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Placental (pro)renin receptor expression and plasma soluble (pro)renin receptor levels in preeclampsia

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ABSTRACT

Introduction: The prorenin receptor ((P)RR) contributes to the regulation of the tissue renin-angiotensin system (RAS) and the function of V-ATPase, which are essential for Wnt signaling. Thus, (P)RRs may be involved in the control both of feto-placental and maternal circulation during pregnancy. This study was conducted to clarify how placental (P)RR expression and plasma soluble (P)RR [s(P)RR] levels are associated with blood pressure elevations and renal function during pregnancy.

Methods: We conducted a cross-sectional study, conducted at Saitama medical center in 2010–2013. Preeclamptic women (n = 16) diagnosed according to the criteria of Japan Society of Obstetrics and Gynecology and normotensive pregnant women (n = 15) participated in the study. We measured the expression of (P)RR in the placenta, plasma s(P)RR levels, systolic blood pressure (SBP), and estimated glomerular filtration rate (eGFR).

Results: Placental expression of (P)RR was significantly higher in preeclamptic women than in normotensive pregnant women. The plasma s(P)RR levels were significantly higher in preeclamptic women than in normotensive pregnant women. Systolic blood pressure (SBP) was positively correlated with placental (P)RR levels ($P = 0.0001$) and plasma s(P)RR levels ($P = 0.005$) in all pregnant women. In preeclamptic women, SBP was positively correlated with placental (P)RR levels ($P = 0.004$), but not with plasma s(P)RR levels ($P = 0.15$). The eGFR was negatively correlated with placental (P)RR levels ($P = 0.02$) and plasma s(P)RR levels ($P = 0.0002$) in all pregnant women. In preeclamptic women, eGFR was negatively correlated with plasma s(P)RR levels ($P = 0.006$), but not with placental (P)RR levels ($P = 0.93$).

Discussion: Placental (P)RR can be involved in blood pressure regulation via the tissue RAS. On the other hand, plasma s(P)RR may be involved in the pathogenesis of decreased renal function in preeclampsia.

1. Introduction

The renin-angiotensin system (RAS) plays an important role in maintaining maternal hemodynamics during pregnancy and in the pathogenesis of preeclampsia [1]. During pregnancy, estrogen increases angiotensin II (ATII) and renin in placenta, and serum levels of prorenin, an inactive renin precursor, are >200 times higher than those of renin [2]. Serum prorenin levels are elevated in patients with preeclampsia and increases further if preeclampsia becomes more severe [3]. Prorenin concentration is also known to be elevated in placental tissue [4], but the pathophysiological significance of prorenin as an inactive substance has been unclear.

In 2002, Nguyen et al. isolated and identified the prorenin receptor [(P)RR] from the human kidney [5]. The (P)RR is a single transmembrane domain protein that enhances enzymatic activity of prorenin by binding to it, and is distributed in important organs and tissues such as the brain, heart, kidneys, liver, vascular smooth muscle, and adipose tissues. (P)RRs have also been found in the placenta [5]. In vivo studies have shown that binding of prorenin to the (P)RR, through activation of the tissue RAS [6], is involved in renal damage, cardiac damage, and retinal lesions [7,8]. In addition,

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(P)RRs, through V-ATPase independent of the RAS [9], are essential for Wnt signaling that is important in fetal growth and development [10]. Therefore, it is possible that (P)RRs, through both RAS-dependent and RAS-independent mechanisms, play a role in the pathogenesis of preeclampsia during pregnancy.

(P)RR is cleaved by furin to generate soluble (P)RR [s(P)RR]. The s(P)RR is secreted into extracellular space and is ultimately found in blood. We recently developed an s(P)RR enzyme-linked immunosorbent assay (ELISA) kit to measure blood s(P)RR levels [11]. Studies measuring plasma s(P)RR concentrations have shown that increased s(P)RR levels are associated with blood pressure elevation [12] and increased rates of gestational diabetes [13], and can predict the development of fetal growth retardation (FGR) [14]. Although it is speculated that levels of placental (P)RR and plasma s(P)RR in addition to blood pressure and renal function may be altered in preeclampsia, it remains to be determined. Therefore, this study was conducted to measure placental (P)RR expression and plasma s(P)RR levels and to clarify how those are associated with blood pressure elevations or renal function in preeclampsia.

2. Materials and methods

2.1. Subjects

This investigation was approved by the Saitama Medical Center Ethics Committee and informed consent was obtained from each participant. Blood pressure measured just before delivery in the supine position at the bedside.

