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Oxidatvive Stress in Rat Model of Preeclampsia and Clinical Correlates

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There are growing evidences suggesting a pivotal role of oxidative stress in the pathophysiology of preeclampsia. We investigated oxidative stress in the rat model of preeclampsia, and in clinical cases. Pregnant female rats were injected intraperitoneally with deoxycorticosterone acetate (DOCA) and given 0.9% saline as drinking water during their pregnancy. We assessed plasma F_2-isoprostane (8-iso-$PGF_{2\alpha}$) and malondialdehyde (MDA) in a rat model, and the same markers in the plasma of maternal blood and fetal cord blood in pregnant women with preclampsia. Blood samples from the umbilical arteries and veins were collected separately. The concentrations of MDA were increased in the preeclampsia groups of animal and humans, compared with the control group; it was significantly increased in the umbilical artery and vein of the preeclampsia group. The concentrations of F_2-isoprostane were elevated in the preeclampsia groups of animal and humans, compared with the control group, and the increase in F_2-isoprostane concentration was prominent in the umbilical vein than umbilical artery of the preeclampsia group. Therefore, it appears that the placenta has an important role in the pathophysiology of preeclampsia, and the F_2-isoprostanes of the umbilical vein may serve as a relatively reliable marker for ischemic/hypoxic injury to the fetus during the perinatal period.

Key Words: Preeclampsia, Oxidative stress, Malonaldehyde, F_2-isoprostane, Fetal cord blood

INTRODUCTION

Preeclampsia is a systemic disorder of pregnancy characterized by maternal hypertension, proteinuria, and edema, causing fetal growth restriction and premature delivery (Myatt et al, 2000). Despite extensive research, preeclampsia remains one of the leading causes of maternal mortality worldwide and, yet, there is no reliable screening test (Condo-Agudelo et al, 2004). The precise cause of preeclampsia remains elusive because there is a dearth of animal models (Podjany et al, 2004). Among the few animal models with preeclampsia, we choose one for evaluating its relevance as an animal model, which applied deoxycorticosterone acetate (DOCA) to pregnant rats whose drinking water had been replaced with saline (Ianosi-Irimie et al, 2005).

The debate on the exact role of oxidative stress in the pathophysiology of preeclampsia continues (Myatt & Miodovnik, 1999; Poston & Mallet, 2002). Increasing evidences suggests that a disruption in the oxidative stress-antioxidant balance in pregnancy is likely to be a contributing factor, and the placenta is likely to play a central role in oxidative stress in preeclampsia (Vanderlelie at al., 2005). A relatively hypoxic placenta caused by inadequate uteroplacental circulation is thought to release placenta-derived factors into the systemic maternal circulation (Parra et al, 2005). It has been suggested that these factors mediate a disturbance of the maternal endothelium which results in the clinical manifestations of preeclampsia, such as hypertension and proteinuria. Among the placenta-derived substances suggested are oxidized lipid products, such as lipid peroxides and isoprostanes, which could initiate the maternal pathophysiological changes of preeclampsia. Free radicals, such as peroxyl- and superoxide radicals, can react with polysaturated fatty acids and form lipid peroxides. Isoprostanes are lipid peroxidation products, and are prostaglandin-like compounds produced in vivo by free radicals' peroxidation of arachidonic acid (Roberts & Morrow, 1997). The isoprostanes represent stable markers of oxidative stress, and has been shown to be a reliable and specific indicator of lipid peroxidation (Roberts & Morrow, 2000). They are formed in situ on cell membrane phospholipids and then released into the circulation in free form, presumably by phospholipase A2. F2-isoprostanes represent a class of isoprostanes, and 8-isoprostane (8-iso-prostaglandin $F_{2\alpha}$) is an F2-isoprostane that is increased in many diseases associated with oxidative stress. As free-radical catalysts, isoprostanes are biologically active prostaglandin-like compounds synthesized from arachidonic acid peroxidation, and are not related to cycloo-

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ABBREVIATIONS: F_2-isoprostane, 8-iso-prostaglandin$F_{2\alpha}$; MDA, malondialdehyde; DOCA, deoxycorticosterone acetate; BP, blood pressure; COX, cyclooxygenase; ELISA, enzyme-linked immunosorbent assay.

xygenase (COX) under abnormal conditions, as reported by Jason *et al.* in 1993.

