Human neutrophil collagenase (matrix metalloproteinase 8) in parturition, premature rupture of the membranes, and intrauterine infection

Eli Maymon, MD, Roberto Romero, MD, Percy Pacora, MD, Ricardo Gomez, MD, Neil Athayde, MD, Sam Edwin, BS, and Bo H. Yoon, MD, PhD

OBJECTIVES: The mechanisms by which microbial invasion of the amniotic cavity leads to membrane weakening and rupture are poorly understood. Recently, endogenous host enzymes have been implicated in this process. Matrix metalloproteinases are a family of potent enzymes that degrade components of the extracellular matrix. Collagen type I provides the main tensile strength of the fetal membranes. Matrix metalloproteinase 8 (MMP-8), or neutrophil collagenase, degrades interstitial collagens, acting preferentially on collagen type I. This study was undertaken (1) to determine whether MMP-8 is present in amniotic fluid and whether its concentrations are changed in preterm and term labor and membrane rupture with and without intra-amniotic infection and (2) to determine whether the amniotic fluid concentrations of MMP-8 in labor at term are different in the lower and upper uterine compartments.

STUDY DESIGN: A cross-sectional study was conducted and transabdominal amniocentesis was performed in women in the following categories: (1) midtrimester (n = 25), (2) preterm labor in the presence and absence of microbial invasion of the amniotic cavity (n = 86), (3) preterm premature rupture of the membranes in the presence and absence of microbial invasion of the amniotic cavity (n = 51), (4) term patients in labor and not in labor (n = 51), and (5) term premature rupture of membranes (n = 20). Additional paired samples of amniotic fluid were retrieved by transabdominal amniocentesis (upper compartment) and transvaginal amniocentesis (lower or forebag compartment) from 14 term patients (28 samples) in spontaneous labor with intact membranes. Amniotic fluid MMP-8 concentrations were determined with a sensitive and specific immunoassay.

RESULTS: MMP-8 was detected in 95.4% (249/261) of all samples. (1) Spontaneous human parturition was associated with a significant increase in amniotic fluid concentrations of MMP-8 in both term and preterm gestation. Term (no labor median, 3.3 ng/mL; range, <0.06-38.6 ng/mL; vs labor median, 16.6 ng/mL; range, 0.33-1650 ng/mL; P < .05). Patients with preterm labor who delivered preterm (in the absence of microbial invasion of the amniotic cavity) had a significantly higher median amniotic fluid MMP-8 concentration than those with preterm labor who delivered at term (preterm labor, term delivery median, 3.1 ng/mL; range, <0.06-415.1 ng/mL; vs preterm labor, preterm delivery median, 32.5 ng/mL; range, <0.06-6006.6 ng/mL; P < .003). (2) Spontaneous rupture of membranes in preterm gestation but not in term gestation was associated with elevated amniotic fluid concentrations of MMP-8. Preterm gestation (preterm labor, intact membranes median, 3.1 ng/mL; range, <0.06-415.1 ng/mL; vs preterm premature rupture of membranes median, 35.1 ng/mL; range, 0.71-1184.1 ng/mL; P < .05). Term gestation (intact membranes median, 3.3 ng/mL; range, 0.29-38.6 ng/mL; vs rupture of membranes median, 5.6 ng/mL; range, 0.22-19.8 ng/mL; P = .9). (3) Microbial invasion of the amniotic cavity was associated with a significant increase in amniotic fluid MMP-8 concentration in patients with preterm labor and intact membranes, as well as in patients with preterm premature rupture of membranes. Preterm labor (no microbial invasion of the amniotic cavity, preterm delivery median, 32.5 ng/mL; range, <0.06-6006.6 ng/mL; vs microbial invasion of the amniotic cavity median, 208.1 ng/mL; range, 4.2-14,600 ng/mL; P < .001). Preterm premature rupture of membranes (no microbial invasion of the amniotic cavity median, 35.1 ng/mL; range, 0.71-1184.1 ng/mL; vs microbial invasion of the amniotic cavity median, 317.9 ng/mL; range, 2.16-14,500 ng/mL; P < .01). (4) The median amniotic fluid MMP-8 concentrations were significantly higher in fluid obtained from the forebag compartment than in that obtained from the upper compartment (median, 66.2 ng/mL; range, 7.4-170 ng/mL; vs median, 13.3 ng/mL; range, 2,170 ng/mL; respectively; P < .01).

