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Original Article

2-Methoxyestradiol deficiency is strongly related to hypertension in early onset severe pre-eclampsia

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ABSTRACT

Objective: 2-Methoxyestradiol (2ME) deficiency leading to placental insufficiency has been related to pre-eclampsia (PE). Here we investigate whether 2ME is related to clinical profiles and vasoactive factors in early onset severe PE patients.
Methods: 28 severe PE patients and 20 uncomplicated normal pregnant women, with gestational weeks between 24 and 32 weeks, were recruited. All cases and controls had singleton pregnancies and were matched for maternal age, parity, body mass index, and gestational weeks. Plasma levels of 2ME, estradiol (E2), soluble Fms-like tyrosine kinase-1 (sFLT-1), endothelin-1 (ET-1), nitric oxide (NO) were determined.
Results: PE patients had significant lower 2ME [906(422–1768) vs. 2032(1400–2910) pg/mL, $P = 0.002$], higher sFLT-1 [5.55(3.24–11.22) vs. 3.13(2.17–5.36) ng/mL, $P = 0.015$] and higher NO [122.40(72.92–168.23) vs. 45.83(25.52–61.46) μmol/L, $P = 0.0008$] levels in their plasma than the controls. In the PE group, plasma 2ME level correlated negatively with systolic pressure ($r = -0.48$, $P = 0.012$), diastolic pressure ($r = -0.52$, $P = 0.007$) and mean arterial pressure ($r = -0.54$, $P = 0.005$) even after controlling for maternal age; 2ME level did not correlate with proteinuria, plasma levels of E2, sFLT-1, ET-1 or NO. In the control group, plasma 2ME level did not correlate with any of the above clinical profiles or laboratory measurements.
Conclusions: 2ME levels were markedly lower in early onset severe PE and they correlated inversely with blood pressure only in women with PE. Although we cannot tell whether lower 2ME level is the causation or the result of PE, our study provides clinical evidences that 2ME deficiency is strongly related to hypertension in early onset severe PE patients.

Introduction

Pre-eclampsia (PE) is a syndrome characterized by hypertension and significant proteinuria developed at or after 20 weeks of gestation in an otherwise normotensive woman. It is one of the most common contributors to both maternal and perinatal morbidity and mortality. The precise pathogenesis of PE remains to be determined [1–5].

2-Methoxyestradiol (2ME) is a naturally occurring metabolite of endogenous 17-β estradiol (E2), generated via the sequential actions of cytochrome P450 and catechol-O-methyltransferase (COMT) [6]. The concentration of 2ME increases throughout the three trimesters of

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normal pregnancy [7]. In 2008, Kanasaki et al. first reported deficiencies of COMT and 2ME in cases of pre-eclampsia. In addition, COMT knockout mice develop hypertension and proteinuria when pregnant. 2ME ameliorated pre-eclampsia-like features in the $COMT^{-/-}$ pregnant mice and reversed the increases in placental hypoxia-inducible factor-1α (HIF-1α) expression and plasma soluble Fms-like tyrosine kinase-1 (sFLT-1) levels. Kanasaki et al. suggested that 2ME may be a diagnostic marker or even a therapeutic supplement for PE [8]. Worldwide interest was evoked by their report [9–11], and Lee et al. subsequently reported that 2ME could induce cytotrophoblastic invasion *in vitro* under hypoxic conditions; they suggested that 2ME is necessary for cytotrophoblast invasion in the first trimester and therefore prevents PE [12]. However, although these reports regard the role of 2ME in the pathogenesis of PE, evidence linking 2ME levels to features of PE is still limited.

On the other side, as a stable metabolite of E2, 2ME has been reported to have direct effects on vascular system. 2ME has been found to inhibit vascular smooth muscle cell growth and protect against atherosclerosis and vascular dysfunction [13,14]. 2ME was also reported to inhibit rat aortic smooth muscle contraction [15]. 2ME may thus prevent PE, not only by protecting the placental trophoblasts, but also by promoting maternal vascular health directly.

In this paper, we provide clinical evidence that 2ME deficiency is related to hypertension in cases of PE. We compared the plasma levels of 2ME between early onset severe PE patients and normal pregnant women, analyzed the correlation of plasma 2ME level with clinical profiles and some known vasoactive factors in PE patients. Up till now, this is the largest study of plasma level of 2ME in severe PE patients, and is the first to relate 2ME level to clinical profiles of PE.

Materials and methods

Subjects

In a case-control study, 28 PE patients (PE group) and 20 healthy pregnant women with uncomplicated pregnancies (control group) were recruited from the out-patient or in-patient department of Obstetrics in Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University. The maternal age, parity, BMI, and gestational weeks at blood sampling (all were between 24 and 32 weeks of gestation) were matched. Blood pressures on the first day of recruitment were determined on two occasions longer than six hours apart and the average was recorded. Fasting peripheral blood samples were taken from all the subjects on the day following recruitment.

All the PE patients met the criteria for early onset PE because their diagnosis was made prior to 34 weeks of gestation. All were diagnosed as having severe PE with at least one of the following signs: elevated blood pressure of $\geqslant$160 mmHg systolic or $\geqslant$110 mmHg diastolic; proteinuria $\geqslant$2 g/24 h (without urinary tract infection); abnormal liver or kidney function or thrombocytopenia. In this study, PE superimposed on chronic hypertension was not excluded.

Exclusion criteria for PE and control subjects are as follows: multiple pregnancy, diabetes mellitus diagnosed before or during pregnancy, autoimmune disease, angiopathy, chronic renal disorder, maternal or fetal infection and fetal congenital anomaly.

Blood samples were taken from antecubital veins into tubes containing heparin, and then centrifuged at room temperature at 1000*g* for 15 min. The aliquots of plasma were stored immediately at −80 °C until analyses.

Reagents

Plasma 2ME and E2 concentrations were measured by 2-Methoxyestradiol EIA kit and Estradiol EIA kit (Item No. 582261 and 582251, respectively, from Cayman Chemical Company, USA) according to the manufacture's instruction. Human soluble Fms-like Tyrosine Kinase Receptor 1 (sFLT-1) ELISA Kit (Item No. E01F0103) was from Shanghai BlueGene Biotech CO., LTD, China. Endothelin (ET)-1 Radioimmunoassay Kit (Item No. D11PZB) was from Beijing North Institute of Biological Technology, China. Nitric Oxide (NO) was detected by total NO_2^-/NO_3^- detection Kit (Nitrate reductase, Item No. A012 from Nanjing Jiancheng Bioengineering Institute, China). All the above measurements were performed in duplicate.

Statistical analysis

Data were analyzed using Statistical Analysis System (SAS) version 9.13. All the data were tested for normality. The normal distribution results are expressed as mean values ± standard deviation (SD), while the skewed distribution results are expressed as median values (25–75% quartile). Statistical analysis between two normal distribution groups was performed by *t* or *t*' (equal variance assumed or not assumed) test. Statistical analysis between two skewed distribution groups was performed by Wilcoxon test. Correlation analysis was performed by Spearman rank correlation. In view of multiple comparisons between groups, a threshold of $P < 0.05$ was set for statistical significance of all computed analyses.

Results

Clinical characteristics

There was no significant difference in the maternal age, percentage of nulliparity, BMI, gestational weeks on the day of blood sampling between the PE group and the controls ($P > 0.05$). The PE group had significantly higher systolic pressure, higher diastolic pressure and higher mean arterial pressures than the control group. The PE group had significant higher proteinuria while the controls had negative proteinuria. The PE group had significantly earlier gestational age at delivery, significantly lower birth weight and lower Apgar scores than the controls (Table 1).