Animal models are definitely needed in preeclampsia research for furthering our understanding of the pathophysiology of this disease, but, because preeclampsia occurs spontaneously in only primates, the definitive studies on preeclampsia will, of necessity, be clinical. Therefore, we compared the biochemical markers of oxidative stress in an animal model to that in pregnant women with preeclampsia.

To establish relatively reliable markers for hypoxic injury during pregnancy or delivery, we comparatively monitored the concentration changes of F_2-isoprostane and MDA, as markers of lipid peroxides, in the plasma of the rat model and in the maternal human preeclamptic and normotensive pregnancy. We also compared concentration changes of F_2-isoprostane and MDA in the plasma of the umbilical artery and vein in normal delivery and preeclampsia groups to elucidate the role of the placenta as an oxidative stress mediator in preeclampsia.

METHODS

Rat model with preeclampsia

Female Sprague-Dawley (SD) rats (225~250 g; Central Lab. Animal Inc., Seoul, Korea) were allowed free access to standard rat chow and tap water. They were maintained on a 12 : 12-hr light:dark cycle and acclimatized for one week before being studied. The animals were mated with male SD rats. Pregnancy was confirmed by the presence of vaginal plugs or by examination of vaginal smears. For the preeclampsia model rats (n = 10), 10.0 mg of DOCA were injected intraperitoneally in a depot form, followed by 5.0 mg injections of DOCA on a weekly basis. In these animals, drinking water was replaced with 0.9% saline. For the control group of normal pregnant rats (n = 10), saline was daily injected. Blood levels of malondialdehyde (MDA) and F_2-isoprostane were assessed on day 20 of gestation. Intracardiac blood sampling and a hysterectomy were performed on day 20 of gestation. The mean systolic BP (measured with tail cuff device), level of proteinuria, and the pups' birth weights were recorded.

Human study

We enrolled a total of 39 pregnant women who had delivered single births normally at Dankook University Medical Center in Cheonan, Korea. The study was approved by the Human Research Review Committee of the Dankook University School of Medicine. Venous blood samplings were performed from the antecubital vein before delivery and other blood samples were collected from their umbilical arteries and veins immediately after delivery. The normal delivery group (n=24) and preeclampsia group (n=15) were assigned. Preeclampsia was defined as a rise in blood pressure after 20 weeks' gestation to >140/90 on ≥ two occasions six hours apart in a previously normotensive woman, combined with proteinuria. Proteinuria was defined as a protein dip stick having ≥1+ on ≥ two midstream urine samples 6 hours apart or a 24-hour urine excretion of ≥0.3 g protein, in the absence of a urinary infection.

F_2-isoprostane analysis

Blood samples were collected into cold tubes with 3.8% trisodium citrate (blood: anticoagulant ratio being 9 : 1) and indomethacin 15 μmol/L, kept on ice for 30 min, and centrifuged for 10 min at 2000 rpm at 4°C. The plasma was collected and protected from oxidation by the addition of butylated hydroxytoluene at a final concentration of 20 μmol/L, before storage at −70°C until analysis. Following the extraction method by Kim et al (1998), 0.5 mL of plasma added to 99.9% methanol, 0.5 mL, with 0.1% acetic acid was centrifuged at 15,000 rpm at 4°C for 20 minutes. The supernatant was treated with a threefold volume of 0.1% acetic acid to be the equivalent concentration of 25% methanol. The diluted supernatant was loaded onto a 2 mL Sep-Pak column (pre-washed with 5 mL of methanol containing 0.1% acetic acid and 5 mL of 0.1% acetic acid). The Sep-Pak column was washed with 5 mL of 0.1% acetic acid containing 25% methanol. The fraction containing F_2-isoprostane was extracted using 2.5 mL of 90% methanol with 0.1% acetic acid. The eluate was vaporized to nearly dryness on the SpinVac and resolved to 0.2 mL of 50% methanol. F_2-isoprostane was measured by the 8-iso-$PGF_{2\alpha}$ ELISA Kit purchased from R & D Systems Co. (Minneapolis, MN, USA) and the manufacturer's instructions were followed.

