Epoxyeicosatrienoic acid Metabolism in Preeclampsia

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SUMMARY

Objective: Preeclampsia (PE) is a disease that characterized by hypertension and proteinuria during pregnancy. Epoxyeicosatrienoic acids (EET) are arachidonic acid metabolites which have vasodilator, anti-inflammatory and profibrinolytic effects. Soluble epoxide hydrolase (sEH; EC 3.3.3.2) catalyses the degradation of EETs to their inactive diols (DHETs). Low circulating levels of EETs may be related to high blood pressure in preeclampsia. The aim of this study is to determine the level of 11,12-DHETs, a representative metabolite of sEH-mediated metabolism of EET, in preeclamptic patients.

Method: 11,12-DHET levels were measured by ELISA in plasma samples of 75 PE patients and 75 normotensive pregnant women as controls.

Results: It was found that plasma 11,12-DHET levels of PE patients was significantly increased compared to the control group ($p < 0.05$).

Conclusions: These results and our previous findings suggest that high sEH activities in PE patients may cause to produce more 11,12-DHETs in PE. sEH enzyme with high catalytic activity may play a role in the pathogenesis of PE by contributing to the reduction of vasodilator, anti-hypertensive and anti-inflammatory effects of EETs by rapid degradation of these molecules.

Keywords: Dihydroxy-eicosatrienoic acid, Epoxyeicosatrienoic acid, Preeclampsia, Soluble epoxide hydrolase
INTRODUCTION

Preeclampsia is a pregnancy specific multi-system syndrome characterized by the new onset of hypertension and proteinuria during the second half of pregnancy. It is the leading cause of maternal and fetal mortality and morbidity affecting 3-5% of all pregnancies. Evidence is accumulating that women with history of PE may have increased risk of cardiovascular diseases (CVD) later in life. Although the pathophysiological mechanism of PE is unclear, a substantial amount of evidence indicates that immunological alterations, systemic inflammation, endothelial dysfunction and genetic factors contribute to the pathogenesis of the disease9. Clinical manifestations such as hypertension and proteinuria indicate the endothelium as the target of the disease and it is believed that endothelial dysfunction is a hallmark of PE.

Vascular endothelium releases vasodilators such as nitric oxide (NO), prostacyclin and endothelium derived hyperpolarizing factors (EDHF) and also vasoconstrictors to regulate vascular tone. Endothelial dysfunction, characterized by a disrupted balance in the production and/or degradation between these molecules resulting in higher concentration of vasoconstrictors is a major predisposing factor for PE. Endothelium-dependent vasodilatation mediated by other than prostacyclin and NO has been attributed to EDHF that includes epoxyeicosatrienoic acids (EETs), hydrogen peroxide (H2O2), potassium and probably other factors. Published data provide convincing evidence for EETs action as EDHFs in arteries from a variety of species including humans. EETs are synthesized from arachidonic acid (AA) by CYP 450 epoxygenases localized in endothelial and vascular smooth muscle cells. CYP2C and CYP2J families of CYP epoxygenases convert AA to four biologically active EETs (5, 6-EET, 8, 9-EET, 11, 12-EET, and 14, 15-EET) that have vasoprotective, antiinflammatory and profibrinolytic effects.

In addition to circulating levels of EETs, they are formed in the placenta, trophoblast, amnion, chorion, decidua, and myometrium of the gravid uterus. Growing evidence suggest that EETs contribute to the physiological response to normal pregnancy and the pathophysiology of pregnancy induced hypertension. Jiang et al. reported that EETs may modulate systemic and fetoplacental hemodynamics in normal and preeclamptic pregnancies. Decreased renal EET generation may be associated with the hypertension in preeclampsia. Catella et al. showed an increase in the biosynthesis of EETs in human pregnancy and a further increment in pregnancy induced hypertension. Zhou et al. suggested that EET synthesis in the kidney was elevated during pregnancy and EETs may contribute to the control of blood pressure during pregnancy. Inhibition of EET producing enzymes (CYP2C11, 2C23, and CYP2J2) by PPOH (an epoxygenase inhibitor) caused hypertension in pregnant rats.

