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CORRESPONDENCE

Elevated vasoinhibin derived from prolactin and cathepsin D activities in sera of patients with preeclampsia

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Preeclampsia (PE) is a pregnancy-related disease characterized by high blood pressure and proteinuria. Although the precise pathogenic mechanisms remain unclear, aplasia of the placental helicine artery is considered to be a potential cause of PE. Moreover, several biomarkers, such as soluble fms-like tyrosine kinase and placental growth factor, have been suggested to predict PE.[1,2]

Prolactin, a 23-kDa hypophyseal polypeptide hormone, has an angiogenic function. However, a 16-kDa fragment of N-terminal prolactin, which is cleaved by enzymes, such as cathepsin D (CathD), has anti-angiogenic functions.[3] Cleaved growth hormone and placental lactogen also exert anti-angiogenic effects, and these residues are designated as vasoinhibins.

Recent studies have detected vasoinhibin derived from prolactin (PRL-V) in urine, amniotic fluid, placental and serum samples of patients with PE.[4,5] Placental protein expression of CathD was increased more in PE patients than healthy controls.[6] Thus, PRL-V is suspected as one of the contributing factors to PE. However, PRL-V values and CathD activity in the sera of PE patients remained unknown. The aim of the present study was to quantify PRL-V and to measure CathD activity in the sera of PE patients compared with healthy pregnant women. This study was approved by the Ethical Committee at the National Cerebral and Cardiovascular Center in Osaka, Japan, and was performed with the informed consent of all participants.

Seven healthy pregnant women (control group) and nine patients with PE (PE group) participated in the study. Sera and urine samples were collected from participants in the early morning at three separate time points: antepartum (specifically after the diagnosis of PE in the PE group), soon after delivery and 1 month after delivery. The diagnosis and severity of PE were determined by the physicians' clinical judgments according to the National High Blood Pressure Education Program Working Group Report on high blood pressure in pregnancy.[7]

The quantification method of serum PRL-V was developed in the study on the basis of a previous method.[8] Serum was pretreated with an Albumin/IgG Removal Kit (Merck, Darmstadt, Germany), and immunoprecipitated by the human prolactin polyclonal antibody, anti-hPRL-IC-5, CYTO (National Hormone and Peptide Program, Torrance, CA, USA), according to the protocol recommended by the manufacturer. Immunoprecipitated samples were applied to western blotting with an antibody that detected N-terminal of prolactin (anti-hPRL monoclonal antibody clone 5602, Diagnostics

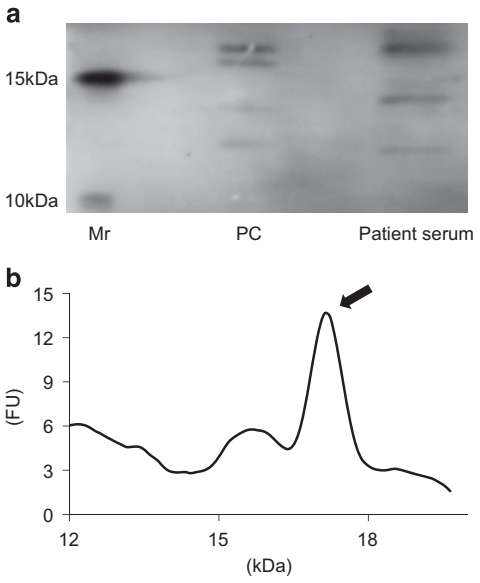

Figure 1 Sample images of vasoinhibin derived from prolactin (PRL-V) measurements. (**a**) Western blot analysis. A western blot analysis of immunoprecipitated serum from a patient with the N-terminal prolactin antibody. Serum bands in the patient serum were detected at ~12, 14.5 and, especially, 17 kDa. Positive control (PC: human prolactin+bovine CathD citrate phosphate buffer, 37 °C, 10 min) bands were detected at 12, 14, 16 and 17 kDa. (Mr, molecular weight marker). (**b**) Electrophoretic waveform by Bioanalyzer. A capillary electrophoresis analysis of the same immunoprecipitated serum in **a**. The vertical axis shows fluorescence units (FU) and the horizontal axis shows the molecular weight of PRL-V. The peak of the electrophoretic waveform was at 17 kDa (arrow). The PRL-V value was quantified with the peak FU. The background FU was subtracted from the peak FU; thus, the PRL-V value was 8.8 FU in this case.

