Is There a Role for Isofurans and Neuroprostanes in Pre-Eclampsia and Normal Pregnancy?

Anne E. Barden,¹ Tomas B. Corcoran,^{1,2} Emilie Mas,¹ Thierry Durand,³ Jean-Marie Galano,³ L. Jackson Roberts II,⁴ Michael Paech,^{1,5} Neil A. Muchatuta,⁵ Michael Phillips,⁶ and Trevor A. Mori¹

Abstract

Pre-eclampsia is a complex disorder of pregnancy that adversely affects the mother and baby. Arachidonic acid and docosahexaenoic acid are essential for fetal development and can undergo free radical oxidation to F₂-isoprostanes (F₂-IsoPs) and isofurans (IsoFs); and F₄-neuroprostanes (F₄-NeuroPs), respectively. These metabolites may be relevant to pre-eclampsia and fetal development. We examined IsoFs, F₄-NeuroPs, and F₂-IsoPs in maternal plasma and cord blood plasma of 23 women with pre-eclampsia and 21 normal pregnancies. Women with pre-eclampsia had significantly elevated maternal IsoFs and F₄-NeuroPs, but not F₂-IsoPs. Cord blood F₄-NeuroPs were elevated among neonates of women with pre-eclampsia. In women with pre-eclampsia, birth weight was predicted by gestation at delivery. The latter was also true in normal pregnancy, but birth weight was negatively related to maternal F₂-IsoPs, IsoFs, and F₄-NeuroPs. We have shown that in women with pre-eclampsia, IsoFs and F₄-NeuroPs are elevated, and cord blood F₄-NeuroPs are increased. The inverse relationship between maternal F₂-IsoPs, IsoFs, and F₄-NeuroPs and birth weight may be relevant as predictors of low birth weight in normal pregnancy. Future studies should examine whether these markers in maternal blood at early stages of pregnancy relate to subsequent maternal, fetal, and neonatal complications. *Antioxid. Redox Signal.* 16, 165–169.

Pregnancy, Pre-Eclampsia, and Oxidative Stress

PRE-ECLAMPSIA IS A COMPLEX DISORDER of unknown etiology. Nulliparity is a major risk factor for pre-eclampsia, but other factors play an important role in the development of pre-eclampsia. For example, in women who subsequently develop pre-eclampsia, total cholesterol and triglycerides are elevated above those of normal pregnancy. F₂-isoprostanes (F₂-IsoPs) are formed from free radical attack on arachidonic acid (AA) and are considered to be good markers of *in vivo* lipid peroxidation (7). We have shown that plasma F₂-IsoP are raised in pre-eclampsia (1). In addition, increased urinary F₂-IsoPs at 16 weeks is associated with a heightened risk of developing pre-eclampsia, thus suggesting that oxidative stress may be important in the pathogenesis of this syndrome (8). Related compounds, such as isofurans (IsoFs) that are also formed from free radical-induced peroxidation of AA but under conditions of high oxygen tension, and F₄-neuroprostanes (F₄-NeuroPs) that are formed from docosahexaenoic acid (DHA, 22:6 ω 3), are likely to be relevant to neonatal

Innovation

Pre-eclampsia is a life-threatening disorder of pregnancy that adversely affects the mother and baby. Oxidative stress may contribute to the pathogenesis of this syndrome. Free radical oxidation of arachidonic acid and docosahexaenoic acid, both essential for fetal development, generates F₂-isoprostanes (F₂-IsoPs) and isofurans (IsoFs); and F₄-neuroprostanes (F₄-NeuroPs), respectively. We examined IsoFs, F₄-NeuroPs, and F₂-IsoPs in maternal plasma and cord blood plasma of 23 women with pre-eclampsia and 21 normal pregnancies. In women with pre-eclampsia, IsoFs and F₄-NeuroPs were elevated and cord blood F₄-NeuroPs increased. These new and important findings may have important clinical implications. Further studies are required to determine how these markers of oxidative stress in maternal and cord blood relate to subsequent maternal, fetal, and neonatal complications.