From the Perinatology Research Branch, National Institute of Child Health and Human Development, the Department of Obstetrics and Gynecology, Wayne State University/Hutzel Hospital, and the Department of Obstetrics and Gynecology, Seoul National University.

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Reprint requests: Eli Maymon, MD, Perinatology Research Branch, National Institute of Child Health and Human Development, Wayne State University/Hutzel Hospital, Department of Obstetrics and Gynecology, 4707 St Antoine Blvd, Detroit, MI 48201.


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CONCLUSIONS: These data suggest a role for a specific interstitial collagenase (MMP-8) in microbial invasion of the amniotic cavity, preterm membrane rupture, and term and preterm labor. The higher concentration of MMP-8 in fluid bathing the cervical region may explain the predilection for membrane rupture to occur close to the lower pole of the uterus. (Am J Obstet Gynecol 2000;183:94-9.)

Key words: Matrix metalloproteinase 8, parturition, premature rupture of fetal membranes, intra-amniotic infection, neutrophil collagenase, forebag

Preterm labor and premature rupture of the fetal membranes are the leading causes of preterm birth. Microbial invasion of the amniotic cavity is involved in 30% to 40% of patients with preterm membrane rupture, and the lower the gestational age, the higher is the association with intrauterine infection. The mechanisms responsible for membrane rupture in the setting of intra-amniotic infection are poorly understood. One view is that bacteria are a source of an enzyme that degrades the extracellular matrix of the membranes. Alternatively, a host response to infection may participate in the mechanism of membrane rupture.

Matrix metalloproteinases are a family of endogenous enzymes with potent extracellular matrix–degrading capabilities. We have reported an increase in matrix metalloproteinase 9 (MMP-9) (gelatinase B) concentrations in amniotic fluid obtained from women with intra-amniotic infection. Similarly, Fortunato et al reported the induction of MMP-9 messenger ribonucleic acid in human fetal membranes in response to bacterial toxins. MMP-9 acts predominantly on collagen type IV and gelatin. However, the predominant collagen in fetal membranes responsible for their tensile strength is collagen type I, which is degraded by the collagenases matrix metalloproteinase 1 (MMP-1), matrix metalloproteinase 8 (MMP-8), and matrix metalloproteinase 15 (MMP-13).

MMP-8, or human neutrophil collagenase, is contained as a proenzyme in the secondary or specific granules of polymorphonuclear leukocytes. It is encoded by a gene different from that of MMP-1, or the fibroblast type of interstitial collagenase. MMP-8 is a 75- to 80-kd glycoprotein that is synthesized as a latent proenzyme during the myelocyte stage of neutrophil development and is released on chemotactic stimulation in vitro or during inflammatory conditions in vivo. The proenzyme is released on stimulation of polymorphonuclear leukocytes by a variety of proinflammatory stimuli, including tumor necrosis factor α, interleukins 1 and 8, granulocyte-macrophage colony-stimulating factor, and arachidonic acid metabolites. The active enzyme is capable of cleaving all three α chains of collagen types I, II, and III, preferentially acting on collagen type I. In contrast, MMP-1 acts predominantly on collagen type I. MMP-8 has been implicated in proteolytic damage to connective tissues in a number of inflammatory conditions, including adult periodontal disease and rheumatoid arthritis. Neutrophils are intimately involved in the host response to infection, and an increase in the amniotic fluid leukocyte count is a diagnostic feature of intra-amniotic infection. The objectives of this study were (1) to determine whether MMP-8 is present in amniotic fluid and whether its concentrations change in preterm and term labor and membrane rupture, with and without intra-amniotic infection, and (2) to determine whether labor at term is associated with different concentrations of amniotic fluid MMP-8 in the upper and lower uterine compartments.

