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Effect of Urinary Trypsin Inhibitor on Preterm Labor with High Granulocyte Elastase Concentration in Cervical Secretions

Masako Hayashi, Atsuko Oya, Hidehiko Miyake,
Akihito Nakai and Toshiyuki Takeshita

Division of Reproductive Medicine, Perinatology and Gynecologic Oncology, Graduate School of Medicine, Nippon Medical School

Abstract

Aims: To explore whether intravaginal treatment with urinary trypsin inhibitor (UTI) prevents preterm delivery in patients in preterm labor with increased levels of granulocyte elastase in cervical secretions.

Methods: The subjects were patients in preterm labor with increased levels of granulocyte elastase in cervical secretions from 16 to 33 weeks gestation. Maternal and neonatal outcomes were compared between patients receiving UTI treatment (UTI group; n=33) and those not receiving UTI treatment (control group; n=40).

Results: In patients receiving UTI, the mean gestational age at delivery was greater than that in the control group (37.8 vs. 35.6 weeks, p=0.003), and the rates of premature delivery before 34 and 37 weeks gestation were lower (3% vs. 20%, p=0.028; and 18% vs. 47%, p=0.008, respectively). The percentage of neonates weighing more than 2,500 g was significantly higher in the UTI group, with no neonates weighing less than 1,500 g. The neonatal hospitalization rate was lower in the UTI group (9% vs. 42%, p=0.001).

Conclusion: In patients in preterm labor with a high elastase concentration in cervical secretions, treatment with UTI reduced the risk of preterm delivery and improved neonatal outcomes.

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Key words: preterm labor, urinary trypsin inhibitor, granulocyte elastase

Introduction

Prematurity is the main cause of neonatal morbidity and mortality and is responsible for half of all neonatal deaths[1]. Therefore, the prediction and prevention of preterm delivery are extremely important.

Recent studies confirm that inflammatory cytokines, such as interleukin 1, tumor necrosis factor, and interleukin 8, play a central role in parturition, including preterm labor. Granulocyte elastase, a neutral serine protease, is assumed to be involved in cervical maturation[2]. Because elastase degrades elastin fibers, collagen cross-links, and type III collagen, which reinforces the strength of cervical tissue[3,4], elastase released from granulocytes likely causes softening of the cervix. Our preliminary

Correspondence to Masako Hayashi, MD, Department of Obstetrics and Gynecology, Nippon Medical School, Tama Nagayama Hospital, 1–7–1 Nagayama, Tama, Tokyo 206–8512, Japan
E-mail: yamaguchi1126@yahoo.co.jp
Journal Website (http://www.nms.ac.jp/jnms/)

study demonstrated that the increased level of granulocyte elastase in cervical secretions was an independent predictive factor for preterm delivery before 34 weeks of gestation in patient in preterm labor[5].

Urinary trypsin inhibitor (UTI)[6], a member of the inter-alpha trypsin inhibitor family, suppresses inflammatory proteases, such as neutrophil elastase[6–8]. UTI is used to prevent several types of acute and chronic inflammation, e.g., acute pancreatitis, diabetes mellitus, acute myocardial infarction, coronary artery disease, kidney diseases, and certain autoimmune disorders[9]. Recent studies suggest that UTI may also be useful for treating preterm labor[7,10,11]. Kanayama et al. have reported that intravaginal treatment with UTI is more effective than ritodrine for inhibiting recurrent uterine contractions and for lengthening pregnancy[7].

Because preterm labor is related to increased levels of granulocyte elastase and because UTI suppresses elastase, we hypothesized that UTI would be effective in patients in preterm labor with increased levels of granulocyte elastase. The objective of the present study was to examine whether intravaginal treatment with UTI prevents preterm delivery in patients in preterm labor and increased levels of granulocyte elastase in cervical secretions.

Patients and Methods

The initial subjects were 322 women admitted for preterm labor from January 2002 through September 2006 at Tama-Nagayama Hospital of Nippon Medical School. Preterm labor was defined as regular uterine contractions at a frequency of 10 minutes or less continue at least 1 hour. Changes in cervical status (dilatation or effacement) were not required for admission. Samples for granulocyte elastase and fetal fibronectin in cervical secretions were obtained from each patient at admission, and patients with increased level of granulocyte elastase from 16 through 33 weeks of gestation were examined in this study. We excluded women with a history of uterine anomaly; clinical evidence of chorioamnionitis (which was defined according to the criteria of Gibbs et al[12]), congenital anomaly of the fetus, clinical diagnosis of abruptio placenta, placenta previa, gross cervical bleeding, rupture of fetal membranes, or any maternal contraindication for the use of tocolytic agents.

