ORIGINAL ARTICLE

Oxidative stress in the fetus of preeclamptic women with fetal growth restriction

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Aim: The aim of this study was to determine whether oxidative stress occurring in the maternal body also affects the fetus in preeclamptic women with fetal growth restriction (FGR).

Methods: We recruited 14 preeclamptic women with FGR, 13 preeclamptic women without FGR, and 10 uncomplicated pregnant women. We measured concentrations of derivatives of reactive oxygen metabolites (d-ROMs) as a marker of oxygen free radicals and biological antioxidant potential (BAP) in a umbilical artery and vein.

Results: Umbilical artery and vein d-ROM levels were elevated in preeclamptic women with FGR compared to the control group. Umbilical artery d-ROM levels were significantly higher than in the vein in preeclamptic women with FGR, but not in those without FGR. Umbilical artery BAP levels did not change significantly in all three groups. Umbilical arterial blood pH was significantly lower and the partial pressure of carbon dioxide (PaCO_2) was significantly higher in preeclamptic women with FGR. Umbilical arterial blood partial pressure of oxygen (PaO_2) tended to be lower in preeclamptic women with FGR.

Conclusions: These results indicate that oxidative stress occurring in the maternal body also affects the fetus in preeclamptic women with FGR.

Introduction

Preeclampsia, a complex generalized syndrome in pregnancy characterized by hypertension and proteinuria, remains a major cause of maternal and fetal morbidity and mortality.¹⁾ Injury to the vascular endothelium is a basic pathologic event in preeclampsia.²⁾ Endothelial injury reduces vasodilator production and may increase vasoconstrictor production, resulting in vasospasms. This endothelial damage is considered to be mediated in part by oxidative stress that results from increased oxygen free radicals and/or decreased antioxidant levels.³⁾ We previously found that oxygen free radical production increased, without a decrease in antioxidant levels, during pregnancy in preeclamptic women. In addition, flow-mediated vasodilation (FMD) of the brachial artery, a known marker of vascular endothelial function, was decreased in preeclamptic women.⁴⁾ There is also a negative correlation between FMD and levels of derivatives of reactive oxygen metabolites (d-ROMs) in all pregnant women. These results support the notion that in preeclamptic women, endothelial damage is associated with oxidative stress, which is caused by increased

oxygen free radical production.

Fetal growth restriction (FGR) frequently accompanies preeclampsia, but its pathogenesis is complex and not fully understood. We previously demonstrated that the uterine artery pulsatility index (PI) measured by Doppler ultrasound was higher in preeclamptic women than healthy women with uncomplicated pregnancy. Furthermore, PI in preeclamptic women with FGR was greater than that of preeclamptic women without FGR.⁵⁾ We also demonstrated that levels of hypoxia-inducible factor-1 α (HIF-1 α), an indicator of placental hypoxia, and 8-hydroxy-2'-deoxyguanosine (8-OHdG), a well-established marker of oxidative DNA damage in nuclei of the placental trophoblast, were significantly higher in preeclamptic women with FGR compared to healthy pregnant women.⁵⁾ These results indicate that impaired utero-placental perfusion due to oxidative stress may cause placental dysfunction, ultimately leading to FGR.

Recent studies established the association between low birthweight and incident coronary heart disease.^{6–8)} The fetal origin hypothesis indicates the possibility that oxidative stress may affect not only the maternal body but also the fetus in preeclamptic women with FGR. We