¹School of Medicine and Pharmacology, Cardiovascular Research Centre, University of Western Australia, Perth, Australia.

²Department of Anaesthesia and Pain Medicine, Royal Perth Hospital, Perth, Australia.

³Faculty of Pharmacy, Institut des Biomolécules Max Mousseron (IBMM) UMR 5247 CNRS/UM I/UM II, Montpellier, France.

⁴Department of Pharmacology, Vanderbilt University, Nashville, Tennessee.

⁵Department of Anaesthesia and Pain Medicine, King Edward Memorial Hospital for Women, Perth, Australia

⁶Western Australian Institute for Medical Research, University of Western Australia, Perth, Australia.

outcomes in complicated pregnancies. DHA is essential for the growth and functional development of the fetal brain and is taken up by the brain, particularly during the last trimester, in preference to other fatty acids. F₄-NeuroPs may be important markers of brain-related oxidative stress (7). The high oxygen requirements of the brain suggest that IsoFs are also likely to be important indicators of brain oxidative stress (7). Although one of the F₂-IsoP isomers, 15-F_{2t}-IsoP, affects vascular and platelet function, the role of F₄-NeuroPs and IsoFs in vascular homeostasis has not been fully characterized. The aim of this study was to compare maternal plasma and cord blood plasma IsoFs, F₄-NeuroPs, and F₂-IsoPs in age-matched women with pre-eclampsia and normal pregnancies.

Measures of Lipid Oxidation in Pre-Eclampsia and Normal Pregnancy

Compared with normal pregnancy, women with pre-eclampsia did not differ with regard to age, body mass index (BMI), or smoking status (Table 1). Women with pre-eclampsia had significantly higher blood pressure, were more likely to be primigravida (43% *vs.* 10%), and their gestation at delivery was shorter. Proteinuria, determined by urinary dipstick, was >1+ in 58% of the women with pre-eclampsia. Birth weight, length, and head circumference of the babies born to these women were significantly smaller than those of normal pregnancies. The ratio of male to female babies in the two groups was not different. Umbilical cord arterial and venous blood gases did not differ between the groups. Ar-

terial and venous bicarbonate (HCO₃⁻) were significantly lower in the pre-eclampsia group, but pH was not different.

Women with pre-eclampsia had increased levels of plasma F₄-NeuroPs and IsoFs compared with normal pregnancy ($p=0.007$ and $p=0.045$, respectively) (Fig. 1A, B). Maternal concentrations of plasma F₂-IsoPs did not differ between the groups (Table 2). Cord blood F₄-NeuroPs (Fig. 1C) were significantly higher in women with pre-eclampsia ($p=0.014$). In contrast, cord blood IsoFs (Fig. 1D) and F₂-IsoPs (Table 2) were not significantly different between the groups. Interestingly, cord blood IsoFs were approximately fivefold higher than levels in maternal plasma.

Univariate regression analyses showed that each of the maternal plasma IsoFs, F₄-NeuroPs, or F₂-IsoPs was not related to levels in cord blood plasma within either the pre-eclampsia or normal pregnancy groups. Cord blood IsoFs, F₄-NeuroPs, and F₂-IsoPs were not significantly correlated with umbilical cord arterial or venous blood gases, birth weight, or head circumference.

