Antithrombin improves the maternal and neonatal outcomes but not the angiogenic factors in extremely growth-restricted fetuses at <28 weeks of gestation.

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2 tables and no figure.

Abstract

Objective: Severe preterm fetal growth restriction (FGR) remote from term is problematic in perinatal medicine. We aimed to investigate the effect of maternally administered antithrombin on maternal and neonatal outcomes.

Study design: A prospective, one-arm, pilot study was performed in 14 women with severe FGR (≤5th centile) at <28 weeks of gestation, without gestational hypertension, preeclampsia, or chronic hypertension. The maternal plasma concentrations of soluble fms-like trypsin kinase-1 (sFlt-1) and placental growth factor (PlGF) were measured and categorized into 3 groups; group 1: low sFlt-1 and high PlGF, group 2; moderate sFlt-1 and low PlGF, and group 3; high sFlt-1 and low PlGF. Antithrombin was administered for 3 days. The incidence of perinatal mortality, infant morbidity and the period of pregnancy prolongation were compared.

Results: In group 1(n=4), their pregnancies were extended for longer period and the maternal and infant outcomes were good. The prolongation periods were shorter in groups 2 (n=3) and 3 (n=7), which resulted in poor maternal and infant outcomes.

Half of the women were diagnosed with severe preeclampsia or HELLP syndrome; fetal or neonatal death occurred in three cases; and neurological complications occurred in 4 cases. Normal development was only observed in 3 infants.

Conclusions: The evaluation of the maternal sFlt-1 and PlGF at 21-27 weeks of gestation is useful in the managements of severe FGR. Antithrombin treatment could prolong the pregnancies with low sFlt-1 and high PlGF without negatively affecting maternal or fetal health.

Key words. Antithrombin; fetal growth restriction; placental pathology; perinatal mortality; preeclampsia.

Introduction

Severe fetal growth restriction (FGR) (≤5th centile) is associated with increased perinatal mortality and morbidity. Premature FGR is extremely critical when the fetal body weight is <500g and birth occurs at <28 weeks of gestation. Obstetricians encounter the medical dilemma of choosing between early delivery, which involves the risks associated with prematurity, and pregnancy prolongation, which may lead to the deterioration of fetal health. At present, there are no useful measures for improving fetal growth once it falls into the severe range of FGR. Thus, we have little choice but to periodically observe fetal growth and fetal well-being until maternal or fetal indications for delivery occur.

In a randomized controlled trial of severely preeclamptic women who received antithrombin or a placebo, we reported that the maternal administration of antithrombin (3000 IU/day for 7 days) significantly improved fetal growth, to the point that standardized growth for gestational age was achieved, and maintained fetal well-being [1]. In our experience, growth restriction is the first clinical manifestation of FGR. This is followed, several weeks later, by preeclampsia . In

addition, the maternal plasma concentration of placental growth factor (PlGF), a pro-angiogenic factor that is mainly assessed in preeclamptic women, is also significantly decreased in women having FGR infants without hypertension during pregnancy [2, 3]. These observations suggest that the maternal administration of antithrombin may improve the fetal growth of severely growth-restricted fetuses in women without hypertension, who may or may not subsequently develop preeclampsia.

We performed this pilot study to examine the effects of antithrombin on fetal growth and the perinatal outcome in severe FGR infants ($\leq 5^{th}$ centile) in women without hypertensive disorders during pregnancy in whom FGR occurred remote from term. We also investigated whether the maternal administration of antithrombin altered the angiogenic imbalance to prevent hypertensive disorders. For this purpose, we measured the maternal concentrations of soluble fms-like trypsin kinase-1 (sFlt-1), placental growth factor (PlGF), and vascular endothelial growth factor (VEGF).

Materials and methods

This was a prospective, one-arm, interventional pilot study. The study was approved by the ethics committee of the University of Miyazaki, Faculty of Medicine (＃652, approved on April 20th, 2010). Written informed consent was obtained from all of the subjects.