Table 1. Clinical characteristics of the study population

	Normal	PE	PE + FGR
Number of patients	<i>n</i> = 10	<i>n</i> = 13	<i>n</i> = 14
Maternal age (year)	31.7 ± 1.3	33.1 ± 1.4	32.2 ± 1.1
Body mass index (kg/m ²)	20.0 ± 1.0	22.6 ± 1.2	21.0 ± 1.0
Gestational age of onset of preeclampsia(week)	—	35.3 ± 1.0	31.6 ± 1.0 ^c
Gestational age at test (week)	35.6 ± 0.5	35.0 ± 0.8	33.5 ± 0.9
Gestational age at delivery (week)	37.4 ± 0.2	37.0 ± 0.7	34.1 ± 0.9 ^{a,d}
Neonatal weight (g)	2,936 ± 75	2,547 ± 149	1,692 ± 136 ^{b,e}
Placental weight (g)	557 ± 19	502 ± 25	347 ± 28 ^{b,e}
Neonatal weight/placental weight	5.21 ± 0.2	5.07 ± 0.2	4.88 ± 0.2 ^b
Systolic BP (mmHg)	112.5 ± 2.0	154.1 ± 4.0 ^b	164.7 ± 3.4 ^{b,c}
Diastolic BP (mmHg)	69.2 ± 2.5	93.0 ± 2.8 ^b	100.3 ± 2.8 ^b

Abbreviations: BP, blood pressure; PE, preeclampsia; FGR, fetal growth restriction.

^a *P* < 0.01, ^b *P* < 0.001 versus Normal, ^c *P* < 0.05, ^d *P* < 0.01, ^e *P* < 0.001 versus PE.

Data are expressed as mean ± S.E.

previously demonstrated that concentrations of d-ROMs in the umbilical artery were significantly increased in preeclamptic women with FGR, but not in those without FGR,⁵⁾ suggesting that the production of oxygen free radicals is increased in both the maternal body and fetus in preeclamptic women with FGR. We speculate that oxidative stress may impair utero-placental perfusion and cause placental dysfunction, thereby leading to FGR. Accordingly, oxidative damage may also affect the fetus in preeclamptic women with FGR.

In the present study, we measured oxygen free radical concentrations in the umbilical artery and vein in preeclamptic women and healthy women with uncomplicated pregnancy to determine whether oxidative stress also affects the fetus in preeclamptic women with FGR.

Materials and methods

Study participants

Between April 1, 2010 and March 31, 2012, we consecutively recruited 14 preeclamptic women with FGR, 13 preeclamptic women without FGR, and 10 healthy pregnant women with uncomplicated pregnancy. Enrollment criteria for preeclampsia included systolic blood pressure (BP) ≥ 140 mmHg or diastolic BP ≥ 90 mmHg after 20 weeks gestation, and proteinuria (≥ 300 mg protein/24 hrs). None of the preeclamptic women presented with hemolysis, elevated liver enzymes, or low platelet syndrome. Women with uncomplicated pregnancy, matched as a group for maternal age and gestational age, served as controls. FGR was defined as newborn weight < 10th percentile for gestational age in the study population. All control women had a normal course of pregnancy and full-term delivery. None of

the participants smoked, used caffeine or alcohol, had a history of thyroid disease, liver disease, diabetes mellitus, hypertension, or hyperlipidemia, or were currently taking any medication known to influence lipoprotein metabolism. All participants delivered by cesarean section prior to the onset of labor. All control group participants underwent cesarean section this time was for the previous cesarean section. Written informed consent was obtained from each participant. This study was approved by the Ethics Committee of Aichi Medical University School of Medicine.

Derivatives of reactive oxygen metabolites (d-ROMs) and biological antioxidant potential (BAP)

Blood samples were collected from a maternal vein between 9:00 am and 11:00 am after a 12-hour fast before delivery. They were also collected from the umbilical artery and vein at delivery. The pH, partial pressure of oxygen in arterial blood (PaO₂), partial pressure of carbon dioxide in arterial blood (PaCO₂), and base excess (BE) of umbilical arterial blood collected at delivery were determined. We analyzed that d-ROMs, a marker of oxidant status, and BAP using a Free Radical Analytical System (FRAS) 4 (Diacron, Grosseto, Italy).⁹⁾ This assay measures serum levels of organic hydroperoxides, which reflect levels of free radicals from which they were formed. In an acidic buffer, hydroperoxides react with iron, a transition metal liberated from proteins, and are converted to alkoxyl and peroxy radicals. These radicals are able to oxidize an additive to the corresponding radical cation. The concentration of this persistent species was determined by spectrophotometry (505 nm). Results of the d-ROM test are expressed as Carratelli units (CARR U). The BAP serum levels were also measured

to determine the antioxidant levels. The BAP test is based on the ability of a colored solution containing a source of ferric ions bound to a special chromogenic substrate to decolorize when ferric ions are reduced to ferrous ions, which occurs when a reducing/anti-oxidant source is added. The concentration is then measured by spectrophotometry (505 nm).

