activity upon prolonged exposure (Ito et al., 1996; Schonbeck et al., 1998). It was suggested, therefore, that the balance of the respective MMPs and the precursor IL-1β is an important regulator of the long-term appearance of IL-1β activity. Although IL-1β is also known as an autoregulated protein, inducing its own expression (Dinarello et al., 1987; Manson et al., 1989), it is not known whether the autoregulatory mechanism of IL-1β also acts in human myometrial smooth muscle cells. It can be reasonably suggested, however, that the production of proMMP-9 in the human myometrium is up-regulated by the action of IL-1β on myometrial cells from the fact that IL-1β concentration in the lower uterine segment increases during labour (Winkler et al., 1998).

We have shown significantly increased activities of proMMP-9 in the lower uterine segment during labour, compared to before labour. The increased production of proMMP-9 in the myometrium would play a great role in the formation of the lower uterine segment during labour and uterine involution in the puerperium. The results of in-vitro experiments indicate that the production of proMMP-9 is up-regulated by IL-1β and TNF-α in myometrial smooth muscle cells. In human myometrium, cytokines are very likely to play an essential role in up-regulating the production of proMMP-9, which was finely regulated during pregnancy, contributing to the tissue remodelling of uterus at parturition.

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References


Mackay, A.R., Ballin, M., Pelina, M.D. et al. (1992) Effect of phorbol ester and cytokines on matrix metalloproteinase and tissue inhibitor of metalloproteinase expression in tumor and normal cell lines. Invasion Metastasis, 12, 168–84.