Pregnant women, whose estimated fetal body weight was less than or equal to the 5th centile at registration, without gestational hypertension, preeclampsia, chronic hypertension, or apparent collagen diseases, and whose gestational age was <28 weeks were enrolled. Cases with fetal anomalies or known congenital abnormality syndromes were excluded. The gestational age was confirmed by the crown-rump length (7-10 weeks of gestation) and biparietal diameter (12-14 weeks of gestation) by ultrasonography.

We tried to prolong the pregnancies until maternal or fetal indications for delivery occurred. The maternal indications included severe gestational hypertension, severe preeclampsia, or HELLP syndrome (hemolysis, elevated liver

enzymes, and low platelet count). Fetal indications included combinations of the following: a deterioration of fetal heart rate, a low biophysical profile score <8, abnormal Doppler velocimetry, a lack of growth of the fetal head circumference for 2 weeks, or a lack of fetal growth for 3 weeks or more. The fetal indications were applied to fetuses whose estimated body weight was >400g because of the limitations of our neonatal intensive care unit.

After obtaining informed consent, the women were hospitalized and told to minimize daily activity with a hope of increasing the fetal body weight. We also administered antithrombin. Briefly, when minimizing daily activity for one week did not result in any increase in fetal growth (on ultrasonography), Antithrombin (1,500 U; Anthrombin P, CSL Behring, Tokyo, Japan) was administered intravenously for 3 consecutive days. The maternal blood concentrations of sFlt-1, PlGF, VEGF, antithrombin activity (%), thrombin-antithrombin complex (TAT), plasmin-alfa2 plasmin inhibitor complex (PIC) and fibrinogen were measured (SRL, Japan) before, and at 4 and 7 days after the administration of antithrombin. The results of these parameters were concealed until the end of the study so that the

information would not be used for clinical decision-making.

The fetal body weight was estimated by ultrasonographic measurements of the biparietal diameter, the abdominal circumference and femur length (1/week). Fetal well-being was assessed by fetal heart rate monitoring (1/day), biophysical profile scoring (2/week) and Doppler velocimetry of the umbilical artery (1-2/w).

The infant neurological outcome was determined at 18 months of age or older by pediatric neurology specialists. Mental retardation was defined as a DQ of <70, using the Kyoto Scale of Psychological Development 2001 [4], which is strongly correlated with the corresponding composite Bayley III score in very low-birth-weight infants at 18 months of age [4]. Infants with moderate or severe types of cerebral palsy were included in the present study.

The data are expressed as the mean ± SD. Incidence was compared by the chi-squared test and Fisher's exact test. Multiple mean values were compared by a one-way ANOVA and a post-hoc test (the Tukey HSD and Dunnett's *t*-test). P values of <0.05 were considered to indicate statistical significance.

Results

A total of 14 pregnancies were included in the present study. They had severe FGR ($\leq 5^{th}$ centile) at <28 weeks of gestation (Table 1). At registry, the blood pressure of all of the subjects was within the normal limits (systolic blood pressure <140 mmHg and diastolic blood pressure <90 mmHg), the results of a 75 g glucose tolerance test were normal, and antiphospholipid antibodies (lupus anticoagulant and anticardiolipin antibodies) were also negative. Maternal screening for toxoplasmosis, rubella, cytomegalovirus, and herpes simplex were negative.

Retrospectively, we arbitrarily classified these 14 pregnancies into 3 groups according to the maternal concentrations of sFlt-1 at registry: group 1, <2,000 pg/ml (arbitrarily defined as low); group 2, 5,000-10,000 pg/ml (middle); and group 3, >10,000 pg/ml (high) (Table 1). Accordingly, the PlGF levels were significantly higher in group 1 (214 ± 86 pg/ml, $p<0.05$) than in group 2 (38 ± 25 pg/ml) and group 3 (28 ± 9 pg/ml) ($p<0.01$, one-way ANOVA with Tukey HSD).

In group 1 (n=4), where the sFlt-1 level was low and the PlGF level was high, pregnancy was extended for ≥60 days (range: 62-98) and the neonatal

outcomes were all favorable (Table 1). The growth centile of two fetuses was improved after the administration of antithrombin; however, that of the other two fetuses worsened to below the 1st centile.

