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Concentrative relationship between polymorphonuclear elastase and urinary trypsin inhibitor in amniotic fluid

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Abstract In the amniotic fluid, urinary trypsin inhibitor (UTI) seems to inhibit polymorphonuclear elastase (PMNE) activity. The PMNE and UTI concentrations in normal amniotic fluid at 16–20 and 38–40 gestational weeks were measured. The PMNE concentration increased significantly at 38–40 weeks, whereas UTI concentration decreased significantly. According to concentrative relationships between both substances, PMNE may be activated more at the full term pregnancy. Since PMNE-induced tissue injury potentially causes degradation of amniotic collagen, the present result suggests that the quotient of PMNE and UTI concentrations is a reliable index to estimate the occurrence of rupture of the membranes.

Key words Urinary trypsin inhibitor · Polymorphonuclear elastase · Amniotic fluid · Pregnancy · Relationship

Introduction

Degradation of amniotic collagen may be caused by the intrinsic amniotic trypsin derived from fetal meconium or extrinsic polymorphonuclear elastase (PMNE) released from the neutrophils. Trypsin concentration in the amniotic fluid increases at the second trimester, but shows low values at the third trimester [21]. PMNE concentration in the amniotic fluid potentially increases at the full term pregnancy, because the neutrophils invade the cervix during this period [8]. Furthermore, higher PMNE concentration in the amniotic fluid has been reported in parturients with preterm labor than in those with term labor [22]. α_1-protease inhibitor and urinary trypsin inhibitor (UTI) are physiological substances which can inhibit these collagenolytic enzymes, of these the former substance is predominant [19]. Although α_1-protease inhibitor exists abundantly in the blood, its amniotic concentration seems to be decreased at the full term pregnancy [5]. At the last stage of pregnancy, therefore, the amniotic UTI may become an alternative inhibitor for the amniotic PMNE activity. In this report, we measured PMNE and UTI concentrations in the amniotic fluid as well as in the maternal and neonatal plasma.

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Patients and methods

After approval of the Institutional Committee and obtaining informed consent, 7 healthy parturients at 16–20 gestational weeks with normal pregnancy undergoing artificial abortion or amniocentesis for chromosomal examination, and 8 healthy parturients at 38–40 gestational weeks with normal pregnancy, Bishop score 0 and no evidence of fetal distress undergoing elective cesarean section were studied.

In the former group at 16–20 gestational weeks, 2 mL amniotic fluid was collected during artificial abortion or amniocentesis. Artificial abortion was performed after thiamylal injection, and amniocentesis was performed under local anesthesia. Subsequently, 10 mL urine was collected. In the latter group undergoing cesarean section, the anesthesia was performed by spinal anesthesia in all cases, and 2 mL arterial blood was collected from the femoral artery after anesthesia, followed by start of the surgery. At amniotomy during surgery, 2 mL amniotic fluid was collected. Immediately after delivery, 2 mL of either umbilical venous or arterial blood was collected from the double clamped segment of the umbilical cord, and 10 mL urine was collected via the urinary catheter.

Amniotic and blood samples were immediately centrifuged and the supernatant and plasma were obtained. Amniotic and plasma PMNE were measured by enzyme immunoassay (15689 PMN Elastase, MERCK Immunoassay, Chelles France). Normal plasma range of this as-

say kit is 21–165 ng/mL. Amniotic, plasma and urinary UTI were measured by radioimmunoassay (an assay kit from Sumitomo Kinzoku Bioscience, Tokyo, Japan). One unit (U) of UTI inhibits 50% activity of 2 μg trypsin. Furthermore, urinary creatinine concentration was measured and urinary UTI concentration (U/mL) was divided by this creatinine concentration (mg/dL). The revised value (U/mg) obtained was regarded as the systemic UTI concentration.

Data are expressed as the mean±SD (range). Statistical comparison between the groups was made by unpaired t test. Correlation between the amniotic PMNE concentration and the maternal arterial, umbilical venous or arterial plasma PMNE was analyzed by Pearson's correlation coefficient. $P<0.05$ was considered significant.

Results

The age and gestational duration of parturients at 16–20 weeks and 38–40 weeks were 30±4 (26–37) years and 31±5 (22–40) years, and 17±1 (16–20) weeks and 39±1 (38–40) weeks, respectively. There was no significant difference in age between the groups. 5 parturients at 16–20 gestational weeks received artificial abortion and others received amniocentesis for chromosomal examination, all of which consequently showed normal chromosomes. The cause of cesarean section was repeated cesarean section in all cases. No parturients showed any clinical problems during and after spinal anesthesia, and their neonates also presented sufficient Apgar Scores and no clinical problems since delivery.