Multiple regression analysis was used to explore the relationship between markers of lipid peroxidation and birth weight. Gestation at delivery was a significant predictor of birth weight in both women with normal pregnancies ($p=0.001$) and those with pre-eclampsia ($p=0.0001$) (Table 3). In normal pregnancy, but not pre-eclampsia, inclusion of maternal plasma IsoF, or F₄-NeuroP or F₂-IsoP concentrations significantly improved the model (adjusted $R^2=0.252$ with gestation at delivery alone and $R^2=0.410$ with inclusion of maternal plasma IsoFs, $R^2=0.402$ with F₄-NeuroPs and $R^2=0.496$ with F₂-IsoPs). The model that best explained the variance in birth weight in normal pregnancy included a positive relationship with length of gestation ($\beta=0.596$, $p=0.001$) and a negative relationship with the sum of maternal plasma concentrations of IsoFs, F₄-NeuroPs, and F₂-IsoPs ($\beta=-0.544$, $p=0.002$). This model explained 53.6% of the variance in birth weight in normal pregnancy (Table 3). This relationship was also independent of smoking status, age, BMI, gravida status, and neonatal gender. In pre-eclampsia, gestation at delivery accounted for 81.6% of the variation in birth weight, and inclusion of the lipid oxidation measures did not significantly alter the model.

Do IsoFs, F₄-NeuroPs, or F₂-IsoPs Have a Role in Pre-Eclampsia and Pregnancy?

This study has shown for the first time that women with pre-eclampsia have significantly elevated levels of maternal plasma IsoFs and F₄-NeuroPs formed from free radical oxidation of AA and DHA, respectively. In addition, cord blood F₄-NeuroPs, but not IsoFs, from women with pre-eclampsia are significantly elevated compared with normal pregnancy. Maternal and cord blood F₂-IsoP were not different between the groups.

We have previously shown that plasma F₂-IsoPs were significantly elevated in women with proteinuric pre-eclampsia, matched for age and gestation with normal pregnancies (1, 2). These data were confirmed in several subsequent (4, 5), but not all, studies (6). The finding in our current study that plasma F₂-IsoPs were not significantly increased in women with pre-eclampsia relative to normal pregnancy likely relates to differences in the severity of the condition. In previous reports, we had shown that plasma F₂-IsoPs were increased in women who had been specifically

TABLE 1. CHARACTERISTICS OF WOMEN WITH NORMAL PREGNANCY OR PRE-ECLAMPSIA AND NEONATAL OUTCOMES

	Normal pregnancy (n=21)	Pre-eclampsia (n=23)
Age (year)	30.4±1.1	28.9±1.2
BMI (kg/m ²)	32.7±1.5	32±1.7 ^a
Primigravida (n)	2	10 ^a
Smokers (n)	6	3
Proteinuria ≥1+ dipstick (n)	0	14 ^b
Systolic BP at delivery (mmHg)	129±3	152±3 ^a
Diastolic BP at delivery (mmHg)	80±3	92±1 ^a
Gestation at delivery (weeks)	38.8±0.1	33.9±0.9 ^a
Neonatal Outcomes		
Gender M/F	9/12	11/13
Birth length (cm)	49.0±0.3	43.0±1.2 ^a
Head circumference (cm)	35.2±0.3	30.6±0.8 ^a
Umbilical blood gases		
PaO ₂ (mmHg)	15.2±1.5	15.7±1.0
PvO ₂ (mmHg)	27.5±3.0	23.8±1.6
PaCO ₂ (mmHg)	56.7±2.1	54.4±1.3
PvCO ₂ (mmHg)	45.4±1.4	46.5±1.2
Arterial HCO ₃ ⁻ (mmol/L)	25.9±0.5	22.1±0.7 ^a
Venous HCO ₃ ⁻ (mmol/L)	24.1±0.4	22.1±0.6 ^a
Arterial pH	7.28±0.01	7.28±0.01
Venous pH	7.34±0.01	7.32±0.01

Values are mean±SEM.

^a $p<0.05$.

^b $p<0.01$.

BMI, body mass index; BP, blood pressure.