In group 2 (n=3), where the sFlt-1 was in the middle range and the PlGF level was low, pregnancy was extended for 38 to 57 days (this was significantly different from group 3 but not from group 1; Table 1). The fetal body weights remained below the 1st centile both before and after the administration of antithrombin. Three pregnancies were terminated because two fetuses showed non-reassuring fetal heart rate patterns and the remaining one fetus did not show any increase in fetal head size. The maternal outcome was poor in one woman who developed severe preeclampsia at 5 weeks. One neonate died and the remaining two neonates had mental retardation; thus, the perinatal outcomes were poor in all of the group 2 cases.

In group 3 (n=7), where the sFlt-1 level was high and the PlGF level was low, the duration of pregnancy prolongation ranged from 5 days to 38 days. There was no improvement in the growth centile of any of the fetuses after the

administration of antithrombin (Table 1). The maternal complications included the development of severe preeclampsia (n=2) and HELLP syndrome (n=2); another mother was confirmed to have systemic lupus erythematosus in the late postpartum period. Fetal death occurred in two cases because the fetuses were too light (<400g) to deliver. One infant had mental retardation, one infant (in a case that was complicated by cytomegalovirus infection) had unilateral hearing loss; the three remaining were neurologically normal.

There were significant differences in the pregnancy prolongation period among the 3 groups ($p<0.01$; one-way ANOVA and Dunnett's *t*-test, Table 1). The mean increase in body weight (g/week) was significantly greater in group 1 than in groups 2 and 3 ($p<0.01$; one-way ANOVA and Dunnett t-test). Fetal deterioration (fetal death or non-reassuring fetal status) occurred more frequently in group 2 (3/3) than in group 1 (0/4) ($p<0.05$; Fisher's exact test). Similarly, maternal deterioration (severe preeclampsia, HELLP syndrome or SLE) occurred more frequently in group 3 (4/7) than in group 1 (0/4) ($p<0.05$, Fisher's exact test).

From a chronological viewpoint, maternal deterioration toward severe

preeclampsia or HELLP syndrome occurred earlier in groups 2 and 3 (n=5, median, 28 weeks; range, 24-29 weeks) than in group 1, in which maternal hypertensive disorders did not occur. Similarly, fetal death or non-reassuring fetal status occurred earlier in group 3 (n=3; median, 26 weeks; range, 24-26 weeks), followed by group 2 (n=3; median, 28 weeks; range, 27 to 35 weeks), and the infants of group 1 did not show any signs of fetal deterioration.

Maternal vessel thrombosis, which occurred more frequently in groups 2 and 3 than group 1, was the most common finding (n=7) in the pathological examinations of the placenta, followed by infarction (n=6), chorangiosis (n=4), and fetal vessel thrombosis (n=2).

The incidence of poor outcomes (death or neurological damage) was compared between the most severe FGR infants (<1st centile at registry) and the other FGR infants (2nd to 5th centile); however, the difference did not reach statistical significance (6/9 versus 1/5, p=0.27).

The changes in the maternal blood concentrations of sFLT-1, PlGF, and antithrombin are shown in Table 2. The antithrombin concentrations increased

significantly at the 4th day of administration. However, the levels of sFLT-1 and PlGF did not change after the administration of antithrombin in any of the groups. Furthermore, there were no changes in the VEGF concentrations or in the d-dimer, thrombin-antithrombin complex, and plasmin-plasmin inhibitor complex concentrations after the administration of antithrombin.

Discussion

At present, obstetricians have few treatment options to improve the outcomes in cases of severe FGR (≤5th centile) at <28 weeks of gestation. We therefore encounter the dilemma of choosing between prematurity and restricted fetal growth. In the present pilot study, we investigated the effects of antithrombin on fetal growth, fetal well-being, perinatal mortality and morbidity, and maternal complications among the 3 groups of women who were divided according to their concentrations of sFlt-1 and PlGF.

