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Neurokinin B and urotensin II levels in pre-eclampsia

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Abstract

Objective. To verify neurokinin B (NKB) and urotensin II (UII) plasma levels in pre-eclampsia (PE) and to determine the relationship between plasma NKB and UII levels.

Method. A total of 60 women in the third trimester of pregnancy were recruited, 40 women with PE (study patients) and 20 age- and BMI-matched normotensive women (healthy controls). They were divided into three groups: the 20 normotensive pregnant women (Group 1); 20 women with mild PE (Group 2); 20 women with severe PE (Group 3). The plasma levels of NKB and UII were measured simultaneously by enzyme-linked immunosorbent assay.

Results. Compared with controls, levels of NKB were significantly higher in women with mild or severe PE ($p < 0.01$ for both groups), levels of UII were significantly higher in women with mild or severe PE ($p < 0.01$ for both groups). Moreover, there was a positive correlation between plasma levels of NKB and UII in pre-eclamptic women ($r = 0.783$, $p < 0.01$).

Conclusions. These findings suggest that there was an elevation of NKB and UII in the plasma of pre-eclamptic women. NKB and UII may be involved in the pathophysiology of PE and associated with the development of severe disease.

Keywords: *Pre-eclampsia, neurokinin B, urotensin II, plasma, enzyme-linked immunosorbent assay*

Introduction

Pre-eclampsia (PE) occurs in 7–10% of pregnancies and remains a leading cause of maternal mortality and morbidity [1]. Previous studies indicated that defective trophoblastic invasion was a leading consistent feature in PE, in which the spiral arteries retain their musculo-elastic properties and responsiveness to vasoactive substances, resulting in trophoblastic ischemia and hypoxia.

Traditionally, neurokinin B (NKB) has been classified as neurotransmitters being found in discrete neurons and immune cells [2,3]. These neuropeptides have been implicated in a variety of biological functions, such as smooth muscle contraction, vascular reactivity, pain transmission, neurogenic inflammation and the activation of the immune system [4–8]. Recently, this assumed dogma was challenged when the placenta, a tissue devoid of nerves, was found to be a source of NKB gene expression [9,10]. Page et al. [9,11,12] proposed a hypothesis that excessive secretion of NKB by the placenta could induce PE, the damaged endothelial cell in PE could also lead to reduced vasodilator and elevated vasoconstrictor. Urotensin II (UII) is the most potent endogenous vasoconstrictor identified to date, whose vasoconstriction ability is superior to endothelin, it is abundant in vascular endothelial cells and coupled with its specific high-affinity receptor GPR14 could induce vascular smooth muscle contraction [13]. This study was conducted to determine the relationship between PE and NKB, UII levels in pregnant women.

Patients and methods

Patients

After giving informed consent, 60 women admitted to the Department of Gynecology and Obstetrics of Tongji Hospital were enrolled in the study from October 2006 to February 2008. Forty patients had a

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diagnosis of PE according to the criteria of the International Society of the Study of Hypertension in Pregnancy (ISSHP); they were divided into mild PE and severe PE [14]. PE was defined as sustained pregnancy-induced hypertension with proteinuria. Hypertension was defined as sustained blood pressure (BP) readings of 140/90 mmHg or higher, with readings taking place 6 h or more apart or a sustained 15 mmHg rise in diastolic BP from the first-trimester value or a 30 mmHg rise in systolic BP also from the first-trimester value. The ISSHP defined proteinuria as a protein concentration of 30 mg/dl or greater (or 1+ on a urine dipstick) in two or more random urine specimens collected at least 4 h apart; it defined severe PE as a blood pressure reading of 160/110 mmHg or higher, with either 3+ or 4+ on a dipstick in a random urine sample, or a proteinuria greater than 0.5 g over 24 h. Women who met the criteria for PE but not severe PE were diagnosed as mild PE. The other 20 healthy pregnant women acted as controls; they were all recruited at the same day as for each of the PE case.

There were no differences in age, pregnancy duration, weight and body mass index (BMI) between the PE and the normal pregnant women (Table I). From careful history taking, physical examination and routine laboratory tests, the study participants were found to be non-smokers and had no clinical evidence of diabetes, genetic disorders, infections or autoimmune disease. The PE group's mean $\pm$ SD systolic and diastolic BP at admission were 156 ± 11 and 106 ± 5 mmHg, respectively, on a standard mercury sphygmomanometer, and their mean urinary protein was 1.8 ± 0.2 g/dl. The controls' mean systolic and diastolic blood pressure was 109 ± 7 and 72 ± 6 mmHg, and their mean urinary protein was 0.2 ± 0.0 g/dl. All the patients in the PE (gestational age at delivery: 37.8 ± 0.6 weeks) required cesarean delivery, and the patients in the control group (gestational age at delivery: 37.4 ± 0.3 weeks) underwent an elective caesarean section for other indications (the common reasons were as follows: a previous cesarean section, breech presentation or a narrow pelvis).

