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Endothelin-1 production via placental (pro)renin receptor in a mouse model of preeclampsia

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ABSTRACT

Introduction: Preeclampsia (PE) pathogenesis is explained by the two-stage disorder theory. However, mechanisms underlying hypertension and proteinuria in PE remain unclear. The role of (pro)renin receptor (PRR) in PE pathology has received special attention. We examined endothelin-1 (ET-1) production via placental PRR in a PE mouse model.

Methods: At 14.5 day-post-coitum (DPC), we performed a reduced uterine perfusion pressure (RUPP) operation, ligating the uterine arteriovenous vessels in female mice. We also infused these mice with a PRR inhibitor, decoy peptide in the handle region of prorenin (HRP) for mice (NH2-RIPLKKMPSV-COOH). At 18.5 DPC, blood, urine, and placenta were collected; fetus and placenta were weighed. We evaluated placental hypoxia using quantitative polymerase chain reaction (PCR), with hypoxia-inducible factor-1α (HIF-1α) as index. We also evaluated PRR, transforming growth factor-β1 (TGF-β1), and ET-1 expression in the placenta using quantitative PCR and western blotting. ET-1 concentration in blood plasma was assessed using enzyme-linked immunosorbent assay.

Results: Blood pressure and proteinuria significantly increased, and fetal and placental weights decreased in RUPP mice. HIF-1α, PRR, TGF-β1, and ET-1 expressions considerably increased in RUPP mice placentas. ET-1 concentration in RUPP mice blood plasma was markedly increased. PRR inhibitor suppressed these changes.

Discussion: In PE model mice that underwent RUPP treatment, placental hypoxia increased PRR and ET-1 expression suggesting a causative relationship between ET-1 and intracellular PRR signaling. RUPP treatment, when combined with HRP, reversed the effect of elevated ET-1 levels in the model. This study may help to elucidate the pathogenesis of PE considering PRR and ET-1.

1. Introduction

Preeclampsia (PE) is a potentially fatal disease that affects 2–8% of all pregnancies, causing serious complications in both the mother and fetus, including death [1,2]. PE is defined as maternal hypertension accompanied by maternal albuminuria, other maternal organ dysfunctions, such as liver involvement and kidney injury, and uteroplacental dysfunction, such as fetal growth restriction and abnormal umbilical artery doppler wave, occurring between 20 weeks of gestation and 12 weeks postpartum in pregnant women [3,4]. PE pathogenesis is explained by the two-stage disorder theory proposed by Roberts [5]. Suppression of trophoblast invasion leads to spiral artery remodeling failure and decreased placental blood flow (Stage 1), which results in placental hypoxia/ischemia and collapse of the balance between angiogenic and anti-angiogenic factors (Stage 2) [5,6]. However, the mechanisms underlying hypertension and proteinuria in PE are not fully understood.

In recent years, an association between PE and prorenin has been reported [7]. Prorenin is an inert precursor of renin and exhibits enzymatic activity by binding to the (pro)renin receptor (PRR) [6]. PRR is a 38 kDa protein comprising a transmembrane domain and an extracellular domain. It is expressed in organs throughout the human body,

Abbreviations: PRR, (pro)renin receptor; PE, preeclampsia; ET-1, endothelin-1; ICR, Institute of Cancer Research; DPC, day-post-coitum; RUPP, reduced uterine perfusion pressure; HIF-1α, hypoxia-inducible factor-1α; TGF-β1, transforming growth factor-β1.

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including the kidneys, heart, brain, eyes, placenta, and immune system, and plays numerous physiological roles via intracellular signaling pathways by binding to prorenin/renin [8,9]. However, it has been pointed out that the decoy peptide in the handle region of prorenin (HRP) may competitively bind to the PRR and inhibit the intracellular signaling pathway induced by PRR [10,11]. Recently, we found that endothelin-1 (ET-1), which is a potent vasoconstrictor, might be produced by PRR-mediated intracellular signaling in the placenta of patients with PE [12]. However, this study was only performed *in vitro*, and the precise role of ET-1 in maternal hypertension and proteinuria in patients with PE is still unknown. Additionally, further *in vivo* studies are required to confirm whether suppression of PRR-mediated intracellular signaling and ET-1 production inhibit hypertension and proteinuria.

The reduced uterine perfusion pressure (RUPP) model is widely used in various pregnant animals, such as rats [13–15] and baboons [16]. RUPP mice are a well-characterized animal model of PE that can replicate the symptoms associated with PE, including elevation of maternal blood pressure, proteinuria, increased serum sFlt-1, and fetal growth restriction [17,18]. Thus, the RUPP mouse model is suitable for understanding the pathophysiology of PE associated with placental ischemia.