Statistical analysis

We used SPSS Statistical Software, version 14.0 for Windows (SPSS Inc., Chicago, IL, USA), to perform statistical analysis. Data are expressed as mean $\pm$ standard error (SE). We compared patient characteristics and serum concentrations of d-ROMs by one-way analysis of variance, followed by Scheffe's multiple comparison procedure. Concentrations of d-ROMs and BAP in the umbilical artery and vein were compared with the Student's paired *t*-test. $P < 0.05$ was considered significant.

Results

Maternal age and gestational age at test did not differ significantly between preeclamptic and control groups; however, neonatal and placental weights were significantly lower and the ratio of neonatal weight to placental weight were significantly higher in preeclamptic women with FGR than in healthy or preeclamptic women without FGR. Gestational age at preeclampsia onset and at delivery was significantly earlier for preeclamptic

Figure 1. Concentrations of d-ROMs in umbilical artery (black column) and vein (white column) in the control group and preeclamptic women with and without FGR. PE, preeclampsia; FGR, fetal growth restriction; U.V., umbilical vein; U.A., umbilical artery; N.S., not significant. Data are expressed as mean $\pm$ SE.

women with FGR than those without FGR (Table 1).

Concentrations of d-ROMs in both umbilical artery and vein were significantly increased in preeclamptic women with FGR, but not in those without FGR (Table 2A). Concentrations of d-ROMs in the umbilical artery were significantly higher than that in the umbilical vein in preeclamptic women with FGR ($P < 0.05$); this was not the case for preeclamptic women without FGR (Figure 1). Concentrations of BAP in umbilical artery were similar in all three groups (Table 2A).

Umbilical arterial blood pH was significantly lower in preeclamptic women with FGR, but not in those without FGR (Table 2B). Umbilical arterial blood PaO_2 tended to be lower in preeclamptic women with FGR ($P = 0.08$), but not in those without FGR (Table 2B). Umbilical arterial blood PaCO_2 was significantly higher in preeclamptic women with FGR, but not in those without FGR (Table 2B). Umbilical arterial blood BE was similar in all three groups (Table 2B).

Discussion

Oxygen free radical production is increased in preeclamptic women.^{10–13} We previously demonstrated that maternal serum concentrations of d-ROMs were increased in preeclamptic women with or without FGR, confirming that oxidative stress is increased in preeclamptic women.⁵ Our previous findings also indicated that increased d-ROMs originate from the placenta in preeclamptic women, as that these levels tended to decrease after delivery.⁵ In the present study, we measured concentrations of d-ROMs in the umbilical artery and vein and found that they were elevated only in preeclamptic women with FGR, but not in those without FGR. These results suggest that increased d-ROMs in the umbilical artery and vein may originate in the placenta, umbilical cord, and fetus in preeclamptic women with FGR. Furthermore, concentrations of d-ROMs in the umbilical artery were significantly higher than that in the umbilical vein in preeclamptic women with FGR, whereas this was not the case in those without FGR. These results indicate that oxygen free radical production is not only increased in the maternal body, but also in the fetus in preeclamptic women with FGR.