Table I. Characteristics of the study participants.*

Variable	Normal pregnancy ($n=20$)	Preeclampsia ($n=40$)	p value
Age (year)	27.9 ± 8.2	28.3 ± 5.1	NS
Weight (kg)	74.4 ± 4.2	73.5 ± 4.3	NS
BMI	22.9 ± 0.6	23.0 ± 0.4	NS
Gestational age (week)	37.8 ± 0.6	37.4 ± 0.3	NS
Proteinuria (g/dl)	0.2 ± 0.0	1.8 ± 0.2	<0.01
Systolic blood pressure (mmHg)	109 ± 7	156 ± 11	<0.01
Diastolic blood pressure (mmHg)	72 ± 6	106 ± 5	<0.01

BMI, body mass index.
*Values are given as mean $\pm$ SD.

The study was approved by the Ethics Committee of the Tongji Medical College and in compliance with their guidelines.

Methods

Fasting blood samples were collected from study patients soon after the disease became manifest. Samples were collected into tubes containing ethylenediamine tetraacetic acid (EDTA), gently rocked the tubes several times immediately for anti-coagulation, transferred the blood from the tubes to centrifuge tubes containing aprotinin (0.6 TIU/ml of blood) and gently rocked several times to inhibit the activity of proteinases, centrifuged the blood at 1600*g* for 15 min at 4°C and collected the plasma. Plasma kept at -70°C may be stable for 1 month.

Plasma concentrations of NKB and UII were determined by enzyme-linked immunosorbent assay (ELISA). The assays were conducted according to manufacturer's protocols. Neurokinin B EIA Kit (Phoenix Pharmaceuticals, US) was used for the measurement of human NKB (sensitivity, 0.4 ng/ml; interassay coefficient of variation [CV], 5.0%). Human Urotensin II ELISA kit (Cusabio, CN) was used for the measurement of plasma UII levels (lower limit, 0.04 pg/ml; interassay CV, 3.2%).

All continuous data (e.g. age, NKB and UII) were presented as mean and standard deviation. Continuous data of two sub-groups were tested for significant difference by the unpaired *t*-test for normally distributed data or, where data were not distributed normally, the Mann–Whitney *U*-test. The correlation was analysed with Pearson correlation coefficients in PE and control groups separately. Statistical differences were based on a significance level of 0.05. All statistical analyses were performed with the software package SPSS for Windows, version 12.0J (SPSS, Chicago, IL).

Results

Results of this study are shown in Tables I, II and Figure 1. As shown in Table I, there were no significant differences in age, weight, BMI or pregnancy duration at blood sampling between the PE and control group. Compared with the control group, a significant increase in the mean serum levels of NKB and UII was found in the PE group ($p < 0.01$ for both) (Table II).

There was an evident positive correlation between plasma NKB and UII levels in PE ($r=0.783$,

Table II. Plasma NKB and UII levels in cases of MPE and SPE subjects.

Variable	Controls	Mild PE	Severe PE
Plasma NKB (ng/ml)			
Mean ± SD	33.82 ± 5.42	41.77 ± 6.34	50.48 ± 4.93
p Value*	–	<0.01	<0.01
p Value**	–	–	<0.01
Plasma UII (pg/ml)			
Mean ± SD	3.17 ± 0.13	4.44 ± 0.36	5.89 ± 0.15
p Value*	–	<0.01	<0.01
p Value**	–	–	<0.01

*Statistical analysis of difference between mild or severe PE values vs. control values.
**Statistical analysis of difference between mild PE values vs. severe PE values.

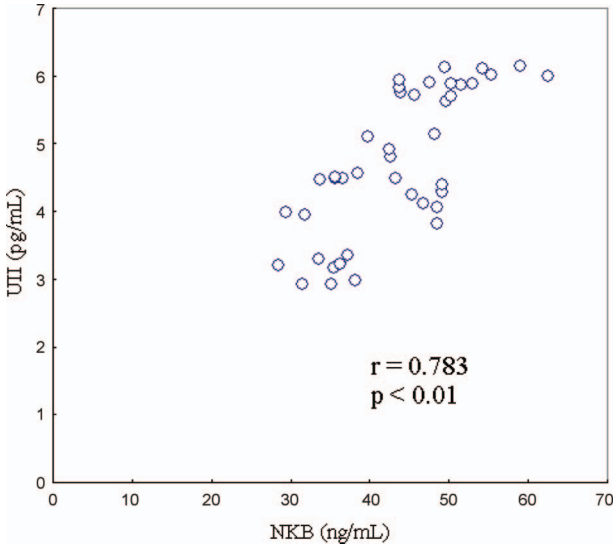

Figure 1. Correlation between plasma NKB and UII concentrations in PE group.

$p < 0.01$) (Figure 1), whereas no significant correlation was observed in the control group.