In this study, we examined the significance of PRR-mediated intracellular signaling in the placenta of a pregnancy-induced PE mouse model using RUPP mice to elucidate the pathogenesis of PE with a focus on PRR and ET-1.

2. Materials and methods

2.1. Animals

All animal experiments were performed in compliance with the protocol approved by the Animal Care and Use Committee of Okayama University (OKU-2020244 and 2021246). Female and male Institute of Cancer Research (ICR) mice were purchased from CLEA Japan, Inc. (Tokyo, Japan). Animals were housed at 23 °C ± 1 °C on a 12:12 h light-dark cycle with free access to food and water.

2.2. Reduced uterine perfusion pressure operation

To induce placental ischemia, the RUPP model was adopted with reference to Fushima et al. [18]. At 8–12 weeks of age, ICR female mice were pair-housed with male mice. The presence of a vaginal plug was designated as 0.5 day-post-coitum (DPC). At 14.5 DPC, the pregnant mice were anesthetized with isoflurane. The mice were placed in the supine position, the limbs were fixed, and the abdomen was disinfected with 10% povidone-iodine solution (Meiji Seika KK, Tokyo, Japan). A midline incision was made to reach the abdominal cavity and identify the uterine artery. Both sides of the uterine arteries and veins were ligated with 6–0 silk; RUPP group (n = 21), as described in Fig. 1A and B. The abdominal wall was closed with skin clips, and buprenorphine was injected subcutaneously. The control group mice (n = 11) did not undergo these surgeries. The schedules are shown in Fig. 1C.

2.3. Administer PRR inhibitor and placebo treatment

Mouse HRP (NH2-RIPLKKMPSV-COOH) was purchased from Greiner Bio-One (Germany). At 14.5 DPC, pregnant mice underwent RUPP operation, and at the same time, mini osmotic pumps (model 2001, ALZET Scientific Corporation, USA) were implanted for the administration of mouse HRP (1 mg/kg/day); R + H group (n = 12). Some mice were implanted with pumps for saline administration; R + saline group (n = 8). The schedules are shown in Fig. 1C.

2.4. Sample collection

After warming and calming the mouse, the blood pressure (BP) of the mouse was measured at 15.5 and 18.5 DPC by the tail-cuff method using a non-invasive fixation device (BP-98E, Softron Co., Ltd, Tokyo, Japan). At 18.5 DPC, under isoflurane anesthesia, 2 mL of blood was collected from the inferior vena cava and urine were collected with 0.8 mL plastic tubes.

At 18.5 DPC, urine protein concentration was measured in urine immediately after collecting using the test strip analysis; Uro-paper III (Eiken, Tokyo, Japan). Results were expressed according to the manufacturer's recommendation.