Malondialdehyde analysis

After 1.0 mL of blood was treated with 0.5 mL of 0.5 M perchloric acid in a water bath and protein was removed, 0.5 mL of supernatant was treated with 0.2 mL of 8.1% sodium laurylsulfate, 1.5 mL of 20% acetic acid, 1.5 ml, 1.5 mL of 0.8% 2-thiobarbituric acid (2-TBA) and distilled water, to be 4 mL in total volume. The tube was sealed in a vacuum and heated in a 100°C heating block for one hour. After cooling the tube, the mixture of n-butanol and pyridine (15/1, v/v) 5 mL was added in a water bath, vortexed for one minute and was centrifuged at 4,000 rpm for 10 minutes. Absorbance was measured at 532 nm.

Statistical analysis

Data are presented as mean±SEM. Statistical analysis was performed with an ANOVA (analysis of variance). Fisher's protected least significant difference test (PLSD) test was used as a post hoc test. A probability level less than 0.5 was considered significant.

RESULTS

Rat model with preeclampsia

The results of these studies are provided in Table 1. Mean BP increased from 106±5 to 135±6 mmHg ($p<0.05$) in the animals that received DOCA+saline. The DOCA+saline model animals, when compared with the normotensive controls, showed higher mean arterial pressure (135±6 versus 107±7 mmHg, respectively; $p<0.05$). The DOCA+saline model animals showed many phenotypic characteristics of preeclampsia, including the development of hypertension, proteinuria, and intrauterine growth retardation. Plasma F_2-isoprostane concentrations were signifi-

cantly higher in DOCA+saline rats when compared with the control pregnancy group (587.9±70.7 vs. 288.3±28.6 pg/ml, $p<0.01$). Plasma MDA concentrations were also significantly higher in DOCA+saline rats when compared with the control pregnancy group (4.12±1.16 vs. 1.78±0.31 μmol/ml, $p<0.01$).

Human preeclampsia

Plasma F_2-isoprostane concentrations were significantly higher in preeclampsia pregnancy group when compared with normotensive pregnancy group (66.5±6.9 vs. 42.2±5.4 pg/ml, $p<0.05$). Plasma MDA concentrations were also significantly higher in preeclampsia pregnancy group when compared with normotensive pregnancy group (203.4±24.1 vs. 128.9±20.8 nmol/ml, $p<0.05$) (Table 2).

Concentration changes of F_2-isoprostane in the umbilical artery and vein

This study monitored the concentration changes of F_2-isoprostane in the umbilical artery and vein in each group. In the normal delivery group, the concentrations of 8-iso-$PGF_{2\alpha}$ were 85.4±8.4 pg/mL in the umbilical artery and 81.7±8.9 pg/mL in the umbilical vein, respectively. In the preeclamptic delivery group, the concentrations of F_2-isoprostane were markedly increased in the umbilical artery to 2.8 times the concentrations in the normal delivery group; they were also markedly increased in the umbilical vein to 3.3 times that of the normal delivery group, respectively (Fig. 1).