Material and methods

Study design. A cross-sectional study was constructed by searching our clinical database and bank of biologic specimens and included women in the following groups: Group 1, Women in the midtrimester (15-17 weeks) of pregnancy (n = 25) who underwent amniocentesis for genetic indications. All these women had normal outcomes (ie, term delivery of infant appropriate in size for gestational age). Group 2, Women with preterm labor and intact membranes. Preterm labor was defined by the occurrence of regular uterine contractions at a minimum frequency of 2 every 10 minutes, in combination with documented cervical changes in either effacement or dilatation, or both, to 37 weeks’ gestation. These women were subdivided for study purposes into the following categories: (a) women who delivered at term (n = 34), (b) women who delivered prematurely in the absence of intra-amniotic infection (n = 33), and (c) women with intra-amniotic infection who delivered prematurely (n = 19). Microbial invasion of the amniotic cavity (intra-amniotic infection) was identified when an amniotic fluid culture result into those with (n = 27) and without (n = 22) intra-amniotic infection. Premature rupture of the fetal membranes was defined as amniorrhesis before the onset of spontaneous labor. Membrane rupture was diagnosed with the use of vaginal pooling, ferning, or a Nitrazine paper test. The indications for amniocentesis in women in groups 2 and 3 were for the detection of intra-amniotic infection and the determination of fetal lung maturity. Group 4, Women with term gestation (ie, at >37 weeks’ gestation). These women
were subdivided into (a) women with intact membranes in labor \((n = 26)\) and not in labor \((n = 25)\) and (b) women with rupture of membranes not in labor \((n = 20)\). Women at term not in labor underwent amniocentesis for assessment of fetal lung maturity before cesarean delivery, whereas those in labor or with membrane rupture underwent amniocentesis because of labor at an uncertain gestational age or for the diagnosis of intra-amniotic infection. To exclude the effect of gestational age on the concentrations of MMP-8, we matched the groups for gestational age.

To determine whether parturition at term is associated with topographic differences in the concentrations of amniotic fluid MMP-8 in the uterine cavity, we obtained paired samples of amniotic fluid from transvaginal and transabdominal amniocentesis at the time of hysterotomy. Fourteen patients with intact membranes who were in spontaneous labor at term and who had repeated cesarean delivery consented to participate in this study. A speculum was inserted into the vagina, and a spinal needle was used to puncture the membranes under direct visualization.

Many of these samples were used previously in studies of amniotic fluid matrix metalloproteinases, cytokines, chemokines, and arachidonic acid metabolites. All women provided informed consent before collection of amniotic fluid, and the study was conducted under protocols approved by the institutional review boards.

**Amniotic fluid.** We collected amniotic fluid by transabdominal amniocentesis in all cases. Additionally, 14 patients in labor at term also underwent transvaginal amniocentesis. Amniotic fluid not required for clinical purposes was centrifuged for 10 minutes at 4°C to remove cellular and particulate matter. Aliquots of amniotic fluid were stored at –70°C until assay. A sample of amniotic fluid not required for clinical purposes was centrifuged for 10 minutes at 4°C to remove cellular and particulate matter. Aliquots of amniotic fluid were stored at –70°C until assay. A sample of amniotic fluid was transported to the laboratory for aerobic, anaerobic, and *Mycoplasma* cultures, except for patients in the midtrimester of pregnancy.

**Amniotic fluid MMP-8 quantitation.** We determined MMP-8 concentrations in duplicate, using a commercially available enzyme-linked immunosorbent assay (Amersham Pharmacia Biotech, Inc, Arlington Heights, Ill). We have validated this assay system for determining amniotic fluid concentrations of MMP-8 conducting spike and recovery experiments. To investigate matrix effects in these experiments, we added graded amounts of human recombinant MMP-8 to pooled amniotic fluid obtained from women in the midtrimester. Curves parallel to that obtained with assay buffer were observed. The sensitivity of the assay in our laboratory was 0.06 ng/mL, and the interassay and intra-assay coefficients of variation were 4.6% and 3.7%, respectively.

**Statistical analysis.** Nonparametric statistical tests were used for analysis of differences between groups: Kruskal-Wallis test, Mann-Whitney \(U\) test, Wilcoxon test for censored observations, paired \(t\) tests, and analysis of covariance. The statistical package used was SPSS 7.5 (SPSS Inc, Chicago). We considered \(P < .05\) as significant.