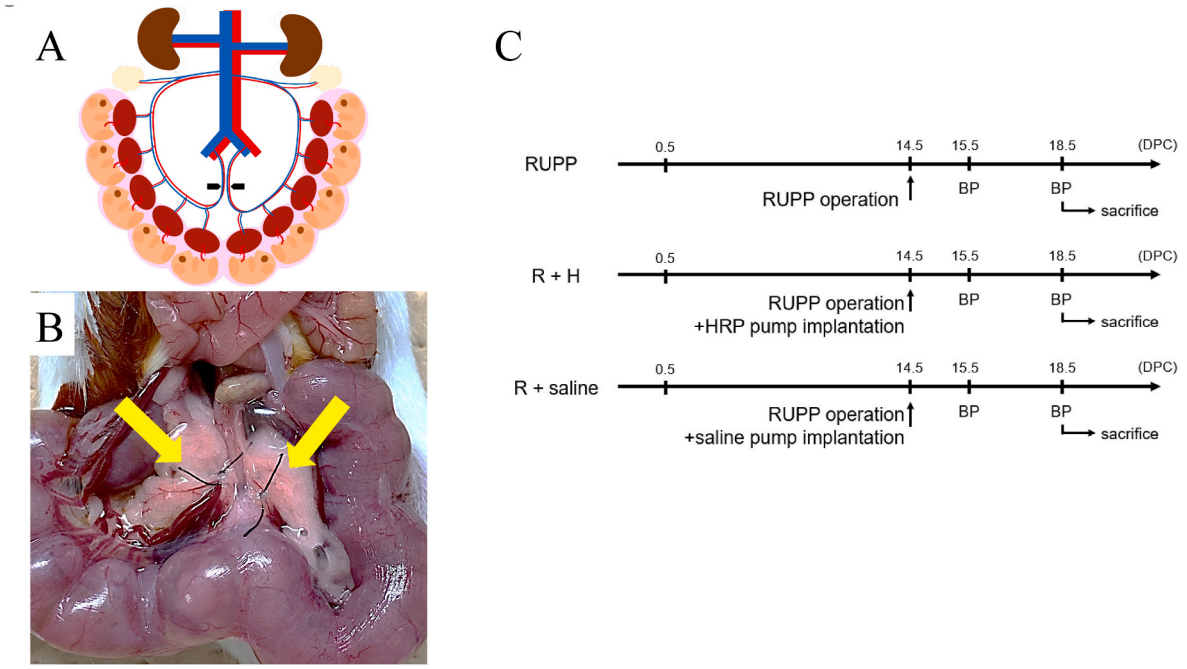

Fig. 1. Ligation of bilateral uterine artery and vein was performed in pregnant mice. (A, B) RUPP operation of mice. (C) Study scheme. Experimental groups included the RUPP operation group, RUPP operation plus HRP pump implantation group (= R + H), and RUPP operation plus saline pump implantation group (= R + saline).

Simultaneously, the placentae and fetuses were removed during pregnancy and weighed.

Thereafter, all female mice were sacrificed by isoflurane inhalation and pups by vertebral dislocation.

2.5. Immunohistochemistry

Immediately after excision, the tissue samples were fixed in 10% phosphate-buffered formaldehyde (pH 6.7) for 24 h and then embedded in paraffin. Antigen retrieval was performed using the Target Retrieval Solution (pH 6.0; Dako, Tokyo, Japan). Immunohistochemical staining of 5-μm thick sections was performed using primary antibodies against ATP6AP2 (1:100, polyclonal, rabbit, 1-926-1-AP, Proteintech, USA) followed by incubation with an avidin-biotin-blocking system (Dako), secondary antibodies, and a peroxidase-labeled avidin-biotin complex system (EnVision + System-HRP Labeled Polymer; Dako). The ATP6AP2 level in placental tissues was evaluated and compared with that in the tissue from the control group. Two examiners independently conducted microscopic analyses in a blinded manner; four sections per group were stained, and the brown-positive staining intensity was evaluated twice.

2.6. Western blotting

Placental tissues were added at a ratio of 1 mL RIPA lysate (Thermo Scientific, USA) to 100 mg. Tissues were homogenized using a Q55 probe-type ultrasonic homogenizer (QSonica, USA), centrifuged at 10 000–16 000×*g* for 20 min, and the supernatant was collected for testing. The remaining supernatant was stored at −80 °C. One hundred micrograms of total protein were loaded and separated on a NuPAGE bis-Tris 4–12% gradient gel electrophoresis and electro-transferred onto polyvinylidene fluoride (PVDF) membranes (Invitrogen PVDF, 0.45 μm, Invitrogen, USA). After being blocked in 3% bovine serum albumin diluted in phosphate-buffered saline with Tween-20 for 1 h, the antibodies were added and membranes were incubated overnight at 4 °C. The following antibodies were used: anti-extracellular signal-regulated kinase (ERK)1/2 (1:7000, 11257-1-AP, Proteintech, USA), anti-phospho (p)-ERK1/2 (1:2500, 28733-1-AP, Proteintech), anti-ATP6AP2 (1:2500, 1-926-1-AP, Proteintech), anti-transforming growth factor-β1 (anti-TGF-β1) (1:7000, 21898-1-AP, Proteintech), anti-ET-1 (1:2000, 12191-1-AP, Proteintech), and anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:4000, PA1-988, Thermo Scientific, USA). GAPDH was used as a loading control. Subsequently, the membranes were incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG antibody (1:4000, SA00001-2, Proteintech) or goat anti-rabbit IgG (H + L) (1:4000, 4050-05, Southern Biotech, USA) for 1 h at room temperature. Images were captured using the ECL Prime Western Blotting Detection Reagent (Cytiva, Tokyo, Japan) and LAS 500 (Fujifilm, Japan), and quantified with ImageQuant TL software (GE Healthcare, Little Chalfont, UK).

2.7. Quantitative reverse transcription polymerase chain reaction (RT-qPCR)

Total RNA was extracted from placental tissues using the RNeasy Mini Kit (Qiagen, UK) and stored at −80 °C until analysis. RNA was reverse-transcribed to complementary deoxyribonucleic acid (cDNAs) using a reverse transcription kit (Invitrogen). RT-qPCR was performed using gene-specific primers for HIF-1α (Bio-Rad PrimePCR SYBR Green Assay, Hif1a, Cat#10025636), ATP6AP2 (Bio-Rad PrimePCR SYBR Green Assay, Atp6ap2, Cat#10025636), TGF-β1 (Bio-Rad PrimePCR SYBR Green Assay, Tgfb1, Cat#10025636), and ET-1 (Bio-Rad PrimePCR SYBR Green Assay, Edn1, Cat#10025636), SYBR green PCR Supremix (SsoAdvanced Universal SYBR Green PCR Supermix; Bio-Rad), and a Step One Plus Real-Time PCR instrument (Applied Biosystems, Foster City, CA, USA). RT-qPCR data were analyzed using the $2^{-\Delta\Delta Ct}$ method relative to the housekeeping gene, *GAPDH* (Bio-Rad PrimePCR SYBR Green Assay, Gapdh, Cat#10025636).