The results of PMNE and UTI concentrations in the amniotic fluid, systemic UTI concentration, and maternal or umbilical plasma PMNE and UTI concentration are shown in Table 1. In the amniotic fluid, PMNE concentration increased significantly at 38–40 gestational weeks, whilst UTI concentration decreased significantly. The PMNE/UTI ratio in the amniotic fluid increased significantly at 38–40 gestational weeks. Systemic UTI concentration increased significantly at 38–40 gestational weeks. The PMNE/UTI ratios in the maternal arterial, umbilical venous and umbilical arterial plasma showed similar values.

There was no significant correlation between the amniotic PMNE concentration and either the maternal ($P=0.6499$), umbilical venous ($P=0.4154$) or arterial ($P=0.9602$) plasma PMNE concentration.

Discussion

The UTI is an acid-stable glycoprotein and a physiological and multipotential protease inhibitor [7, 17], which inhibits not only a variety of serine proteases such as trypsin, α-chymotrypsin, PMNE, plasmin, and cathepsin G, but also hyaluronidase and collagenase [19, 20]. Furthermore, a protective effect against interleukin-1β and tumor necrosis factor-β [3], prostaglandin-E_2 and uterine myometrial contraction [4], or endothelin, prostaglandin-$F_2\alpha$ and oxytocin [14] has been reported. The UTI is produced in the liver and rapidly excreted into urine, probably from circulating blood via the kidney [7, 17, 23, 24], and its generation is accelerated after the administration of steroids [1, 6, 7, 23] or in inflammatory diseases, malignant diseases, pregnancy, and postsurgical states [7, 11, 18]. Since UTI is quickly excreted into the urine [24], urinary excretion of UTI has been regarded as the index of systemic UTI concentration, in which the re-

Table 1 Results of polymorphonuclear elastase (PMNE) and urinary trypsin inhibitor (UTI) concentrations

	16–20 gestational weeks (n=7)	38–40 gestational weeks (n=8)	P value
Amniotic fluid			
PMNE (ng/mL)	106±40	753±539	0.0076
UTI (U/mL)	31±18	14±10	0.0385
PMNE/UTI (ng/U)	6±6	91±99	0.0409
Systemic UTI concentration (U/mg)	8±15	53±8	0.0029
Maternal arterial plasma			
PMNE (ng/mL)		397±418	
UTI (U/mL)		94±44	
PMNE/UTI (ng/U)		5±5	
Umbilical venous plasma			
PMNE (ng/mL)		154±51	
UTI (U/mL)		54±29	
PMNE/UTI (ng/U)		4±2	
Umbilical arterial plasma			
PMNE (ng/mL)		174±83	
UTI (U/mL)		50±26	
PMNE/UTI (ng/U)		5±4	

Values are mean±SD. Systemic UTI concentration is calculated by dividing urinary UTI concentration (U/mL) by urinary creatinine concentration (mg/dL)

vised value (U/mg) calculated by dividing urinary UTI concentration (U/mL) by urinary creatinine concentration (mg/dL) is commonly used [12, 18], particularly when causal or short-time urine is sampled. As demonstrated in this study, systemic UTI concentration was increased at 38–40 gestational weeks, which is consistent with above report [7]. The UTI has also been detected in the bile, abdominal fluid, bronchial mucus, cerebrospinal fluid and amniotic fluid [10, 12, 14, 18, 25]. Regarding amniotic fluid, Kanayama [14] reported that amniotic UTI concentrations were 88, 78 and 42 U/mL of a mean value at 20–29, 30–36 and 37–41 gestational weeks, respectively. Although the tendency for UTI depression as gestational weeks increase was similar with the present results, the values reported by Kanayama [14] were greater than our values. The reason for this may be attributable to a difference in measurement system, in which he used a competitive enzyme immunoassay and we used a radioimmunoassay. The disadvantage of this study was that the populations at 16–20 and 38-40 gestational weeks were different. However, the age and pregnant course were similar and normal in both groups, and the chromosomes and neonates were normal and healthy. Therefore, it would be possible to compare the amniotic fluid between the groups.