As shown in Table 1, the determination of the concentrations of sFlt-1 and

PlGF in women with severe preterm FGR without hypertension was useful for predicting the maternal and fetal outcomes, and the women who should receive antithrombin. In group 1, where the sFlt-1 concentration was relatively low and the PlGF concentration was relatively high, the pregnancies could be extended for a period that was sufficient to allow good maternal and fetal outcomes. In group 2, where the sFlt-1 concentration was moderate and the PlGF concentration was low, the pregnancy could be extended until the point of fetal deterioration. In group 3, where the sFlt-1 concentration was high and the PlGF concentration was low, half of the mothers developed severe preeclampsia or HELLP syndrome, while fetal deterioration occurred in the other half within 5 weeks.

It is reported that the level of sFlt-1 increases, while that of PlGF decreases during the last 2 to 3 months before term in normotensive pregnancies [5]. On the other hand, women who later develop preeclampsia show these changes 5 to 8 weeks earlier. In addition, women with clinical preeclampsia have a higher level of sFlt-1 and lower level of PlGF, irrespective of gestational age [5]. Our results are compatible, in part, with previous reports which indicated that the sFlt-1

concentration was elevated and the PlGF concentration was decreased in women from groups 2 and 3 who later developed preeclampsia or HELLP syndrome. The difference was that the level of sFlt-1 was already as high as that in women who already had clinical preeclampsia and that the level of PlGF was much lower than that in normotensive women; however, they had severe FGR alone and did not exhibit any clinical signs of preeclampsia. Our results suggest that 50% of the women, with high levels of sFlt-1 (>5000 pg/ml) and low levels of PlGF (<100 pg/ml) went on to develop preeclampsia when they had severe FGR (<5th centile) at 21–27 weeks of gestation. Other investigators also showed that a potential PlGF level of 90 pg/ml at 13-16 weeks of gestation was a possible threshold for the prediction of preeclampsia [6]. However, a large-scale multicenter study by the WHO showed that angiogenic factors, including the sFlt-1 and PlGF levels at ≤20 weeks of gestation did not perform well enough in the prediction of subsequent preeclampsia [7].

Recently, the maternal plasma concentration of PlGF was found to be significantly decreased in women having FGR infants without hypertension during

pregnancy [2, 3]. Thus, placental FGR can be antenatally differentiated from constitutional FGR by the extremely low level of PlGF (<12 pg/ml) in maternal circulation [2]. In the present study, 13 of the14 cases had vascular pathologies, such as maternal vessel thrombosis, fetal vessel thrombosis and chorangiosis, which is consistent with our previous observation that the prevalence of infarction, fetal vessel thrombosis and chronic villitis were higher in 257 FGR placentas than they were in controls [8]. The findings of low PlGF concentrations and placental pathology suggest that, in these cases, FGR appeared to be of placental origin. On the other hand, a half of the mothers developed severe preeclampsia or HELLP syndrome within several weeks, suggesting that in those cases, the origin of FGR was pre-eclampsia. Our observation suggests that a low level of PlGF (<100 pg/ml) in maternal circulation at 21-27 weeks of gestation may be associated with either placental FGR or subsequent pre-eclampsia.

Taking these results into consideration, we hypothesize that the severe FGR infants in group 1 may have benefited from the maternal administration of antithrombin, which was administered to prolong pregnancy, for the sake of both

maternal and fetal health. Thus, the determination of the maternal concentrations of sFlt-1 and PlGF during the second trimester is useful. However, since this pilot study did not compare antithrombin with placebo controls, further comparative studies are required for group 1. On the other hand, antithrombin administration failed to improve the maternal and fetal outcomes in groups 2 and 3, which suggests that other therapeutic modalities should be applied.

This study did not reveal the mechanisms through which the administration of antithrombin improved the growth of severe FGR infants in group 1. The maternal concentrations of angiogenic factors, including sFlt-1, PlGF, and VEGF, did not change after the administration of antithrombin, suggesting that some other mechanisms are involved in the improvement of fetal growth and in sustaining fetal well-being.