2.8. Plasma assays

Blood was drawn into tubes containing EDTA and centrifuged at 4 °C for 20 min at 2000×*g* (MRX-150, TOMY). Plasma was then aliquoted and immediately placed in a −80 °C freezer. Commercially available enzyme-linked immunosorbent assay (ELISA) kits were used to measure plasma concentrations of ET-1 (DET100, Endothelin-1 Quantikine ELISA Kit; R&D Systems, Minneapolis, MN, USA). Samples were analyzed in undiluted form. As indicated by the manufacturer, the range of determinability ranged from 0.4 to 25 pg/mL. The assay had an intra-assay precision with a percent coefficient of variation of less than 5% and an interassay accuracy of less than 10%.

2.9. Statistical analysis

Statistical analyses were performed using GraphPad Prism 8.2.0 (GraphPad Software Inc., La Jolla, CA, USA). Data of all groups were compared among all other groups using the Kruskal–Wallis test or one-way ANOVA. When conducting Kruskall Wallis test a post Hoc test was performed by Dunn's multiple comparison test. When conducting one-way ANOVA a post Hoc test was performed by Holm-Sidak's multiple comparison test. We have verified the data using Grubbs test to exclude statistical outliers. The normality of the data was assessed by using Gaussian distribution. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Hypertension and proteinuria

We measured the systolic BP (SBP) and diastolic BP (DBP) of the female mice before pregnancy (preg-), at 15.5 DPC, and 18.5 DPC. We compared the SBP and DBP among all groups using the Kruskal–Wallis test. At preg- and 15.5 DPC, there were no changes to the SBP and DBP values of each group. At 18.5 DPC, the RUPP and the R + saline groups showed significantly higher SBP than the control group ($p = 0.001$ and $p = 0.002$, respectively). There was no significant difference in the BP at any point in the R + H group, compared to the control group and RUPP group. In addition, there was no difference in the DBP between any of the groups (Table 1). In the RUPP group, urine protein level was significantly higher than that in the control group ($p = 0.003$). The R + saline group also had a significantly higher urine protein level than the control group ($p = 0.03$). However, there was no significant difference between the urine protein level of the R + H group and that of the control group ($p = 0.89$). The R + H group had significantly lower proteinuria than RUPP and R + saline groups ($p = 0.004$ and $p = 0.03$, respectively). Statistical significance was determined using one-way ANOVA test (Fig. 2).

3.2. Fetal weight and placental weight

In the RUPP, R + H, and R + saline groups, the fetal weights at 18.5 DPC were significantly lower than those in the control group ($p < 0.001$, $p < 0.001$, and $p < 0.001$, respectively). In addition, R + H group had higher fetal weight than RUPP and R + saline groups ($p < 0.001$ and $p < 0.001$, respectively) (Fig. S1A). The placenta weights of RUPP and R + H groups were greatly lower than those of the control group ($p = 0.002$, $p = 0.01$, respectively). In contrast, the placenta weight of the R + S group did not differ significantly from that of the control group ($p = 0.06$) (Fig. S1B).

3.3. Immunohistochemical staining of PRR in placental tissues of mice

PRR staining intensity appeared to be higher in the labyrinth zone of the placentas of mice in the RUPP group compared to that of the same

Table 1
Median tail-cuff blood pressure of mice (control group (n = 11), RUPP group (n = 21), R + H group (n = 12), and R + saline group (n = 8)). Data were compared all groups among each other groups using the Kruskal–Wallis test. When conducting Kruskal-Wallis test a post Hoc test was performed by Dunn's multiple comparison test. In RUPP and R + saline groups, blood pressure values were significantly higher than in control group (p = 0.001, p = 0.002, respectively). * indicates a significant difference between the median SBP of the RUPP group and that of the control group (p = 0.001). ** indicates a significant difference between the median SBP of the R + saline group and that of the control group (p = 0.002).