Table 1. Blood pressue, number of fetus, maternal plasma concentration of F_2-isoprostane and malondialdehyde (MDA) from pregnant control and DOCA and saline administered preeclampsia groups (PE) of Sprague-Dawley rats on 20 days of gestation

	Controls (n=10)	PE (n=10)
Blood pressure (mm Hg)	107±7	135±6*
Number of fetus	11.8±3.5	7.1±3.1*
Plasma F_2-isoprostane concentration (pg/mL)	288.3±28.6	587.9±70.7**
Plasma MDA concentration (μmol/mL)	1.78±0.31	4.12±1.16**

Data represented as mean±SEM. *$p<0.05$, **$p<0.01$, control pregnancy group versus preeclampsia pregnancy group.

Table 2. Maternal plasma concentration of F_2-isoprostane and malondialdehyde (MDA) from pregnant control and preeclampsia pregnancy groups (PE) at term

	Controls (n=24)	PE (n=15)
Plasma F2-isoprostane concentration (pg/mL)	42.2±5.4	66.5±6.9*
Plasma MDA concentration (nmol/mL)	128.9±20.8	203.4±24.1*

Data represented as mean±SEM. *$p<0.01$, control pregnancy group versus preeclampsia pregnancy group.

Concentration changes of malondialdehyde in the umbilical artery and vein

In the normal delivery group, the concentrations of MDA were 189.7±33.7 nmol/mL in the umbilical artery and 176.9±23.4 nmol/mL in the umbilical vein, respectively. In the preeclamptic delivery group, the concentrations of MDA were 312.5±53.9 nmol/mL in the umbilical artery and 293.1±46.4 nmol/mL in the umbilical vein, respectively, being significantly higher both in the umbilical artery and vein than that in the normal delivery group (Fig. 2).

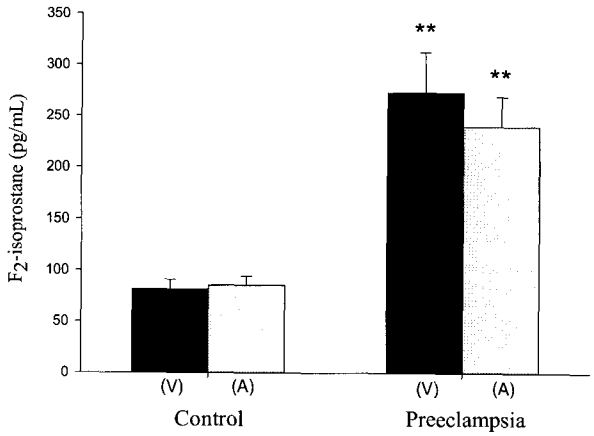

Fig. 1. Plasma concentration of 8-isoprostane in umbilical vein (V) and artery (A) immediately after delivery. Control, normotensive pregnancy; preeclampsia, preeclamptic pregnancy. **$p<0.01$ preeclampsia delivery group versus their respective control delivery group.

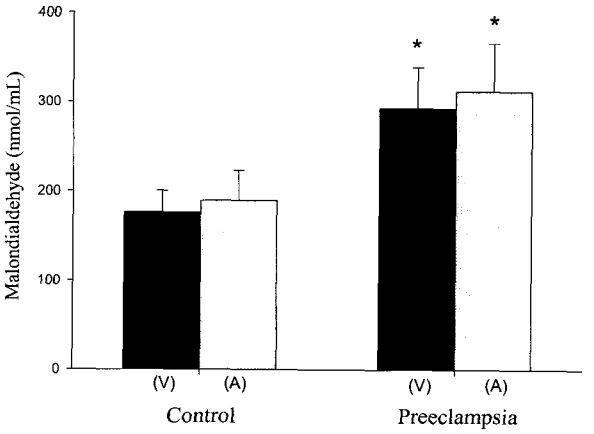

Fig. 2. Plasma concentration of malondialdehyde in umbilical vein (V) and artery (A) immediately after delivery. Control, normotensive pregnancy; preeclampsia, preeclamptic pregnancy. *$p<0.05$ preeclampsia delivery group versus their respective control delivery group.