Preeclampsia was diagnosed when hypertension (systolic blood pressure $\geq$140 mmHg or diastolic blood pressure $\geq$90 mmHg) and proteinuria ($\geq$300 mg/day) appeared after 20 weeks gestation in previously normotensive women, according to the criteria of the Japan Society of Obstetrics and Gynecology [15]. Medical and obstetric histories, including delivery data, were obtained for each woman. The standard deviation (SD) score for birthweight was computed, correcting for gestational age, sex, maternal parity, by comparison with the Japanese standard birthweight curve [16]. Patients were excluded from the study if they had a history of diabetes, renal disease, hepatic disease, hypertension, cardiovascular illness, multiple pregnancies, hemolysis, elevated liver enzymes, autoimmune diseases, or with a fetus having structural or genetic anomalies or infections.

2.2. Sample collection

Plasma samples were obtained from blood collection just before delivery. Plasma prorenin concentration was measured by an ELISA assay (Human Prorenin Elisa Kit, Innovative Research, Novi, USA). Plasma s(P)RR concentration was measured by an ELISA assay (soluble (pro)renin Receptor Assay Kit, Takara Bio, Otsu, Japan) [11]. Using the ELISA kit, Nguyen et al. showed the accurate detection of s(P)RR in the plasma [17]. The strong mono-peak (29 kDa) obtained by the antibodies of the ELISA kit suggested s(P)RR but not renin (42 kDa), prorenin (48 kDa), or full-length (P)RR (39 kDa). Placental samples were taken within 1 h after delivery, membranes were removed, and the tissue was washed once in ice cold PBS in order to minimize maternal blood contamination. The washed tissue block was snap frozen in liquid nitrogen and stored at −80 °C for (pro)renin receptor analysis. Estimated glomerular filtration rate (eGFR) (mL/min/1.73 m^2) was calculated using the equation proposed by the Japanese Society of Nephrology [18].

2.3. Western blot analysis

Placental tissue samples were minced and homogenized. The lysates were centrifuged at 7000 × *g* for 30 min and the resulting supernatants were analyzed. Proteins were electrophoretically separated using 10% SDS-polyacrylamide gels at 100 V for 120 min. Proteins were subsequently transferred electrophoretically onto a Hybond-ECL polyvinylidene difluoride membrane (GE Healthcare, Buckinghamshire, UK). Transfer was conducted at 100 V for 90 min. Blots were blocked for 60 min at room temperature with 5% skim milk. The membrane was incubated with anti-(P)RR antibody (Immuno-Biological Laboratories Co., Tokyo, Japan) overnight at room temperature. The protein bands were visualized with an ECL plus system (GE Healthcare).

2.4. Immunohistochemistry analysis

Placental sections were processed by immunohistochemistry in order to localize the (P)RR using a primary antibody from Immuno-Biological Laboratories Co., (Tokyo, Japan). After deparaffinization, sections were blocked for endogenous peroxidase activity with 3% hydrogen peroxide in methanol for 5 min. Antigen retrieval was performed by incubation in Target Retrieval Solution (Dako, UK) for 15 min at 121 °C. To block non-specific binding, sections were incubated in blocking solution (Protein Block serum, Dako, UK) for 30 min at room temperature. Sections were then incubated at room temperature for 16 h with the primary antibody diluted in blocking solution. The sections were then incubated for 30 min with a biotinylated secondary antibody followed by incubation with streptavidin-conjugated horseradish peroxidase. The site of antibody binding was visualized with diaminobenzidine and counterstained with Weigert's hematoxylin. Negative controls employed antibody diluent in place of the primary antibody.

2.5. Statistical analysis

Results are expressed as mean ± S.D. Data were tested by the Wilcoxon test in order to establish significant differences between groups. Correlations were assessed by Spearman's rank correlation coefficients. A value of $P < 0.05$ was considered to be statistically significant. All statistical analyses were performed using the JMP (Ver. 8) statistical program.

3. Results

3.1. Subject characteristics

As shown in Table 1, the systolic and diastolic blood pressure levels were significantly higher in preeclamptic women than in normotensive pregnant women. The preeclamptic women had a significantly lower gestational age at delivery, birthweight, and SD score for birthweight than normotensive pregnant women. However, body mass index was similar in preeclamptic and normotensive pregnant women. Only one subject in the preeclamptic group and in the normotensive group had gestational diabetes mellitus.

3.2. Expression of (P)RR in the placenta and plasma s(P)RR levels

Placental expression of (P)RR was significantly higher in preeclamptic women (84.9 ± 34.6 arbitrary units) than in normotensive pregnant women (55.9 ± 15.0) (Fig. 1A). The plasma s(P)RR level was significantly higher in preeclamptic women (32.0 ± 10.6 ng/mL) than in normotensive pregnant women (24.9 ± 3.7) (Fig. 1B). Placental expression of (P)RR was localized to the villous syncytiotrophoblast cells both in normotensive pregnant women (Fig. 1C) and preeclamptic women (Fig. 1D). No significant correlation was observed between plasma s(P)RR levels