Systolic Blood Pressure (mmHg)				
	n	preg-	15.5 DPC	18.5 DPC
control	11	95.7 (88.7–102.5)	97.3 (84.7–105.3)	101.7 (89.7–108.5)
RUPP	21	95.3 (86.0–104.7)	97.7 (76.3–117.5)	114.8 (95.3–135.7)*
R + H	12	91.5 (77.3–105.7)	90.1 (75.3–115.0)	105.0 (93.0–115.7)
R + saline	8	85.5 (61.3–109.7)	90.5 (81.7–98.7)	115.6 (111.3–119.0) **

Diastolic Blood Pressure (mmHg)				
	n	preg-	15.5 DPC	18.5 DPC
control	11	64.0 (64.7–78.0)	63.9 (48.0–79.3)	72.4 (59.3–86.0)
RUPP	21	62.0 (68.0–66.0)	62.4 (43.3–79.7)	77.5 (50.7–98.3)
R + H	12	61.8 (54.3–69.3)	60.5 (43.0–78.7)	68.6 (55.0–87.0)
R + saline	8	50.0 (43.0–80.3)	58.4 (46.0–70.3)	75.5 (68.3–87.3)

* control vs. RUPP p = 0.001.
** control vs. R + saline p = 0.002.

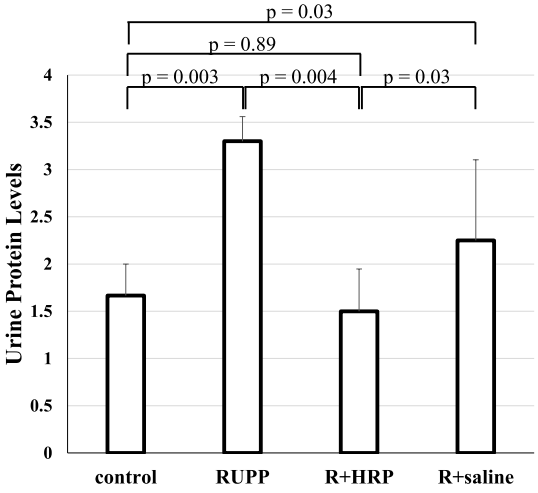

Fig. 2. Proteinuria levels of the mice. Results are shown as the mean ± SEM of data. The results among the groups were analyzed using one-way ANOVA. When conducting one-way ANOVA a post Hoc test was performed by Holm-Sidak's multiple comparison test. Differences were considered significant at p < 0.05. In the RUPP group (n = 10), urine protein level was significantly higher than that in the control group (n = 4) (p = 0.003). The R + saline group was also significantly higher in the control group (p = 0.03). However, there was no significant difference between the urine protein level of the R + H group (n = 5) and that of the control group. The R + H group had significantly lower proteinuria than RUPP and R + saline groups (p = 0.004 and p = 0.03, respectively).

zone of the control group placentas (Fig. S2).

3.4. Expression of HIF-1α, PRR, TGF-β1, and ET-1 in the placenta

The HIF-1α, ATP6AP2 and TGF-β1 mRNA expression levels were significantly higher in the RUPP group than in the control group (p = 0.006, p < 0.001, and p = 0.02 respectively). Additionally, the HIF-1α and ATP6AP2 mRNA expression levels were significantly lower in the R + H group than in the RUPP group (p = 0.02 and p = 0.006, respectively). There were no differences among control, R + H and R + saline groups. The ET-1 mRNA expression was significantly higher in the RUPP and R + saline groups (p < 0.001 and p < 0.001, respectively) than in the control group, and was significantly lower in the R + H group than in the RUPP and R + saline groups (p = 0.001 and p = 0.002, respectively). (Fig. 3).

The ATP6AP2 protein expression level was significantly higher in the RUPP, R + H, and R + saline groups than in the control group (p = 0.005, p = 0.02, and p = 0.002, respectively). There were no differences among RUPP, R + H, R + saline groups. The protein expression levels of phosphor-ERK1/2, TGF-β1, and ET-1 were significantly higher in the RUPP group than in the control group (p < 0.001, p < 0.001, and p < 0.001, respectively), but not in the R + H group. In the R + saline group, the protein expression levels of phosphor-ERK1/2 and ET-1 were significantly higher than in the control group (p = 0.02 and p < 0.001, respectively). In the protein expression levels of phosphor-ERK1/2, there were no differences among R + H, R + saline groups. The protein expression levels of TGF-β1 were not significantly different among RUPP, R + H and R + saline groups. The protein expression level of ET-1 was significantly lower in the R + H group than in the RUPP and R + saline groups (p = 0.001 and p = 0.002, respectively). (Fig. 4). As for ERK1/2 expression, there were no statistically significant difference (data not shown).

3.5. Plasma ET-1 level

A significant elevation in plasma ET-1 level was observed in the RUPP group (p = 0.001) and R + saline group (p < 0.001) compared to the control group. There were no remarkable differences between the R + H groups (Fig. 5).

4. Discussion

Our study revealed that PE model mice, produced by the RUPP protocol, showed increased placental PRR expression and intracellular signaling. We also reported elevated ET-1 concentrations in the plasma and higher ET-1 expression in the placenta of the RUPP mice. In contrast, PE model mice infused with HRP did not show elevated BP. In the placenta, PRR expression was increased, but intracellular signaling associated with PRR, ET-1 concentration in plasma and ET-1 expression in the placenta were not increased. This result suggests that ET-1 production via intracellular signaling of PRR in the placenta may be involved in PE development in this PE model.

