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The role of soluble epoxide hydrolase in preeclampsia

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ABSTRACT

Preeclampsia is a serious complication of pregnancy characterized by the development of vasospasm, hypertension and often associated with proteinuria after the 20th week of gestation. Because termination of pregnancy results in the most efficacious resolution of preeclampsia, it is a leading cause of premature delivery worldwide. In pregnancy, 14,15-epoxyeicosatrienoic acids (EETs) have been shown to facilitate uterine blood flow during preeclampsia, in which the classic vasodilator agents such as nitric oxide and prostacyclin are reduced. EETs are converted to dihydroxyeicosatrienoic acids (DHETs) by the activity of soluble epoxide hydrolase (sEH). We tested the hypothesis that sEH activity is increased in preeclampsia by measuring urinary 14,15-DHET in healthy and preeclamptic pregnant women. Urine samples were collected and incubated with or without β-glucuronidase to enable the measurement of both the glucuronidated and free forms of 14,15-DHET, which were quantified using a 14,15-DHET ELISA. Levels of total (free + glucuronidated) 14,15-DHET, which is a measurement of EET-dependent sEH activity, were higher in urine samples obtained from preeclamptic women compared to healthy pregnant women. Considering the fact that free + glucuronidated 14,15-DHET levels are increased in urine of preeclamptic women, we hypothesize that sEH expression or activity is augmented in these patients, reducing EET and increasing blood pressure. Moreover we suggest that novel anti-hypertensive agents that target sEH might be developed as therapeutics to control high blood pressure in women with preeclampsia.

Introduction

Preeclampsia, a common gestational disorder characterized by the development of hypertension and proteinuria after the 20th week of gestation, is a leading cause of morbidity and mortality for pregnant women and their infants worldwide [1,2]. According to the World Health Organization (WHO) 7.5% of pregnancies are complicated by preeclampsia. The incidence of preeclampsia in the US is 3.5% [1]. Because termination of pregnancy is the most definitive treatment, preeclampsia remains a leading cause of premature delivery worldwide [2–5]. Apart from premature delivery, there are very few effective therapies to remedy the typically relentless course of preeclampsia and therefore knowledge of the mechanism behind its origin and progression is key to the development of targeted therapies.

Studies show that uteroplacental insufficiency, which is the failure of the placenta to deliver adequate nutrients and oxygen, triggers events that lead to endothelial and vascular smooth muscle cell dysfunction, increasing vascular resistance and high blood pressure [6–8]. When the "endothelial cell dysfunction" hypothesis of preeclampsia was first put forward [9], it was primarily thought to be an effect of circulating factors on the maternal microvasculature, but increasingly, ultrastructural studies demonstrate that vessels in fetuses of preeclamptic women also manifest evidence of injury, indicating that these factors can cross the placenta and might directly harm the offspring [10]. So far, the exact link between vascular cell dysfunction and preeclampsia remains unknown. Epoxyeicosatrienoic acids (EETs) act as endothelial-derived hyperpolarizing factors that are involved in vascular relaxation and reduced blood pressure [11–13]. In pregnancy, EETs have been shown to support uterine blood flow during preeclampsia in the face of reduced vasodilator agents such as nitric oxide and prostaglandins [6,11,12]. Therefore maintaining suitable EET levels could be a strategy to control blood pressure during pregnancy. EETs are converted to dihydroxyeicosatrienoic acids (DHETs) by the activity of soluble epoxide hydrolase (sEH) [13]. The majority of DHET is further metabolized to the glucuronidated form by the activity of UDP-glucuronosyltransferase (UGT) and excreted to the urine [14]. Levels of total (free + glucuronidated) 14,15-DHET were higher in plasma and urine of hypertensive patients [11,15], suggesting that sEH might play a role in preeclampsia by converting EET to DHET. In this study we test the hypothesis that

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urinary total (free + glucuronidated) and glucuronidated-DHET serve as markers for preeclampsia.

Methods

Samples

Urine samples from healthy (n = 9) and preeclamptic (n = 7) pregnant women at term were collected, under institutional review board (IRB) approval from the University of California, San Francisco (UCSF). Preeclamptic and healthy pregnant subjects were matched for maternal age, duration of pregnancy and systolic and diastolic blood pressure before 20 weeks' gestation (Table 1). Preeclampsia was defined as new onset hypertension (at least 140/90 mmHg manifested on two readings at least 6 h apart) and proteinuria (>30 mg/dl) consistent with recommendations by the American College of Obstetricians and Gynecologists [16] (Table 1).

Glucuronidated 14,15-DHET analysis

The coded samples were sent to Detroit R&D laboratory and the experiment was carried out in a double-blind fashion. β-Glucuronidase treatment was carried out as described by Newman et al. [14]. Briefly, urine samples were diluted 5-fold with tris-buffered saline (TBS). β-Glucuronidase was dissolved in 1 M sodium citrate solution (pH 5.5) and added to urine samples following a 5 min pre-incubation at 37 °C. Aliquots were removed as soon as the glucuronidase was added (0 h digestion) and 3 h after addition of the glucuronidase (3 h digestion) and immediately frozen on dry ice. Samples were neutralized and further diluted with TBS and 14,15-DHET levels were measured using a commercially available ELISA kit (Detroit R&D, Inc.). Net glucuronidated DHET levels were calculated by subtracting the 14,15-DHET level obtained with the 0 h sample from the 14,15-DHET level obtained from 3 h digested sample (Fig. 1). Free DHET levels were obtained by the ELISA using 4-fold diluted urine samples. Levels of 14,15-DHET were normalized to creatinine levels that were measured using the commercially available ELISA kit (Cayman Chemicals, Ann Arbor, MI).