**Results**

MMP-8 was detected in 95.4% \((249/261)\) of amniotic fluid samples assayed in this study. The amniotic fluid concentrations of MMP-8 did not change with gestational age. The median amniotic fluid concentration of MMP-8 in patients in the midtrimester of pregnancy was not different from that of patients at term not in labor \((P = .2;\) Table I). Similarly, no significant relationship was found between amniotic fluid concentrations of MMP-8 and gestational age when the analysis included patients with intact membranes in the midtrimester of pregnancy, those with preterm labor who subsequently delivered at term, and women at term not in labor \((P = .06)\).

Spontaneous human parturition was associated with a significant increase in amniotic fluid concentrations of MMP-8 in both term and preterm gestation. Patients in term in labor had significantly higher median amniotic fluid concentrations of MMP-8 than patients at term not in labor \((P < .05)\), and patients in preterm labor with intact membranes who delivered prematurely \((P < .05)\). In contrast, the median amniotic fluid concentration of MMP-8 was not significantly higher in patients with spontaneous rupture of membranes at term than in patients at term not in labor \((P = .9;\) Table I).

Spontaneous rupture of membranes in preterm but not in term gestation was associated with an elevated amniotic fluid concentration of MMP-8. The median amniotic fluid concentration of MMP-8 was significantly higher in patients with preterm premature rupture of membranes than in patients with preterm labor and intact membranes matched for gestational age and in the absence of intra-amniotic infection \((P < .05)\). In contrast, the median amniotic fluid concentration of MMP-8 was not significantly higher in patients with spontaneous rupture of membranes at term than in patients at term not in labor \((P = .9;\) Table I).

Microbiologically proven invasion of the amniotic cavity was associated with a significant increase in the amniotic fluid concentration of MMP-8 in patients with preterm labor and intact membranes \((P < .001)\), as well as in patients with preterm premature rupture of membranes \((P < .01;\) Table I).

Topographic differences in the amniotic fluid concentrations of MMP-8 between the upper uterine compartment and the forebag compartment were observed in patients in spontaneous labor at term \((P < .01;\) Fig 2).

Because MMP-8 exists as a proenzyme in the secondary or specific granules of polymorphonuclear leukocytes, we also explored the correlation between leukocytes in amniotic fluid and the presence of MMP-8. A significant cor-
relation was found between MMP-8 and amniotic fluid leukocytes (Spearman $\rho = 0.4$; $P < .001$). We previously reported MMP-9 concentrations in the same group of women; therefore we explored the correlation between the concentrations of both MMP-8 and MMP-9 in amniotic fluid and found that a significant correlation existed between these matrix metalloproteinases (Spearman $\rho = 0.67$; $P < .001$).

Analysis of covariance was conducted to determine whether the age of the sample could have accounted for

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**Table I.** Amniotic fluid concentrations of MMP-8 in study groups

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>MMP-8 concentrations (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Midtrimester</td>
<td>25</td>
<td>2.1 (&lt;0.06-32.7)</td>
</tr>
<tr>
<td>Preterm labor, term delivery</td>
<td>34</td>
<td>3.1 (&lt;0.06-415.1)</td>
</tr>
<tr>
<td>Preterm labor, preterm delivery, not infected</td>
<td>33</td>
<td>32.5 (&lt;0.06-6906.6)</td>
</tr>
<tr>
<td>Preterm delivery, infected</td>
<td>19</td>
<td>208.1 (4.2-14600)</td>
</tr>
<tr>
<td>Preterm membrane rupture, not infected</td>
<td>22</td>
<td>35.1 (0.71-1184.1)</td>
</tr>
<tr>
<td>Preterm membrane rupture, infected</td>
<td>29</td>
<td>317.9 (2.16-14500)</td>
</tr>
<tr>
<td>Term, no labor</td>
<td>25</td>
<td>5.3 (&lt;0.06-38.6)</td>
</tr>
<tr>
<td>Term, in labor</td>
<td>25</td>
<td>16.6 (0.33-1650)</td>
</tr>
<tr>
<td>Term membrane rupture, no labor</td>
<td>20</td>
<td>5.6 (0.22-19.8)</td>
</tr>
</tbody>
</table>

Values are median and range.
the results reported herein. Our analysis indicated that clinical group (eg, preterm labor, infection), but not age of the sample, was the factor explaining the significant changes in amniotic fluid concentrations of MMP-8 in this study.