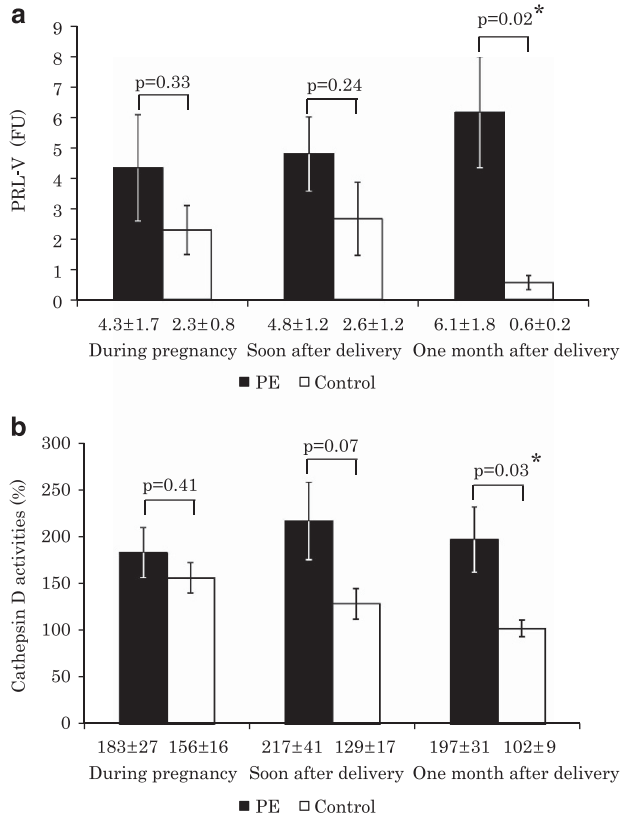

Figure 2 Vasoinhibin derived from prolactin (PRL-V) values and cathepsin D activities in the study participants. (**a**) PRL-V values in the preeclampsia (PE) and control groups. The white bar shows PRL-V values in the control group and the black bar shows those in the PE group. The vertical axis shows the fluorescent unit of PRL-V (mean ± s.e.m., $*P<0.05$ *vs.* control). (**b**) Cathepsin D activity in the PE and control groups. The white bar shows cathepsin D activity in the control group and the black bar shows that in the PE group (mean ± s.e.m., $*P<0.05$ *vs.* control).

Biochem Canada, Dorchester, Ontario, Canada; Figure 1a). Moreover, the same samples were also applied to the Agilent Protein 80 Kit (Agilent Technologies, Santa Clara, CA, USA) and Bioanalyzer (Agilent Technologies) to quantify PRL-V. The peak height of the electrophoretic waveform was indicated as the PRL-V amount (Figure 1b). Each assay was performed in duplicate and the mean values were used for the analysis.

CathD activity was measured by the SensoLyte 520 Cathepsin D Activity Assay Kit (Anaspec, Fremont, CA, USA). The serum of a healthy non-pregnant woman was used as the calibrator in all assays, and CathD activity in participants is presented as a percentage of the calibrator activity. Each assay was performed in triplicate and the mean values were used for the analysis.

Data are expressed as the mean ± s.e.m. Student's *t*-test and the Pearson correlation coefficient were performed.

The average blood pressures of control and PE groups were 108 ± 3/66 ± 3 and 155 ± 4/95 ± 4 mm Hg in antepartum, 113 ± 5/73 ± 5 and 127 ± 4/75 ± 3 mm Hg soon after delivery and 113 ± 4/70 ± 3 and 129 ± 4/81 ± 3 mm Hg 1 month after delivery, respectively (seven patients in the PE group were treated with anti-hypertensive medication soon after delivery; one patient was treated with methyldopa; four patients were treated with nifedipine and two patients were treated with nicardipine). The average proteinuria of control and PE groups were 85 ± 23 and 205 ± 91 mg dl^{-1} in antepartum. The PRL-V values were slightly higher in the PE group than the control group in antepartum and soon after delivery. However, these values were significantly higher in the PE group than the control group 1 month after delivery (Figure 2a).

CathD activities were slightly higher in the PE group than the control group in antepartum and soon after delivery. CathD activity remained significantly higher in the PE group, whereas it had decreased in the control group 1 month after delivery (Figure 2b).

PRL-V values in antepartum were higher in patients with severe PE (5.62 ± 2.61 fluorescence unit (FU), $n=6$) than patients with mild PE (2.15 ± 1.33 FU, $n=3$) but were not significant ($P=0.37$). There were positive correlations between PRL-V and total protein ($r=0.75$, $P=0.05$) and albumin ($r=0.75$, $P=0.05$) in pooled urine. CathD activities correlated with systolic ($r=0.77$, $P=0.07$) and diastolic blood pressures ($r=0.75$, $P=0.16$).

Quantitative detection of PRL-V has been considered to be technically difficult because of the very small amount of PRL-V in serum. This was the first study to quantify serum PRL-V in PE patients, and these results were consistent with previous findings by western blotting analyses.[5,6] Because PRL-V has been investigated in other diseases, such as diabetic retinopathy[8] and peripartum cardiomyopathy,[9] our method may be applicable for elucidating the pathogenic mechanisms for these diseases as well as PE.

PRL-V values and CathD activities were higher in PE patients than healthy controls at all periods, particularly at 1 month after delivery. Although both values 1 month after delivery were decreased in the control group, they remained at a high level in the PE group. PE is usually improved after delivery, whereas vascular dysfunction has been observed late after delivery.[10] Moreover, PE is known as a major risk factor of peripartum cardiomyopathy, which often develops after delivery. Thus, PRL-V may be involved in such vascular disorders or cardiac dysfunction in postpartum women.

As one of the study limitations, the number of samples in this study was small. Further larger studies are required to confirm these results.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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