Table II show the mean levels of plasma NKB and UII in each PE severity group. Women with mild or severe PE had significantly higher plasma levels of NKB ($p < 0.01$ for both) and UII ($p < 0.01$ for both) than women with normal pregnancies. Significant changes were noticed when PE severity increased, NKB and UII levels increased significantly ($p < 0.01$ for both).

Discussion

This study reveals a significant elevation of NKB and UII in the plasma of PE women compared to controls, and with the PE severity increased, NKB and UII levels increase significantly, implying the alteration of these two peptides in maternal blood circulation plays an important role in this disease. It is also noted that plasma NKB concentrations correlated significantly and positively with plasma UII concentrations. These findings reveal the importance of NKB and UII in the pathophysiology and the development of the disease.

Our results are in accordance with the previous reports about NKB plasma behaviour in PE: Page et al. [9] reported extremely high NKB levels in plasma of hypertensive or pre-eclamptic women in the third trimester of pregnancy and low or undetectable protein levels in normotensive patients [9]. Sakamato et al. [15] found that NKB concentration increases as pregnancy advances, decreases rapidly after delivery and its concentration during pregnancy correlates with gestational week, thus confirming that NKB is derived mainly from the placenta. Zulfikaroglu et al. [16] also showed significantly higher levels of NKB in umbilical cord blood than in maternal blood in late pregnancy of patients with PE, it may imply that NKB passes into fetal blood at a significant rate and may have some physiological effects also on the fetus. However, other reports do not support the results of this study. Schlembach et al. [17] reported higher levels of NKB in the serum of healthy, normotensive pregnant women compared with PE, indicating that NKB may not play a role in the pathogenesis of PE and the higher NKB levels in healthy pregnant women may be due to advanced gestational week and/or be a result of a negative interaction of other vasoactive substances. A possible reason for this difference is that their fewer cases (14 PE and 8 normal pregnant women) or other unknown reasons. Although reports of NKB levels in PE are paradoxical and controversial, NKB is attracting considerable interest as a potential biochemical indicator.

Recent and consistent evidences suggest that NKB may play an important role in utero-placental hemodynamic adaptation by inducing uterine and placental vasodilatation, thereby increasing placental blood flow [9,10,18–20]. As the effects of NKB are relatively selective for the venous side of the mesenteric vascular bed, it is feasible that at low dose the only cardiovascular impact would be a modest rise in venous return and hence cardiac output. In this way, release of NKB from the normal placenta could contribute to the hyperdynamic circulation of normal pregnancy. However, on the other hand, the defective trophoblastic invasion could induce excessive secretion of NKB, high-dose NKB responsible for the down-regulation of about 20 proteins secreted by the term placenta trophoblast, which include cytokeratin, thioredoxin and annexin, this deficient could result in peroxidation, the endothelial cell damage and the placenta's ischemia or hypoxia [21]. *In vitro* test, the activation of NKB would cause neutrophilic granulocyte adhere to endothelial cell, white cell and lymphocyte aggregation, accelerate some cytokine's synthesis and release such as interleukin (IL)- 1, -2 and -6, tumor

necrotic factor α (TNFα) and then induce damage to endothelial cell, dysfunction of vascular smooth muscle and release of endothelin (ET) [22–25], these could cause hypertension, edema and proteinuria – all PE's syndrome.

At present, it is considered that the lesion and (or) dysfunction of the endothelial cells is the center ringjoint of the pathogenesis in hypertensive disorder complicating pregnancy. The vascular endothelial cells can excrete many vasoactive substances, and the vasoconstrictor substances predominate and cause the blood vessels contract. However, what relationship does NKB have with hypertension? And who is the intermediary? The latest research hot point to the UII, it is the most potent vasoconstrictor to date, (amounting to some 1- to 110-fold the potency of endothelin-1) [13].

Our data confirm a significant positive correlation between NKB and UII levels in the PE group; to the authors' knowledge, this is the first report concerning this correlation in PE. Defective trophoblastic invasion results in trophoblastic ischemia and hypersecretion of NKB. The endothelial cell damage leads to a reduction in vasodilator and an increase in vasoconstrictor substances in PE [12], such as UII. Interacted with specific high-affinity receptor GPR14, it could induce continued vascular smooth muscle contraction, reduction of tissue perfusion, hypoxia and ischemia – all potentially hypertensive.

In conclusion, our data demonstrated that NKB and UII levels were increased in PE, and that their levels were associated with disease severity. In pathological pregnancies complicated by PE, it seems that placental damage induces NKB production, with the aim of improving blood flow in the utero-placental circulation [19], but high dose NKB responsible for the endothelial cell injury that induce excessive synthesis and secretion of UII. High-dose NKB and UII eventually lead to a series of clinical symptoms of PE. Therefore, the NKB and UII could be used as a serologic indicator of disease progression and severity; they also have important diagnostic and therapeutic implications. This is why it should be explored in future research.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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