Statistical analysis

Data are expressed as mean ± SE. Statistical analysis was carried out using commercially-available software (Salstat2). Shapiro–Wilk tests revealed the data were not normally distributed. The Mann Whitney test was performed to compare DHET levels in normal vs. preeclamptic subjects. Two-tailed tests with $p < 0.05$ were considered statistically significant. The Extreme Studentized Deviate test (ESD- GraphPade software) with the significance set as 0.01 was used to detect outliers and only one outlier in the control group for each glucuronidated 14,15-DHET and total 14,15-DHET was detected. These two values were excluded in the analysis. Power analyses, "Satterthwaite's *t* test assuming unequal variances" and "estimated power for a two-sample means test", were performed using STAT3 software using an alpha value of 0.05. The estimated sample size needed for the experimental group based on the mean and standard deviation of measurements of glucuronidated 14,15-DHET and total 14,15-DHET was 7, confirming that the analyses were performed with an appropriate number of subjects.

Table 1
Characteristics of pregnant women. Data are presented as mean ± SD.

Characteristic	Preeclampsia (n = 7)	Control (n = 9)
Maternal age (y)	28 ± 7	26 ± 6
Weeks of gestation at delivery	38 ± 2	39 ± 2
Blood pressure at <20 weeks gestation (mm Hg)		
Systolic	117 ± 10	110 ± 4
Diastolic	65 ± 6	68 ± 3
Predelivery blood pressure (mm Hg)		
Systolic	152 ± 13**	120 ± 4
Diastolic	95 ± 7**	74 ± 7
Proteinuria (Dipstick "+")	2 ± 1**	0
Hematocrit (%)	36 ± 3	35 ± 3
Serum Creatinine	0.9 ± 0.1	0.8 ± 0.1
Serum Uric Acid (mg/dl)	6.4 ± 1.1	3.9 ± 1.4

** $p < 0.01$ compared with healthy pregnant women.

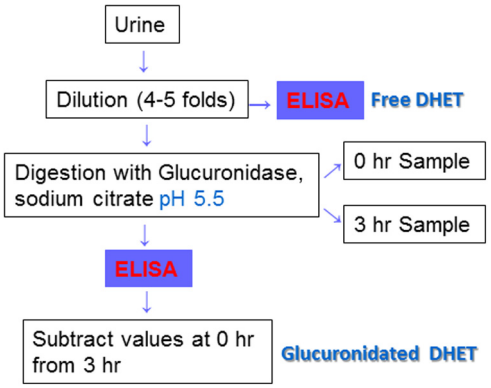

Fig. 1. Protocol for detection of free and glucuronidated DHETs after β-glucuronidase treatment using 14,15-DHET ELISA.

Results/discussion

In the present study, we demonstrate for the first time that the majority of 14,15-DHET excreted into the urine of pregnant women is glucuronidated (Fig. 2). Moreover, the results confirmed our initial hypothesis that urinary levels of 14,15-DHET, total and glucuronidated, were increased in preeclampsia.

Over the past two decades, researchers have demonstrated that EETs have protective cardiovascular effects by acting to maintain vascular homeostasis [11,12,17,18]. Increased levels of EETs, in

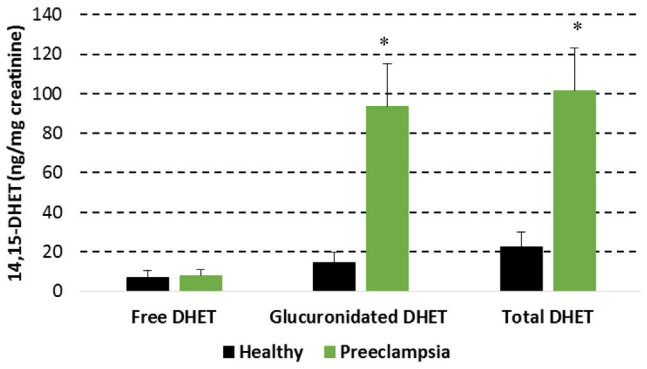

Fig. 2. Levels of 14,15-DHET in normal and preeclamptic pregnant women. Urine specimens of normal and preeclamptic pregnant women were either treated with β-glucuronidase for 0 or 3 h or left untreated. Non-treated samples (no glucuronidase) were used to measure free 14,15-DHET. Levels obtained from 0 h glucuronidase-treated samples were subtracted from levels from glucuronidase-treated samples to measure glucuronidated 14,15-DHET. Total 14,15-DHET was calculated by adding the values of free 14,15-DHET to glucuronidated 14,15-DHET. Levels of 14,15-DHET were normalized by creatinine levels. Data are presented as mean ± SEM. * $p < 0.05$ compared with healthy pregnant women.