FIG. 1. Maternal blood F₄-NeuroPs (A) and IsoFs (B), and cord blood F₄-NeuroPs (C) and IsoFs (D) in women with pre-eclampsia or normal pregnancy. Values are geometric mean and 95%CI. * $p \leq 0.05$, † $p \leq 0.001$. F₄-NeuroPs, F₄-neuroprostanes; IsoFs, isofurans.

recruited on the basis of having proteinuria $>2+$ on dipstick testing (mean proteinuria >2 g/day). Proteinuria was less pronounced in the women with pre-eclampsia in the current study, with 58% having a dipstick reading $>1+$. Another reason for the divergent results between studies may be the different timings of measurements. Our previous studies measured plasma F₂-IsoPs in pre-eclampsia and normal pregnancy at the same gestational age of ~ 30 weeks. The collection of cord blood samples in the current study necessitated all samples being taken at delivery, which was ~ 34 and 39 weeks in pre-eclampsia and normal pregnancy, respectively.

We have shown for the first time that cord blood F₄-NeuroPs from the oxidation of DHA were significantly elevated in pre-eclampsia relative to normal pregnancy. DHA is an essential fatty acid for fetal development. Studies have shown that apart from using DHA and AA from maternal fat

deposits, the fetus is able to synthesize these fatty acids. Our study is not able to determine whether oxidation of DHA occurred within the fetus or the placenta. We found that maternal F₄-NeuroPs were not significantly correlated with cord blood F₄-NeuroPs in either pre-eclampsia or normal pregnancy, thus suggesting that the origin of cord F₄-NeuroPs may be independent of maternal plasma.

In our study, groups were not different for cord blood IsoFs and F₂-IsoPs concentrations. Varma *et al.* (9) also reported no differences in umbilical vein or artery F₂-IsoPs in women with pre-eclampsia and age-matched controls. To our knowledge,

TABLE 2. MATERNAL PLASMA AND CORD BLOOD PLASMA F₂-ISOPROSTANES IN NORMAL PREGNANCY OR PRE-ECLAMPSIA

	Normal pregnancy (n=21)	Pre-eclampsia (n=23)
Maternal plasma F ₂ -IsoPs (pmol/L)	2061 (1844, 2300)	2276 (2052, 2522)
Cord blood plasma F ₂ -IsoPs (pmol/L)	2071 (1799, 2411)	2482 (2126, 2899)

Values are geometric mean and 95% confidence intervals. F₂-IsoPs, F₂-isoprostanes.

TABLE 3. REGRESSION MODELS EXAMINING PREDICTORS OF BIRTH WEIGHT IN NORMAL PREGNANCY OR PRE-ECLAMPSIA

Predictor variable	β	p-value
<i>Normal pregnancy</i>		
Gestation at delivery (weeks)	0.596	0.001
Maternal plasma (F ₂ -IsoPs + IsoFs + F ₄ -NeuroPs)	-0.544	0.002
Adjusted $R^2 = 0.536$; $F_{2,18} = 12.54$, $p < 0.0001$		
<i>Pre-eclampsia</i>		
Gestation at delivery (weeks)	0.908	0.0001
Adjusted $R^2 = 0.816$; $F_{1,23} = 103.3$, $p < 0.0001$		

Maternal plasma (F₂-IsoPs + IsoFs + F₄-NeuroPs) was not a significant predictor in this model.

F₄-NeuroPs, F₄-neuroprostanes; IsoFs, isofurans.

ours is the first study to measure cord blood IsoFs. We showed that although IsoFs were not significantly different between the groups, their concentration in cord blood was approximately fivefold higher than that in maternal plasma. The very high levels of cord blood IsoFs most likely reflect the oxidative challenge presented at birth, when there is a transition from a relatively low intrauterine oxygen environment to a significantly higher extrauterine oxygen environment. This may have masked the ability to show any differences between the groups. An oxidative challenge is likely to be exacerbated by the low efficiency of natural anti-oxidant systems in the newborn.