A total of 73 patients with high granulocyte elastase concentration in cervical secretion were finally enrolled in the study. The subjects were divided into 2 groups. Forty patients admitted from January 2002 through October 2003 were treated without UTI, and 33 patients admitted from December 2003 through September 2006 were treated with a UTI protocol.

Cervical secretions were obtained with a sterile swab stick for granulocyte elastase assessment before digital examination or transvaginal ultrasound assessment. The swab stick was rolled gently across the endocervix. Concentrations of elastase were measured with a kit according to the manufacturer's instruction (granulocyte elastase enzyme-lined immunosorbent assay [ELISA], Sanwa Kagaku Kenkyusho, Nagoya). Briefly, supernatants were added to a 96-well microtiter plate coated with antibodies (antihuman elastase mouse monoclonal antibody) specific for granulocyte elastase. After incubation, the wells were washed. Peroxidase-labeled antibodies (antihuman elastase sheep polyclonal antibody) were then added. After incubation, the wells were washed. Substrate (H_2O_2) and o-phenylenediammonium dichloride were added, and color development was stopped with a H_2SO_4 solution. The color intensity of the reaction mixture was measured with a photometer. Granulocyte elastase concentrations greater 1.6 mg/L in cervical secretions were defined as high[5,13], which agreed with the cut-off value set recommended by the manufacturer.

After the cervical secretions sampling, vaginal secretions were collected to assess fetal fibronectin. A dry swab was rotated in the posterior vaginal fornix for 10 seconds to ensure saturation. The samples for cervicovaginal fetal fibronectin were vortexed, divided, and frozen until assay. Concentration of fetal fibronectin were determined with a commercially available quantitative ELISA assay (Adeza Biomedical, Sunnyvale, CA, USA) with

Table 1 Clinical characteristics and univariate association according to using UTI (n=73)

		UTI group n=33	control group n=40	p value
Maternal age at delivery (years)		32.9 ± 4.75	31.2 ± 3.33	0.080
Parity	Primiparous	15 (46)	23 (58)	0.305
	Multiparous	18 (54)	17 (42)	
Gestational age at admission (weeks)		27.4 ± 3.41	26.6 ± 3.46	0.307
Cervical length (mm)		27.2 ± 12.1	21.9 ± 10.7	0.051
Fibronectin	positive	26 (79)	36 (90)	0.183
	negative	7 (21)	4 (10)	

Continuous variables are given as the mean ± SD, whereas categorical variables are shown as numbers of patients (%) .

specific monoclonal antibody (FDC-6) against the oncofetal domain of fetal fibronectin. Samples with concentrations of at least 50 ng/mL were designated positive, according to previous reports[14].

Ultrasonographic scans of the uterine cervix were performed with a 7.5-MHz transvaginal probe (Hitachi-Medico EUB-515A, Hitachi, Tokyo) with the patient having an empty bladder. By means of real-time imaging, cervical length was measured between the internal os and the external os[15].

The gestational age was based on a reliable menstrual history and confirmed by ultrasound examination before a gestational age of 16 weeks. All subjects provided written informed consent for participation in this study, which was approved by the Ethics Committee.

Subjects in the UTI group received daily intravaginal treatment with UTI (10,000 U/day) as follows: 10,000 U of UTI was dissolved in 10 mL saline, absorbed in a cotton ball with attached thread, and applied gently to the posterior fornix. The cotton ball was removed 2 hours later.

All patients were treated according to standard obstetric indications, with the route and timing of delivery at the discretion of the attending clinician. There were no differences in treatment process other than whether UTI was administered. The characteristics of the subjects, including maternal age, gravidity, parity, and gestational age, were analyzed. The presence of fetal fibronectin and cervical length at admission, and the incidence of other obstetrical managements, such as cerclage and tocolytic agents, were also evaluated in both the UTI and control groups. Gestational age at delivery, neonatal birth weight, Apgar scores, and neonatal hospitalization were analyzed to clarify the clinical effect of UTI.