The EETs are hydrated enzymatically to the corresponding dihydroxy-eicosatrienoic acids (DHETs) by epoxide hydrolases. DHETs are less active than EETs and more readily excreted. The reaction catalysed by soluble epoxide hydrolase (sEH; EC 3.3.3.2) is the main pathway in the metabolism of EETs. Inhibition of sEH prevents EET hydrolysis and prolongs their biological activities. Our previous study including 260 PE patients and 260 healthy pregnant women revealed that women having more active form (K55R) of sEH were more susceptible to develop PE suggesting that decreased bioavailability of EETs due to the higher degradation rate may play a role in development of PE. In the present study, we investigated the association between plasma levels of 11, 12 DHET (an EET metabolite) and PE.

MATERIAL AND METHODS

Study population: This study included 75 pregnant women with PE and 75 normotensive controls that 20 or more week pregnant without cardiovascular diseases and diabetes. PE was defined as the new onset of hypertension (systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg) and either proteinuria (Proteinuria ≥0.3 g, in a 24-hour urine specimen or protein: creatinine ratio ≥0.3) or end-organ dysfunction (platelet count <100,000/microliter, serum creatinine >1.1 mg/dL or doubling of the serum creatinine, elevated serum transaminases to twice normal concentration) after 20 weeks of gestation. Pregnant women with baseline hypertension, diabetes, cardiovascular diseases, proteinuria, or renal diseases were excluded from the study. Plasma samples used in this study are blood samples taken from the study group of the previous study, which has been approved by Ethical Committee of Cumhuriyet University in Sivas, Turkey (The Decision Number; 2010-01/09). Informed consent was obtained from all patients and the controls. This work was supported by the Scientific Research Project Fund of Cumhuriyet University (grant number T-551).
Blood Sampling and Plasma 11, 12 DHET Assay: Blood samples of PE patients and the controls were collected into sitrat containing tubes. The blood samples were centrifuged at 1900g for 10 min at 4 °C. Plasma samples were stored at -20°C until analysed. 11, 12 DHET level in plasma was measured using ELISA kit (Eagle Biosciences, Inc.) according to manufacturer's instructions. The results were expressed as nM 11, 12 DHET in plasma.

RESULTS
The characteristics of the study population were presented Table 1. Mean systolic and diastolic blood pressures were significantly different between patients and controls (p<0.05), although no statistically significant difference was observed in terms of mean age, gravidity and parity (p>0.05).

Table 1. Demographic features of subject

<table>
<thead>
<tr>
<th></th>
<th>Patient (N=75)</th>
<th>Control (N=75)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age±SD ( X ± S)</td>
<td>28.6±7.1</td>
<td>28.1±6.0</td>
<td>0.5900</td>
</tr>
<tr>
<td>Gravida ( X ± S)</td>
<td>2.4 ± 1.8</td>
<td>2.1 ±1.9</td>
<td>0.765</td>
</tr>
<tr>
<td>Parity( X ± S)</td>
<td>1.4±1.4</td>
<td>1.2± 0.8</td>
<td>0.480</td>
</tr>
<tr>
<td>SBP (mmHg; X ± S)</td>
<td>147.4±10.1</td>
<td>116.8±9.1</td>
<td>0.0001*</td>
</tr>
<tr>
<td>DBP (mmHg; X ± S)</td>
<td>98.7 ± 9.4</td>
<td>71.4 ± 7.6</td>
<td>0.0001*</td>
</tr>
</tbody>
</table>

*p<0.05: statistically significant; S: standard deviation; X : Mean; SBP: (systolic blood pressure ); DBP (diastolic blood pressure).

As can be seen in Table 2 plasma 11,12 DHET levels in PE patients were significantly higher than controls.

Table 2. Plasma 11,12 DHET levels in patients and controls.