The results obtained from the present study suggest that the amniotic PMNE concentration increases at the full term pregnancy, whereas amniotic UTI concentration decreases, resulting in increase of the PMNE/UTI ratio. Since 1 U UTI inhibits 174, 17.4 and 1.74 ng PMNE by 10, 56 and 91%, respectively [9], this PMNE/UTI ratio of 91 ng/U at 38–40 gestational weeks may cause acceleration of PMNE activity in the amniotic fluid, if α_1-protease inhibitor in the amniotic fluid is absent. A decrease of α_1-protease inhibitor at the full term pregnancy has been reported [5]. Kanayama and Terao [13] reported a linear correlation between curvival PMNE concentration and cervical ripening expressed as Bishop score. Sine the Bishop scores in the parturients examined in this study showed 0 level, the amniotic PMNE concentrations might be more increased thereafter, if cesarean section was not performed. Therefore, it would be possible that PMNE in the amniotic fluid is activated more during spontaneous delivery period. However, since all of the present parturients showed normal pregnant course, the interaction to this level may be one of the normal physiological reactions at the full term pregnancy. We speculate further that spontaneous delivery, in part, is associated with increased amniotic PMNE activity caused by less inhibitory effect of UTI. Although the origin of amniotic UTI remains unclear, the result that the amniotic UTI concentration decreases in spite of increased systemic UTI concentration at the full term may be explained by a hypothesis that the origin is changed from mother to fetus. The UTI concentration in neonatal urine is reported to be greater than in adult urine [14]. However, the amount of UTI excretion into the amniotic fluid is probably small. We speculate, therefore, that amniotic UTI concentration at the full term pregnancy may be dependent on the fetal condition, the capacity of UTI excretion into the amniotic fluid.

Other concentrative relationship between PMNE and UTI in the maternal and umbilical blood indicated sufficient inhibition against PMNE activity, based on the ability of UTI for PMNE [9]. In addition, α_1-protease inhibitor as the main inhibitor for PMNE exists abundantly in circulating blood, so that the adverse effect of PMNE in circulating blood can not be expected. Thus, blood PMNE concentrations may be expected to estimate the inflammatory condition in the local tissue such as cervix, membrane or amniotic fluid, although the present results showed no correlation with the amniotic PMNE concentrations. This evidence implies that the blood PMNE concentration is not sufficient to estimate the condition of amniotic PMNE. Probably because the origin of PMNE release could not be attributed only to amniotic fluid. Dudenhausen et al. [2] proposed that blood PMNE concentrations above 180 ng/ml potentially cause a premature rupture of the membrane. The PMNE values obtained from 38–40 weeks parturients were greater than this value. However, the parturients examined in this study showed no clinical evidence of membrane infection. Based on these discussions, the blood PMNE concentrations seem not to become a reliable index and the concentrative relationship between the amniotic PMNE and UTI may be reliable to estimate the condition of amniotic membranes.

Consequently, the present study shows some interesting findings that the quotient of PMNE and UTI concentrations in amniotic fluid increases at the full term. Although the increase of PMNE/UTI quotient to some extents is one of the normal physiological reactions, large extent of the quotient may be a possible pathogenetic mechanism for the rupture of the membranes as the result of PMNE-induced tissue injury. To treat the premature rupture of the membranes, chorioamnionitis or preterm labor, antibiotics or β adrenergic agonists have been commonly administered [16]. In addition to these treatments, vaginal suppository of ulinastatin 5000 U (Mochida Pharmaceutical, Tokyo, Japan), which is identical to UTI and purified from human urine, has been applied clinically in Japan [15], although the proposed regimen has not been established. Based on the present study, the concept of PMNE/UTI quotient may be helpful to establish an optimal treatment for these inflammatory pregnancies.

References

1. Dillard GHL (1950) The trypsin inhibitor for the urine in health and disease. J Lab Clin Med 36:266–271
2. Dudenhausen JW, Langner K, Sonmez M (1987) Elastase of polymorphonuclear granulocytes: determination in plasma in pregnancy and following premature rupture of the membranes (in Germany with English abstract). Geburtshilfe Frauenheilkd 47:597–600
3. El Maradny E, Kanayama N, Halim A, Maehara K, Terao T (1994) Urinary trypsin inhibitor has a protective effect on the amnion. Gynecol Obstet Invest 38:169–172