Reports have focused on the relationship between PE and PRR levels in the plasma and placenta. Watanabe et al. reported that high circulating levels of soluble PRR (sPRR) during early pregnancy predicted a subsequent elevation in BP and that high sPRR concentrations at delivery were prominently associated with PE [19]. In women with PE, placental PRR positively correlated with SBP, and plasma sPRR negatively correlated with the estimated glomerular filtration rate [20]. Our research group also showed that the proportion of moderate to strong PRR expression was greatly higher in PE placentas [12].

Trophoblast invasion suppression leads to spiral artery remodeling failure and decreased blood flow to the placenta, resulting in placental hypoxia/ischemia. Some reports have mentioned increased PRR expression in trophoblasts under such hypoxic conditions [12,21,22]. In short, placental hypoxia occurs in mothers with PE, which increases PRR expression in the placenta.

PRR-mediated intracellular signaling activates mitogen-activated protein kinase (MAPK) and ERK1/2 [23–26], subsequently leading to an increase in TGF-β1 expression [27,28], which stimulates the expression of ET-1, a strong vasoconstrictor [29]. In mothers with PE, the plasma concentration of ET-1 is elevated [30], and ET-1 levels

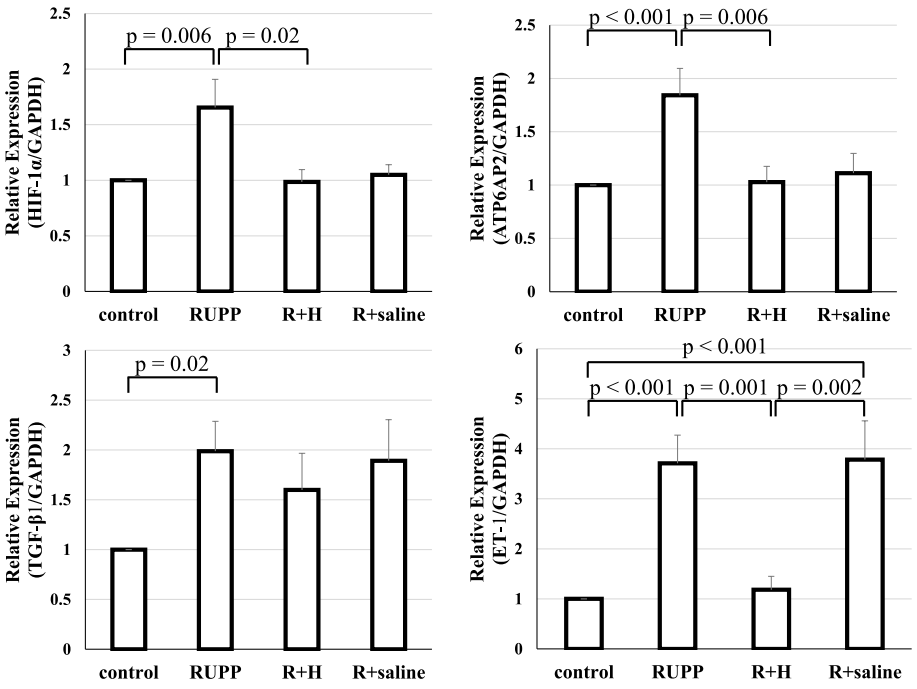

Fig. 3. mRNA expressions in the placenta determined by RT-qPCR. mRNA expression of HIF1-α, ATP6AP2, TGF-β1 and ET-1 are shown. Results of all groups in each target are shown as the mean ± SEM of data. The results were analyzed by one-way ANOVA among each other groups. When conducting one-way ANOVA a post Hoc test was performed by Holm-Sidak's multiple comparison test. Differences were considered significant at $p < 0.05$. The HIF-1α, ATP6AP2 and TGF-β1 mRNA expression levels were significantly higher in the RUPP group (n = 15–58) than in the control group ($p = 0.006$, $p < 0.001$ and $p = 0.02$, respectively). Additionally, the HIF-1α and ATP6AP2 mRNA expression levels were significantly lower in the R + H group (n = 10–28) than in the RUPP group ($p = 0.02$ and $p = 0.006$, respectively). The ET-1 mRNA expression was significantly higher in the RUPP and R + saline groups (n = 12–30) ($p < 0.001$ and $p < 0.001$, respectively) than in the control group, and was significantly lower in the R + H group than in the RUPP and R + saline groups ($p = 0.001$ and $p = 0.002$, respectively).