Pathogenesis of preeclampsia is associated with placental underperfusion and ischemia,^{14,15} and blood flow resistance in uterine arteries increases prior to the onset of clinical signs.^{16–18} We previously found that uterine arterial PI measured by Doppler ultrasound was higher in preeclamptic women than those with uncomplicated pregnancy. Moreover, PI in preeclamptic women with FGR was greater than that of preeclamptic women without FGR. We also found that HIF-1 α and 8-OHdG staining in preeclamptic women with FGR was

Table 2. Derivatives of reactive oxygen metabolite (d-ROMs) and biological antioxidant potential (BAP) concentrations in maternal blood, umbilical artery, and vein

A	Normal	PE	PE + FGR
Concentrations of d-ROMs			
In umbilical artery (CARR U)	29.0 ± 4.2	52.1 ± 7.6	135.6 ± 17.7 ^{c,f}
In umbilical vein (CARR U)	33.0 ± 4.6	65.8 ± 17.4	128.5 ± 13.5 ^{c,f}
Concentrations of BAP			
In umbilical artery (μM)	2,702.9 ± 161.8	2,908.2 ± 111.4	2,819.1 ± 129.0
B	Normal	PE	PE + FGR
In umbilical arterial blood			
pH	7.36 ± 0.01	7.33 ± 0.01	7.28 ± 0.01 ^{c,e}
PaO ₂ (mmHg)	24.9 ± 2.3	23.8 ± 2.7	19.1 ± 2.3
PaCO ₂ (mmHg)	41.0 ± 1.8	41.0 ± 1.7	52.1 ± 2.4 ^{b,e}
BE (mEq/l)	-1.9 ± 0.9	-3.1 ± 0.9	-3.5 ± 0.6

Abbreviations: PE, preeclampsia; FGR, fetal growth restriction; PaO₂, partial pressure of oxygen in arterial blood; PaCO₂, partial pressure of carbon dioxide in arterial blood; BE, Base excess.

Data are expressed as the mean ± S.E.

^a $P < 0.05$, ^b $P < 0.01$, ^c $P < 0.001$ versus Normal, ^d $P < 0.05$, ^e $P < 0.01$, ^f $P < 0.001$ versus PE.

stronger than in preeclamptic women without FGR.^{5,18,19)} Furthermore, almost all preeclamptic women with FGR had early-onset preeclampsia, and almost all preeclamptic women without FGR had late-onset preeclampsia. The mean PI in early-onset preeclamptic women was significantly greater than in late-onset preeclamptic women. HIF-1 α and 8-OHdG staining in nuclei of the placental trophoblast in early-onset preeclamptic women was significantly greater than in late-onset preeclamptic women.²⁰⁾ These results indicate that under prolonged preeclamptic conditions in early-onset disease, long-term exposure to preeclamptic conditions impairs uterine artery flow, reduces blood flow to the placenta, causes placental oxidative DNA damage, and ultimately leads to FGR. In the present study, we demonstrated that oxygen free radical production is increased without change of antioxidants in the fetus in preeclamptic women with FGR. Moreover, umbilical arterial blood pH was significantly lower, and umbilical arterial blood PaO₂ tended to be lower in preeclamptic women with FGR. These results indicate that exposure to oxidative stress is not limited to the maternal body, but also causes hypoxia and acidosis in the fetus in preeclamptic women with FGR.

We previously reported that the antioxidant melatonin reversed ischemia-induced accumulation of 8-OHdG and redox factor-1 (ref-1), an indicator of repair of oxidative damage to DNA, and prevented FGR in rats.²¹⁾ Moreover, maternal administration of melatonin protects against ischemia and reperfusion-induced oxidative

mitochondrial damage in the fetal and neonatal rat brain.^{22,23)} A small-scale randomized controlled trial showed a lower occurrence of preeclampsia in high-risk women who received high doses of vitamin C and E.²⁴⁾ However, two recent randomized studies showed no difference in the incidence of preeclampsia in women taking these vitamins compared to controls.^{25,26)} Although antioxidant therapy may not reduce the incidence of preeclampsia, long-term exposure to oxidative stress may be reduced, and fetal growth, hypoxia, and acidosis may be restored by such therapy in women with early-onset preeclampsia. Further clinical studies are needed to determine whether antioxidant therapy can protect against oxidative placental DNA damage and FGR in preeclamptic women.

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Conflict of interest

None.

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