We examined predictors of birth weight in babies born to women with pre-eclampsia and normal pregnancy by using multiple regression analysis. Gestation at delivery was not surprisingly a major positive predictor of birth weight in both groups. In normal pregnant women, maternal plasma IsoFs, F₄-NeuroPs, and F₂-IsoPs were negatively related to birth weight, and each independently improved the model. Birth weight in normal pregnancy was best predicted by a model that included the sum of maternal IsoFs, F₄-NeuroPs, and F₂-IsoPs, and gestation at delivery. This model accounted for 53.6% of the variation in birth weight. In contrast, in pre-eclampsia, gestation at delivery accounted for 81.6% of the variation in birth weight, and inclusion of the lipid oxidation measures did not significantly contribute. Our data are in accordance with a previous study which showed that increased markers of lipid peroxidation and DNA damage were related to lower birth weight in full-term deliveries (9).

Concluding Remarks and Future Directions

Our study has shown that increased maternal oxidation of both AA and DHA are important determinants of birth weight in normal pregnancy. It is possible that the relationship between these markers of lipid peroxidative stress and fetal growth are present earlier in pregnancy but confounded by other manifestations of pre-eclampsia. Future studies should examine these markers in maternal blood during early stages of pregnancy and relate these to subsequent maternal, fetal, and neonatal complications. Such studies may also elucidate whether measurement of IsoFs and F₄-NeuroPs is more sensitive than measurement of F₂-IsoPs in predicting complications during pregnancy. (A fully referenced discussion may be viewed as Supplementary Data, available online at www.liebertonline.com/ars).

Notes

Patients

Women undergoing caesarian section were recruited at King Edward Memorial Hospital for Women in Perth, Western Australia. All patients gave informed written consent to participate in the study that was approved by the human ethics committee of Women and Newborn Health Service of Western Australia. Twenty three patients with pre-eclampsia, defined using the criteria of International Society for the Study of Hypertension in Pregnancy, were recruited. Normal pregnant women ($n=21$) undergoing elective caesarian section were matched for age and studied in parallel with the cases. Women with preexisting medical conditions or gestational diabetes were excluded from the study. Analysis was confined to those women who had singleton pregnancies, and all women received spinal anesthesia with bupivacaine and fentanyl.

Blood sampling

Maternal venous blood from the antecubital vein and umbilical cord venous blood obtained after delivery was collected into EDTA, reduced glutathione and butylated hydroxytoluene. Samples were centrifuged at 4°C, and plasma was stored at -80°C until analysis.

Measurement of plasma IsoFs, F₄-NeuroPs, and F₂-IsoPs

IsoFs, F₄-NeuroPs, and F₂-IsoPs were measured by gas chromatography-mass spectrometry using electron capture negative chemical ionization and a modification of our previously reported method (3). Samples were hydrolyzed with 1M potassium hydroxide in methanol, acidified, and applied to pre-washed Certify II cartridges (Varian, Lake Forrest, CA). After washing with methanol/water (1:1) and hexane/ethyl acetate (75:25) IsoFs, F₄-NeuroPs and F₂-IsoPs were eluted with ethyl acetate/methanol (90:10) and dried under vacuum. Samples were derivatized with pentafluorobenzylbromide and N,N-diisopropylethylamine (Sigma Chemicals, St. Louis, MO), dried under nitrogen, and treated with N,O-bis-(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (Pierce Chemicals, Rockford, IL). IsoFs, F₄-NeuroPs, and F₂-IsoPs were quantitated by using d₄-15-F_{2t}-IsoP (5 ng) as an internal standard (Cayman Chemicals, Ann Arbor, MI) and monitoring ions at m/z 569, 573, 585, and 593, for F₂-IsoP, d₄-15-F_{2t}-IsoP, IsoFs, and F₄-NeuroPs, respectively. Standards for IsoF and 4(RS)-F_{4t}-NeuroP were synthesized in our laboratory as previously described.

Statistical analysis

Values are expressed as mean $\pm$ SEM or, where data were not normally distributed, geometric mean and 95% confidence intervals. Data were analyzed by using SPSS version 17. Between-group differences in continuous variables were assessed by using a General Linear Model with univariate analysis. Plasma IsoFs, F₄-NeuroPs, and F₂-IsoPs were transformed by using natural logarithms before analysis. Between-group differences in categorical data were analyzed by using a Chi-square test. Regression models were constructed within each group to examine predictors of birth weight. Predictors were selected on the basis that they were significantly correlated with birth weight but not with each other. Birth weight was entered as the dependent variable, and predictor variables (gestation at delivery, and either individual maternal plasma F₂-IsoPs, IsoFs, or F₄-NeuroPs, or Σ F₂-IsoPs, IsoFs, and F₄-NeuroPs) were entered stepwise.