Comment

These are the first data to demonstrate increased availability of MMP-8 in the amniotic fluid of women with spontaneous premature rupture of membranes, intra-amniotic infection, and spontaneous term and preterm labor. Increased protease activity has been proposed as a central mechanism responsible for membrane weakening and rupture; yet the precise enzymes involved in membrane rupture have not been identified. Previous studies have suggested a role for bacterial proteases based on the observation that incubation of membranes with microbial proteases reduces their strength and elasticity. However, there is no in vivo evidence of participation because of the difficulties in assaying the microbial enzymes in biologic samples. Matrix metalloproteinases have been implicated in the mechanisms leading to membrane rupture, parturition, and the host response to intrauterine infection. Human collagenases (MMP-1, MMP-8, and MMP-13) share the unique ability to cleave the triple helical domain of fibrillar collagen types I, II, and III, which form the backbone of the extracellular matrix of the fetal membranes and are responsible for most of the amnion tensile strength. MMP-8 is a secretory product of neutrophils that invade the uterine cervix and myometrium during parturition. It is the only collagenase to be stored in cells rather than being synthesized and released on demand and is also the only matrix metalloproteinase known to cleave aggrecan, a major proteoaminoglycan. Although MMP-8 degrades collagen types I, II, and III, its substrate specificity and greatest activity are on type I collagen. Our observation that term membrane rupture was not associated with an increase in the concentrations of MMP-8 suggests a difference in the participation of this enzyme in the mechanism of membrane rupture in preterm and term parturition.

Our observation that MMP-8 concentrations are elevated during spontaneous preterm and term labor is consistent with the view that degradation of extracellular matrix is part of the common terminal pathway of human parturition, which includes uterine contractility, cervical ripening, and membrane-decidua activation. The term membrane-decidua activation refers to the complex set of biochemical events leading to the separation of the lower pole of the membranes from the decidua and eventually to spontaneous rupture of membranes. Degradation of the extracellular matrix of the fetal membranes and dissolution of the intercellular cement that joins the decidua and the amniochorial membrane are key biochemical events of decidua-membrane activation. In support of this concept is the observation that amniotic fluid concentrations of MMP-9 and MMP-8 are elevated in spontaneous term and preterm parturition and that there was a significant correlation in the concentrations of these enzymes in patients in these groups. Inasmuch as both of these matrix metalloproteinases are produced by neutrophils, our observations may reflect neutrophil chemotaxis into the membranes, uterine wall, and cervical stroma during parturition. A coordinated expression of interleukin 8, a major chemokine, and neutrophil infiltration of the uterine tissues, along with expression of MMP-8, has recently been reported in biopsy specimens taken from the lower uterine segment. Similarly, we have noted that amniotic fluid concentrations of interleukin 8 behaved in a fashion similar to that reported herein. Further studies are required to determine the cellular origin of MMP-8 in the uterus and amniotic cavity.

A major finding of this study was that intra-amniotic infection is associated with a dramatic increase in amniotic fluid concentrations of MMP-8 in patients with intact and ruptured membranes. The most likely explanation is the recruitment and activation of neutrophils in the amniotic cavity in these patients. The correlation between the amniotic fluid leukocyte count and MMP-8 concentrations supports this conclusion. The 9-fold increase in amniotic fluid concentrations of MMP-8 provides a mechanism for accelerated catabolism of collagen type I, the main collagen of amniochorial membranes. The denatured collagen fragments (so-called gelatin) and collagen type IV (present in the amniotic basement membrane and in the spongy layer) are substrates for MMP-9, which is elevated in patients with intra-amniotic infection. Thus the combined actions of MMP-8, MMP-9, and possibly other matrix metalloproteinases of human or bacterial origin may provide a mechanism for weakening of the fetal membranes that is clinically manifested as membrane rupture.

Our finding that the concentration of MMP-8 is consistently higher in fluid obtained from the lower uterine compartment (forebag compartment) than in fluid from the upper compartment suggests that MMP-8 may act on a local level and play a role in decidual membrane activation before rupture of the membranes in the most dependent portion of the membranes.

In conclusion, we have provided in vivo evidence for the involvement of the human neutrophil collagenase MMP-8 in the processes occurring after intra-amniotic infection and in the mechanisms underlying membrane rupture and labor in both term and preterm gestations.

The data are available from the authors on request and at the Perinatology Research Branch Web site.

REFERENCES