4. El Maradny E, Kanayama N, Halim A, Maehara K, Kobayashi T, Terao T (1996) Effects of urinary trypsin inhibitor on myometrial contraction in term and preterm deliveries. Gynecol Obstet Invest 41:96–102
5. Evans HE, Glass L, Mandl I (1975) α_1-antitrypsin concentration in amniotic fluid. Biol Neonate 27:232–234
6. Faarvang HJ (1963) Dose-response between exogenous cortisone acetate and the urinary excretion of trypsin inhibitor in man. Acta Endocrinol 43:484–492
7. Faarvang HJ (1965) Urinary trypsin inhibitor in man ("Mingin"). Scand J Clin Lab Invest [Suppl 83] 17:1–78
8. Fosang AJ, Handley CJ, Santer V, Lowther DA, Thorburn GD (1984) Pregnancy-related changes in the connective tissue of the ovine cervix. Biol Reprod 30:1223–1235
9. Ishikawa A, Fukao K, Tsuji K, Osada A, Yamamoto Y, Iwasaki Y (1993) Can ulinastatin be an effective inhibitor of human polymorphonuclear granulocyte elastase under severe stressed state? N Geka Gakkai Zasshi 94:449–455
10. Iwama H, Kato M, Nakane M (1999) Polymorphonuclear elastase in the intra-abdominal fluid may be activated after major abdominal surgery. Eur J Anaesthesiol 16:737–738
11. Iwama H, Tobishima S, Kaneko T, Komatsu T, Akutsu H (1999) Epidural block during general anesthesia attenuates urinary trypsin inhibitor excretion in lower abdominal surgery. J Anesth 13:150–155
12. Iwama H, Ohmori S, Tsutsumi K (2000) Detectable concentrations of urinary trypsin inhibitor in cerebrospinal fluid. J Neurosurg Anesthesiol 12:29–32
13. Kanayama N, Terao T (1991) The relationship between granulocyte elastase-like activity of cervical mucus and cervical maturation. Acta Obstet Gynecol Scand 70:29–34
14. Kanayama N (1994) Intrauterine defensive mechanism of amniotic fluid and fetal membranes. Acta Obstet Gynaecol Jpn 46:673–685
15. Kanayama N, El Maradny E, Yamamoto N, Tokunaga N, Maehara K, Terao T (1996) Urinary trypsin inhibitor: a new drug to treat preterm labor: a comparative study with ritodrine. Eur J Obstet Gynecol Reprod Biol 67:133–138
16. Maymon E, Chaim W, Sheiner E, Mazor M (1998) A review of randomized clinical trials of antibiotic therapy in preterm premature rupture of the membranes. Arch Gynecol Obstet 261:173–181
17. Muramatsu M, Mori S, Matsuzawa Y, Horiguchi Y, Nakanishi Y, Tanaka M (1980) Purification and characterization of urinary trypsin inhibitor, UTI_{68}, from normal human urine, and its cleavage by human uropepsin. J Biochem 88:1317–1329
18. Nakane M, Iwama H (1999) Intra-alveolar urinary trypsin inhibitor cannot inhibit polymorphonuclear elastase activity in the lung in postsurgical patients with acute respiratory distress syndrome. Surg Today 29:1030–1033
19. Ogawa M, Nishibe S, Mori T, Neumann S (1987) Effect of human urinary trypsin inhibitor on granulocyte elastase activity. Res Commun Chem Pathol Pharmacol 55:271–274
20. Ohnishi H, Kosuzume H, Ashida Y, Kato K, Honjo I (1984) Effects of urinary trypsin inhibitor on pancreatic enzymes and experimental acute pancreatitis. Dig Dis Sci 29:26–32
21. Pocknee RC, Abramovich DR (1982) Origin and levels of trypsin in amniotic fluid throughout pregnancy. Br J Obstet Gynaecol 89:142–144
22. Rivero-Marcotegui A, Larranaga-Azcarate C, Ceres-Ruiz R, Garcia-Merlo S (1997) Polymorphonuclear elastase and interleukin-6 in amniotic fluid in preterm labor. Clin Chem 43: 857–859
23. Shikimi T, Suzuki S, Wessel T, Joh TH, Hattori K, Takaori S (1992) Human urinary trypsin inhibitor (urinastatin)-like substance in mouse liver. Life Sci 50:1399–1406
24. Sugiki M, Sumi H, Maruyama M, Yoshida E, Mihara M (1989) Clearance and distribution of acid-stable trypsin inhibitor (ASTI). Enzyme 2:31–38
25. Yoshida E, Sumi H, Maruyama M, Mihara H, Sakai R (1987) Development and application of an enzyme-linked immunosorbent assay for acid stable trypsin inhibitor (ASTI) in human plasma. Clin Chim Acta 167:155–164