<table>
<thead>
<tr>
<th></th>
<th>Patient (n=75) Median (min-max)</th>
<th>Control (n=75) Median (min-max)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>11,12-DHET (nM)</td>
<td>190.2 (8.6-58031.3)</td>
<td>99.7 (14.4-2692.5)</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

*P<0.05: statistically significant; 11, 12-DHET: 11,12 dihydroxyeicosatrienoic acid.
DISCUSSION

EETs are CYP 450 metabolites of AA that have vasodilatory, antihypertensive, anti-inflammatory properties. It is well established that EETs act as an endothelial-derived hyperpolarizing factor. Recent evidence suggests an important role for EETs in the development and progression of some metabolic diseases. A considerable amount of data indicates their roles in cardioprotective mechanisms. EETs are mainly generated in the liver, kidney and vascular endothelium. In addition to circulating levels of EETs, they are formed in the placenta, trophoblast, amnion, chorion, decidua, and myometrium of the gravid uterus. Therefore in recent years their contribution to physiological response to normal pregnancy and the pathophysiology of pregnancy induced hypertension is pointed out more extensively. EETs are mostly hydrolyzed by sEH into DHETs, which circulate in the blood and are excreted in the urine. Here we investigated the plasma levels of EET metabolite, 11,12 DHET, in preeclamptic patients and healthy pregnant women. We found about 2 fold higher levels of blood 11,12 DHET in preeclamptic women than in healthy pregnant controls. Catella et al. found increased urinary excretion of 11,12 DHET in healthy pregnant women compared with nonpregnant female controls and even further increase in patients with pregnancy induced hypertension. Our results agree with theirs that we found higher plasma 11,12 DHET levels in preeclampsia patients than normotensive pregnant women.

Jiang et al. found increased plasma levels of EETs in both preeclamptic and normotensive pregnancy compared to nonpregnant women and reduced urinary excretion of EETs in the form of DHETs in preeclamptic than normotensive pregnancy. But we found higher plasma 11,12 DHET levels in preeclamptic women than normotensive pregnant women in our study that may reflect the systemic rather than intrarenal levels of EETs and also 11,12 DHETs. In our view this difference is generated by using urine for measurement of 11,12 DHET in their study. Because intrarenal rather than systemic EETs contribute to the level of urinary DHETs and DHETs could be subject to tubular uptake and secretion with the kidney.

From our results, one can conclude that DHET levels increase in plasma as a result of increased plasma EET levels in preeclampsia. Because several studies reported enhanced EET formation in human pregnancy and a further increase in pregnancy induced hypertension. However we bring another point of view to explain the results of the present study that increased sEH activity may be responsible for the elevated plasma 11,12 DHETs in preeclampsia compared to normotensive pregnancy. sEH is the enzyme that converts EETs hydrolytically to DHETs. DHETs are generally thought to be inactivation products of EETs. At least six human sEH variants exist in the human population and that at least four of these may influence sEH-mediated metabolism of endogenous epoxide substrates in vivo. K55R polymorphism results in higher enzyme activity and rapid degradation of EETs by this variant of sEH can cause the higher blood 11,12 DHET levels in PE. An association was reported between K55R polymorphism and hypertension, stroke and CVD. Minuz et al. found reduced ratio of plasma EETs: DHETs in renovascular disease and essential hypertension patients compared to control subjects. They suggest that this reduction may reflect increased sEH activity that will reduce EET levels and, thereby, decrease antipressor activity in renovascular disease and essential hypertension. Because the PE and CVD share many risk factors and pathophysiological features and EETs contribute to the physiological response to normal pregnancy and the pathophysiology of pregnancy-induced hypertension, we think that individual differences in EET catabolism rate may be a susceptibility factor for PE. Moreover in our previous study we reported for the first time that increased sEH activity caused by K55R polymorphism and the promotor hypomethylation of the gene encoding sEH (EPHX2) was significantly associated with PE. These results together with the results of our previous study lead us to conclude that increased activity of sEH may result in increased plasma 11,12 DHET levels in PE and rapid degradation of EETs due to attenuation of beneficial effects of these molecules (anti-inflammatory, vasoprotective and antihypertensive) in pregnancy may play a role in development of the disease. Further epidemiological and mechanistic studies are needed to understand the effect of EET metabolism on the development of PE.

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