Statistical analysis was performed with Student's *t*-test for continuous variables and with likelihood ratios for categorical variables. The level of statistical significance used was a probability value of less than 0.05. Kaplan-Meier survival analysis was applied to evaluate the duration of pregnancy according to the treatment arm. The log-rank test was used to compare differences in the duration of pregnancy between the groups.

Results

For all patients, the mean age was 32 years (range, 23–42). The mean gravidity and mean parity were 1.0 (range, 0–5) and 0.57 (range, 0–2), respectively. The mean gestational age at enrollment was 27 weeks (range, 16–33 weeks). The characteristics of the 2 groups are shown in **Table 1**. There were no significant differences in maternal age, parity, gestational age, cervical length, or fetal fibronectin at enrollment.

Table 2 and **Figure 1** show maternal and neonatal outcomes in the 2 groups. The mean gestational age at delivery in the UTI group (37.8 weeks) was significantly greater than that in the control group (35.6 weeks, p=0.003). Premature delivery rates before 34 and 37 weeks of gestation in the UTI group (n=33) were 3% and 18%, respectively, and were significantly lower than those in the control group (n=40, 20% and 47%, respectively; p=0.028 and p=0.008). With respect to obstetrical management,

Table 2 Outcome of pregnancy according to using UTI (n=73)

		UTI group n=33	control group n=40	p value
Gestational age at delivery (weeks)				
	mean	37.8 ± 2.02	35.6 ± 4.11	0.003 *
	<34	1 (3)	8 (20)	
	≧34	32 (97)	32 (80)	0.019 *
	<37	6 (18)	19 (47)	
	≧37	27 (82)	21 (53)	0.007 *
Apgar score (5 min)		9.09 ± 1.72	8.66 ± 1.73	0.303
Neonatal body weight (g)		2,923.5 ± 1,340.3	2,657.2 ± 847.8	0.078
	<1,500	0 (0)	5 (13)	0.015 *
	<2,500	5 (13)	14 (37)	0.086
Neonatal hospitalization				
	yes	3 (9)	17 (42)	0.001 *
	no	30 (91)	23 (58)	
Obstetrical management				
Cerclage	yes	29 (88)	36 (90)	0.773
	no	4 (12)	4 (10)	
Tocolysis	yes	14 (42)	29 (72)	0.009 *
	no	19 (58)	11 (28)	

Continuous variables are given as the mean ±SD, whereas categorical variables are shown as number of patients (%) .
* p<0.05 compared with control

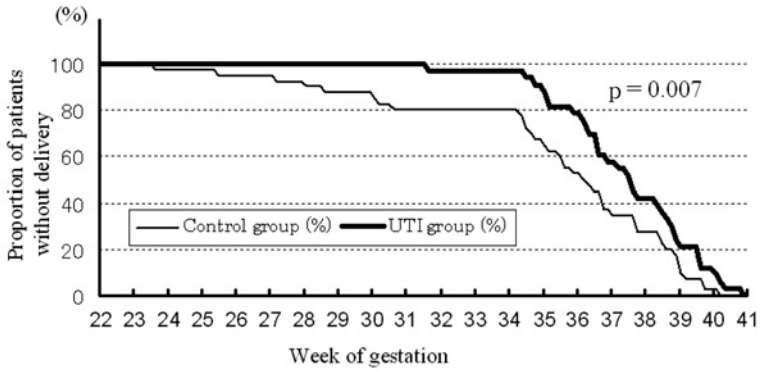

Fig. 1 Survival curves depicting the fraction of women undelivered at each gestational week.

no significant differences were found between the UTI group and the control group in the percentage of patients undergoing cervical cerclage, although in the UTI group the percentage of patients treated with tocolytic agents was significantly lower (p= 0.009). There were no significant differences in 1-minute or 5-minute Apgar scores. The mean neonatal birth weight did not differ significantly between the UTI group (2,923.5 ± 1,340.3 g) and the control group (2,657.2 ± 847.8 g, p=0.078). However, in the UTI group, the percentage of neonates weighing more than 2,500 g was significantly greater (p=0.046), with no neonates with a birth weight less than 1,500 g. Consequently, the neonatal hospitalization rate was lower in the UTI group (9%) than in the control group (42%, p=0.001).