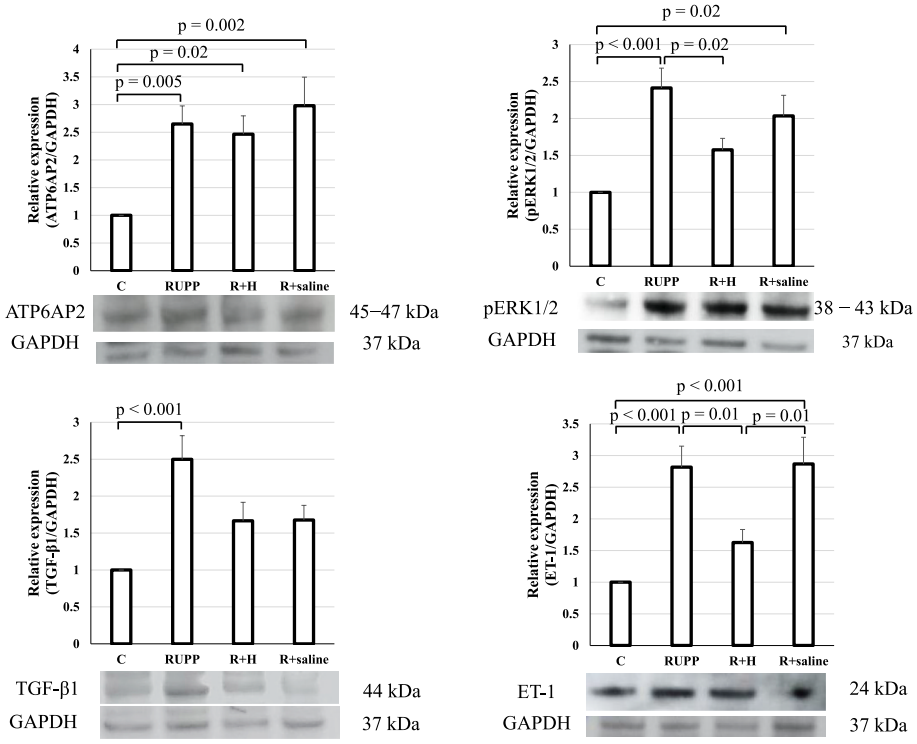

Fig. 4. Protein expression levels in the placenta are determined by western blotting. Protein expression of ATP6AP2, phosphor-ERK1/2, TGF-β1 and ET-1 are shown. Results of all groups in each target are shown as the mean ± SEM of data. The results were analyzed by one-way ANOVA among each other groups. When conducting one-way ANOVA a post Hoc test was performed by Holm-Sidak's multiple comparison test. Differences were considered significant at $p < 0.05$. In RUPP (n = 20–35), R + H (n = 19–30), and R + saline (n = 19–30) group, ATP6AP2 levels were significantly higher than in the control group (n = 20–28) ($p = 0.005$, $p = 0.02$, and $p = 0.002$, respectively). In RUPP and R + saline group, phosphor-ERK1/2 levels were significantly higher than in the control group ($p < 0.001$ and $p = 0.02$, respectively), and was significantly lower in the R + H group than in the RUPP group ($p = 0.02$). TGF-β1 levels were significantly higher in RUPP than in the control group ($p < 0.001$). ET-1 levels were significantly higher in the RUPP and R + saline group than in the control group ($p < 0.001$ and $p < 0.001$, respectively), and was significantly lower in the R + H group than in the RUPP and R + saline groups ($p = 0.01$ and $p = 0.01$, respectively).

significantly correlate with anti-angiogenic factors, such as sFlt-1 [31,32]. Thus, ET-1 is thought to play an important role in PE pathogenesis.

There has been some discussion regarding the effects of HRP administration on PRR-induced intracellular signaling. Kaneshiro et al. showed that MAPK activation and TGF-β1 expression were activated in the kidneys of human PRR-transgenic rats, and HRP infusion appreciably inhibited these changes [33]. Tan et al. reported that the p-p38 expression level decreased in the adipose tissue of mice that were administered HRP and demonstrated that HRP might decrease MAPK signaling [34]. However, some reports have shown the limitations of the HRP effect [26,35–37]. Fedlt et al. showed that HRP did not affect the binding and intracellular signaling of prorenin in human and U937 monocytes [26]. Muller et al. reported that HRP treatment (3.5 μg/kg per day) did not affect hypertension, cardiac hypertrophy, or renal damage in renovascular hypertensive model rats [35]. Mercure et al. also reported that HRP treatment had no effect toward either prorenin or renin in h(P)RR dependency of the enhanced prorenin binding and ERK1/2 activation in h(P)RR cells [36]. In addition, Wilkinson et al. suggested that HRP is a partial (P)RR agonist on retinal function [37]. However, these include room for improvement in the dosage, duration,