Acknowledgment

The authors wish to thank research midwives Mrs Desiree Cavill and Tracy Bingham, who were study coordinators at the hospital site.

Funding Sources

The study was supported by grants from the National Heart Foundation of Australia, and by a grant from the Neurotrauma Research Program of the West Australian Institute of Medical Research and the University of Western

Australia. The synthesis of the IsoFs (Prof Roberts II LJ) was supported by a National Institutes of Health Grant GM42056. The synthesis of the neuroprostanes (Dr Durand T) was supported by a University Montpellier 1 Grant BQR-2008.

References

1. Barden A, Beilin LJ, Ritchie J, Croft KD, Walters BN, and Michael CA. Plasma and urinary 8-iso-prostane as an indicator of lipid peroxidation in pre-eclampsia and normal pregnancy. *Clin Sci* 91: 711–718, 1996.
2. Barden A, Ritchie J, Walters B, Michael C, Rivera J, Mori T, *et al.* Study of plasma factors associated with neutrophil activation and lipid peroxidation in preeclampsia. *Hypertension* 38: 803–808, 2001.
3. Corcoran TB, Mas E, Barden AE, Durand T, Galano J-M, Roberts II LJ, Phillips M, Ho KM, and Mori TA. Are isofurans and neuroprostanes increased after subarachnoid hemorrhage and traumatic brain injury? *Antioxid Redox Signal* 15: 2663–2667, 2011.
4. Harsem NK, Braekke K, and Staff AC. Augmented oxidative stress as well as antioxidant capacity in maternal circulation in preeclampsia. *Eur J Obstet Gynecol Reprod Biol* 128: 209–215, 2006.
5. Harsem NK, Braekke K, Torjussen T, Hanssen K, and Staff AC. Advanced glycation end products in pregnancies complicated with diabetes mellitus or preeclampsia. *Hypertens Preg* 27: 374–386, 2008.
6. Ishihara O, Hayashi M, Osawa H, Kobayashi K, Takeda S, Vessby B, *et al.* Isoprostanes, prostaglandins and tocopherols in pre-eclampsia, normal pregnancy and non-pregnancy. *Free Radic Res* 38: 913–918, 2004.
7. Roberts LJ and Fessel JP. The biochemistry of the isoprostane, neuroprostaglandin, and isofuran pathways of lipid peroxidation. *Chem Phys Lipids* 128: 173–186, 2004.
8. Stein TP, Scholl TO, Schluter MD, Leskiw MJ, Chen XH, Spur BW, *et al.* Oxidative stress early in pregnancy and pregnancy outcome. *Free Radic Res* 42: 841–848, 2008.
9. Varma S, Janesko KL, Wisniewski SR, Bayir H, Adelson PD, Thomas NJ, *et al.* F-2-isoprostane and neuron-specific enolase in cerebrospinal fluid after severe traumatic brain injury in infants and children. *J Neurotrauma* 20: 781–786, 2003.

Address correspondence to:

Prof. Anne E. Barden
School of Medicine and Pharmacology
Cardiovascular Research Centre
University of Western Australia
GPO Box × 2213
Perth 6847
Australia

E-mail: anne.barden@uwa.edu.au

Date of first submission to ARS Central, August 9, 2011; date of acceptance, August 9, 2011.

Abbreviations Used

AA = arachidonic acid
BMI = body mass index
BP = blood pressure
DHA = docosahexaenoic acid
F₂-IsoPs = F₂-isoprostanes
F₄-NeuroPs = F₄-neuroprostanes
IsoFs = isofurans