The durations of pregnancy in both groups are shown with Kaplan-Meier survival curves survival curves in **Figure 1**. The duration of pregnancy was significantly longer in the UTI group than in the control group.

Discussion

In the present study, the rates of premature delivery in patients with high elastase concentration in cervical secretions were compared between those treated with UTI and those not treated with UTI. In patients treated with UTI, the duration of pregnancy was significantly longer, and the rates of premature delivery before 34 or 37 weeks of gestation were significantly lower. Although mean neonatal birth weights did not differ significantly between the groups, in the UTI group the percentage of neonates weighing more 2,500 g was significantly higher and no neonates had a birth weight less than 1,500 g. Consequently, the neonatal hospitalization rate was lower in the UTI group.

Parturition is considered an inflammatory process. During labor, inflammatory cytokines, platelet activating factor, inflammatory proteases, and prostaglandins are released from the fetal membranes, decidua, cervix, and myometrium[7]. The matured cervix is infiltrated by macrophages and neutrophils, which are prominent sources of proteases, collagenases, and elastase[16]. The inflammatory process may induce degradation of collagen fibrils, the main constituent of the uterine cervix, which results in cervical ripening. At the gestational age of 10 weeks the collagen concentration is 70% of that in the nonpregnant cervix, and at term is 30%[17], Simultaneously, the proportion of glycosaminoglycans alters[18]. A rapid increase in interstitial fluid causes edema of the tissue and increases the separation of collagen fibers. The cement substances are also broken down by the action of neutral proteases[16].

In this process, granulocyte elastase, which is a neutral serine protease, is assumed to be an important factor by digesting cross-links between collagen bundles, elastin, fibronectin, and fibrin[2]. Because elastase degrades elastin fibers, collagen cross-links, and type III collagen, which reinforce the strength of cervical tissue[3,4], elastase likely induces softening of the cervix and plays an essential role in cervical maturation. Near term, the collagen cross-links are gradually broken down by neutral proteases, and the solubility of collagen is increased[19,20]. Degranulation of granulocytes in cervical stroma was immunohistologically demonstrated by the irregularity of the staining pattern[2]. A recent study has also demonstrated that polymorphonuclear leukocytes and granulocyte elastase are more abundant in the vaginas of patients with preterm labor than in patients with uncomplicated pregnancies[21]. In addition, Rivero-Marcotegui et al[22], who have found high elastase concentrations in the amniotic fluid of women in preterm labor, have suggested that elastase is a useful maker of amniotic infection and of preterm delivery. These reports would suggest that patient with high concentration of granulocyte elastase is at risk of instantaneous progression of preterm labor.

UTI is widely used to treat human inflammatory diseases. UTI is reported to suppress the production of inflammatory cytokines, including granulocyte elastase, interleukin 1, interleukin 8, trypsin, α-chymotrypsin, plasmin, and cathepsin G, as well as proteases in coagulation cascade[23]. In women with normal pregnancies, trypsin inhibitor activity in urine rises. Additionally, trypsin inhibitor from fetal urine circulates in the amniotic cavity, and UTI levels in the amniotic fluid are much higher than those in adult urine[7]. It may have a protective effect on the chorioamnion and uterine muscles during pregnancy. A recent study has suggested that UTI is effective in reducing uterine contraction, uterine cervical ripening, trophoblastic apoptosis, amniotic permeability, and cytokine levels in plasma and amniotic fluid in an animal model of preterm delivery[11]. Kanayama et al.[7] have reported that intravaginal treatment with UTI was useful for preventing preterm labor. They compared rates of premature delivery between patients treated with UTI and those treated with ritodrine and found that UTI therapy is more effective than ritodrine for inhibiting recurrent uterine contractions. In agreement with these previous reports, our study demonstrates the effect of treatment with UTI for preventing preterm delivery in patients in preterm labor. Treatment with UTI is also associated with lower requirement for tocolytic agents and markedly decreased rate of neonatal hospitalization.

Although the mechanism by which UTI treatment controls uterine contractions remains unclear, UTI may prevent cervical maturation by directly suppressing degranulation of granulocyte elastase. To further investigate the clinical effect of UTI on preterm labor, a double-blind, randomized, controlled trial would be necessary. However, we believe that our study of UTI provides further support for it use in the treatment of preterm labor, with the potential to decrease neonatal morbidity and mortality.

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