DISCUSSION

Studies using animal models with preeclampsia would be of great value in trying to determine the etiology and pathogenesis of the disease and finding a preventive or curative treatment for it. Preeclampsia seems to occur spontaneously in only primates (Baird NJ Jr, 1981), but conditions mimicking the human disease have been induced in various animals in numerous experimental studies (Podjany et al, 2004). Most animal models with preeclampsia seem to produce the second stage of the disease, and the pathophysiologic changes leading to this condition are not identical to the human syndrome. The adapted animal model has a pathogenetic process which includes excessive expansion of the ECF volume, and it produces many of the characteristics of the preeclamptic human patient phenotypically and showed similar levels of oxidative stress as in human preeclampsia. We propose the mentioned animal model may allow direct study of preeclampsia and favor establishment of preventive & curative treatments. Preeclampsia most likely represents a group of diseases with similar phenotypic characteristics, rather than a single disorder (Sibai, 1998; Vatten & Skjaeven, 2004). The common pathogenetic process is the hypoperfusion of the maternal-fetal unit. Increased plasma concentration of F_2-isoprostane supports the hypothesis that poor uteroplacental perfusion is predisposed to an increase in placental free-radical synthesis and, thereby, to maternal oxidative stress (Raijmakers et al, 2004). Our results are in agreement with Chappel et al. (2002) who showed that this marker of lipid peroxidation was increased in women who later had PE. The placenta is known to be the main tissue forming lipid peroxides during pregnancy (Wickins et al, 1981). This finding is associated with elevated levels of lipid peroxides in placental tissue, followed by a sudden decrease at the time of deleivery (Kulkarni & Kenel, 1987). During pregnancy, prostacyclin and vitamin E have vasodilatory actions resulting from their innate anti-oxidative effects and, thereby, play key physiological roles in maintaining pregnancy. In contrast, thromboxane and lipid peroxides, synergistically involved in vasoconstriction and peroxidation, cause derangement in the maintenance of pregnancy (Yuping et al, 1991). Therefore, overdosed lipid peroxides released from the placenta cause the disturbances in vascular endothelial cells, followed by the symptomatic manifestation in preeclampsia (Carl et al, 1989) and the elevation of F_2-isoprostane in fetal cord blood (Yun et al, 2000). Since F_2-isoprostane causes endothelial injury, platelet activation and vasoconstriction, as such, it might well be an etiologic factor as well as a marker for preeclampsia (Anne et al, 1999).

The maternal plasma of the rat model and of that in the human preeclampsia showed consistently high levels of MDA and F_2-isoprostane. These were also increased more in the umbilical artery and vein, therefore, it seems that the placenta has an important role in the pathophysiology of preeclampsia. The measurement of F_2-isoprostane than MDA concentration in the umbilical vein may be used as a reliable and stable marker to assess perinatal outcome for the fetus because F_2-isoprostane have increased more in the umbilical vein than artery.

In summary, the results from the current study suggest that oxidative stress is a important factor in the pathogenesis of preeclampsia. We expect this finding and antioxidant treatment will be promising in the prevention and cure of the disease. Moreover, the effects of antioxidants on preeclampsia might be evaluated by assessing the changes of F2-isoprostane in various tissues.

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REFERENCES

Anne CS, Bente H, Trine R, Tore H. Elevated level of free 8-iso-prostaglandin F_{2a} in the decidua basalis of women with PIH. *Am J Obstet Gynecol* 181: 1211-1215, 1999

Baird NJ Jr. Eclampsia in a lowland gorilla. *Am J Obstet Gynecol* 141: 345-346, 1981

Carl AH, James MR, Robert NT, Thomas JM, George MR, Margaret KM. Lipid peroxidation in pregnancy: new perspectives on PIH. *Am J Obstet Gynecol* 161: 1025-1034, 1989