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Table 1. The characteristics of severe growth-restricted fetuses and their perinatal outcomes

	Maternal age (years)	Para	At the start of AT administration					Delivery							Maternal outcomes	Neonatal outcomes	Placenta
			sFlt-1 * (pg/ml)	PlGF * (pg/ml)	GA (w+d)	EFBW (g)	Percentile	Sex	GA (w+d)	BW (g)	Growth centile	Prolongation # (d)	BW increase * (g/w)	Indications			
Group 1	22	0	1,140	150	27+6	795	5	f	36+5	1728	<1	62	115	HC arrest		Normal	FVT
	30	0	1,390	223	23+1	387	5	f	39+1	2608	25	98	159	Labor onset		Normal	FVT, MVT,C
	33	1	1490	151	20+6	258	3	f	32+4	1210	<1	82	81	In labor		Normal	C
	31	0	1,710	332	26+0	431	<1	f	40+0	2456	10	94	145	Labor onset		Normal	CAM
Average±SD			1,430±120	214±43								84±8	125±17				
Group 2	31	2	5,720	15.7	21+0	165	<1	f	28+1	433	<1	49	38	rLD		NND	MVT, C, Inf
	38	1	5,860	65.2	27+4	557	<1	f	35+0	978	<1	52	57	HC arrest		MR, Coarctation	C
	33	1	9,050	31.8	22+2	233	<1	m	27+4	438	<1	37	39	PD, rLD,	sPE	MR	MVT
Average±SD			6,880±1,090	38±15								48±5	45±6				
Group 3	35	0	11,300	18.6	25+2	320	<1	m	26+4	358	<1	9	29	IUFD		IUFD	chr Inf
	33	0	13,700	26.0	24+3	495	5	f	29+6	870	<1	38	69		sPE	CMV, rt. hearing loss	CMV
	29	0	16,400	45.9	25+4	488	<1	f	29+3	626	<1	27	37		HELLP	MR	Inf
	26	0	>20,000	27.7	23+3	315	<1	–	24+2	305	<1	6	0	IUFD	SLE	IUFD	MVT, Inf
	35	2	>20,000	19.5	25+4	462	<1	f	27+0	540	<1	10	55		sPE	Normal	MVT, Inf
	27	0	>20,000	31.4	25+4	410	<1	f	28+5	582	<1	22	54		HELLP	Normal	MVT, Inf
	31	0	>20,000	24.9	25+3	537	2	f	26+1	539	2	5	3	rLD, sVD		Normal	MVT, Inf
Average±SD			17,300±1,370	28±3								16±4	35±10				

Abbreviations: AT, antithrombin; sFlt-1, soluable Flt-1; PlGF, placental growth factor; GA, gestational age; TAT, thrombin-antithrombin complex; PIC, plasmin-alfa2 plasmin inhibitor complex; HC, head circumference; rLD, recurrent late decelerations; PD, prolonged deceleration; IUFD, intrauterine fetal death; sVD, severe variable deceleration; NND, neonatal death; sPE, severe preeclampsia; HELLP, hemolysis; elevated liver enzymes; and low platelet; SLE, systemic lupus erythmatosus.
MR, mental retardation; CMV, cytomegalovirus; FVT, fetal vessel thrombosis; MVT, maternal vessel thrombosis; C, chorangiosis; CAM, choriamnionitis; Inf, inflammation.
Statistically significant difference by a one-way ANOVA with post-hoc tests. *, $p<0.05$ in group-1 vs. group-2, and group-1 vs. group-3; and #, $p<0.01$ among the 3 groups (Mean + SD).

Table 2. The changes in the maternal blood concetrations of AT, sFlt-1, PlGF, and VEGF before and after the administration of AT.

	AT % activity			sFLT-1			PlGF			VEGF	D-Dimer	TAT	PIC
	Pre	4-day	14-day	Pre	4-day	14-day	Pre	4-day	14-day				
Group 1	95±14	139±3	100±10	1430±240	1380±310	1580±260	214±86	210±54	285±72	<20	1.1±0.5	5.3±0.9	0.5±0.2
Group 2	83±3	124±14	103±32	6880±1880	6700±1500	6880±2790	38±25	40±25	40±26	<20	1.7±1.0	6.6±2.6	0.5±0.4
Group 3	82±7	132±15	106±28	17340±3630	16630±4460	16440±4690	28±9	27±12	28±11	<20	1.2±0.5	5.1±1.5	0.5±0.1

Mean ± SD