Chappell LC, Seed PT, Briley A, Kelly FJ, Hunt BJ, Charnock-Jones DS, Mallet AI, Poston L. A longitudinal study of biochemical variables in women at risk of preeclampsia. *Am J Obstet Gynecol* 187: 127-136, 2002

Conde-Agudelo A, Vilar J, Lindheimer M. World Health Organization systemic review of screening tests for preeclampsia. *Obstet Gynecol* 104: 1367-1391, 2004

Ianosi-Irimie M, Vu HV, Whitbred JM, Pridjian CA, Nadig JD, Williams MY, Wrenn DC, Pridjian G, Puschett JB. A rat model of preeclampsia. *Clin Exp Hypertens* 27: 605-617, 2005.

Jason DM, Tanya AM, Chetan RM. Free radical-induced generation of isoprostanes in vivo. *J Biol Chem* 269: 4317-4326, 1993

Kim HG, Huh YN, Park KS. Simultaneous HPLC analysis of arachidonic acid metabolites in biological samples with simple solid phase extraction. *Kor J Physiol Pharmacol* 2: 779-785, 1998

Kulkarni AP, Kenel MF. Human placental lipid peroxidation. some characteristics of the NADPH-supported microsomal reaction. *Gen Pharmacol* 18; 155-161, 1987

Myatt L, Kossenjans W, Sahay R, Eis A, Brockman D. Oxidative stress causes vascular dysfunction in the placenta. *J Matern Fetal Med* 9: 79-82, 2000

Myatt L, Miodovnik M. Prediction of preeclampsia. *Semin Perinatol* 23: 45-57, 1999

Parra M, Rodrigo R, Barja P, Bosco C, Fernandez V, Munoz H, Soto-Chacon E. Screening test for preeclampsia through assessment of uteroplacental blood flow and biochemical markers of oxidative stress and endothelial dysfunction. *Am J Obstet Gynecol* 193: 1486-1491, 1991

Podjarny E, Losonczy G, Baylis C. Animal models of preeclampsia. *Semin Nephrol* 24: 596-606, 2004

Poston L, Mallet A. No evidence for lipid peroxidation in severe preeclampsia. *Am J Obstet Gynecol* 187: 1118, 2002

Raijmakers MT, Dechend R, Poston L. Oxidative stress and preeclampsia: rationale for antioxidant clinical trials. *Hypertension* 44: 165-170, 2004

Roberts LJ, Morrow JD. Measurement of F(2)-isoprostanes as an index of oxidative stress in vivo. *Free Radic Biol Med* 23: 505-513, 2000

Roberts LJ, Morrow JD. The generation and action of isoprostanes. *Biochim Biophys Acta* 1345: 121-135, 1997

Sibai BM. Prevention of preeclampsia: a big dissapointment. *Am J Obstet Gynecol* 179: 1275-1278, 1998

Vanderlelie J, Venardos K, Clifton VL, Gude NM, Clarke FM, Perkins AV. Increased biological oxidation and reduced antioxidant enzyme activity in pre-eclamptic placentae. *Placenta* 26: 53-58, 2005

Vatten LJ, Skjaerven R. Is preeclampsia more than one disease? *Br J Obstet Gynecol* 111: 298-302, 2004

Wickins D, Wikins MH, Lunec J, Ball G. Dormandy TL. Free-

radical oxidation (peroxidation) products in plasma in normal and abnormal pregnancy. *Ann Clin Biochem* 18: 158–162, 1981

Yun Q, Chi CW, Hartmut K, Jorg R, Chi PP, Michael SR. Determinants of umbilical cord arterial 8-iso-prostaglandin F2 α concentrations. *Br J Obstet Gynaecol* 107: 973–981, 2000

Yuping W, Scott WW, Jingde G, Junyan Z. Maternal levels of prostacyclin, thromboxane, vitamin E, and lipid peroxides throughout normal pregnancy. *Am J Obstet Gynecol* 165: 1690–1694, 1991