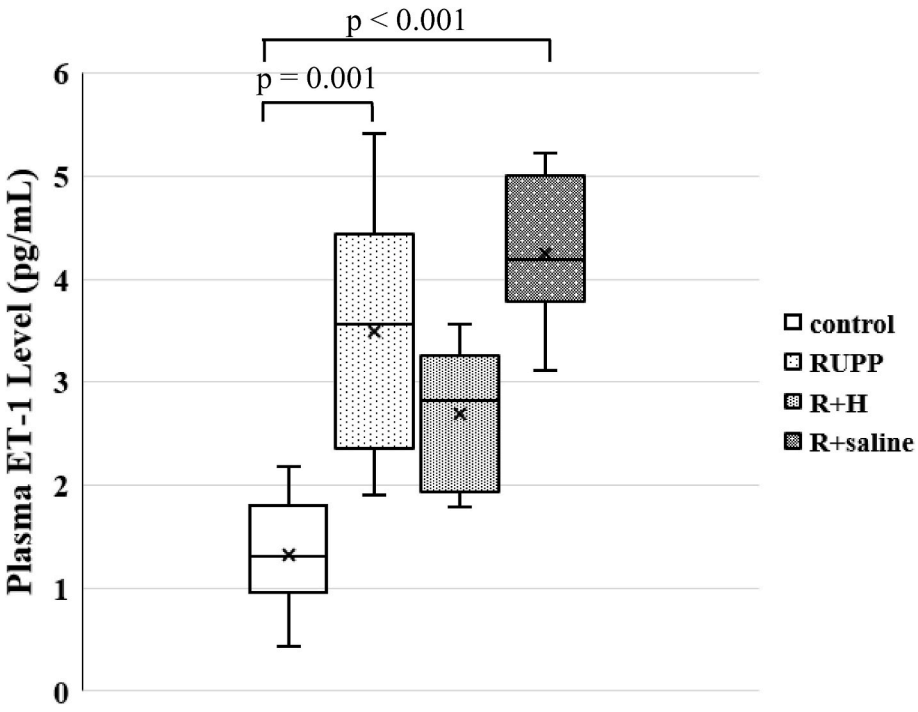

Fig. 5. ET-1 level in plasma. The results were analyzed by Kruskal–Wallis test among each other groups. When conducting Kruskal-Wallis test a post Hoc test was performed by Dunn's multiple comparison test. Differences were considered significant at $p < 0.05$. A significant elevation of ET-1 level was observed in the RUPP group ($n = 17$) and R + saline group ($n = 7$) compared to the control group ($n = 8$) ($p = 0.007$, and $p < 0.001$, respectively). In the R + H group ($n = 6$), no difference was observed.

and timing of administration [34]. On the contrary, Li et al. infused a newly designed putative (P)RR antagonist, PRO20 (corresponding with the first 20 amino acids of the prosegment of mouse prorenin) and inhibited prorenin-induced hypertension [38]. However, this is a local effect and there is skepticism about its effectiveness [39]. HRP has the potential to inhibit PRR-induced intracellular signaling, thereby potentially suppressing the symptomatic progression of PE.

Early-onset PE is centered on placental hypoxia due to uterine spiral artery remodeling failure, as indicated by the 2-stage disorder theory. In this study, RUPP operation was performed after the placentation period, but mimics the early-onset type in that it reduces placental blood flow. HRP may inhibit ET-1 production in the placenta, which is enhanced by placental hypoxia. Conversely, the pathogenesis of late-onset PE is based on vascular endothelial damage associated with systemic chronic inflammation, such as maternal obesity. It is unclear whether HRP is expected to improve symptoms of late-onset type.

HRP might have potential to prolong gestation because it prevents elevations in maternal BP and improves FGR in this research. Prolonged gestation may reduce the incidence of acute neonatal complications. In addition, preterm infants are at a higher risk of non-communicable diseases, such as future diabetes and hypertension [40]. Therefore, this treatment could potentially diminish their susceptibility to these illnesses. Based on this, we postulate that HRP may improve fetal outcome.

This study had limitations that should be acknowledged. We measured proteinuria using test strip analysis with a semi-quantification of the protein level by color changes of test strip. It is very important that we measure soluble PRR levels and circulating prorenin/renin or Ang peptides and reveal that the HRP is working not only on the placenta, but also on the renin-angiotensin systems. However, this study focused on the intra-cellular signaling pathways of the PRR of placenta during the pregnancy, and is the first report, to our knowledge, focused on these aspects during pregnancy. We aim to investigate the renin-angiotensin system in future research.

5. Conclusion

In summary, the placental expression of PRR and PRR-mediated intracellular signaling was increased in PE model mice, as was the ET-1 concentration in the plasma and the expression of ET-1 in the placenta. On the contrary, BP elevation and increased proteinuria were not observed in HRP-administered PE model mice, and the placental expression of PRR-mediated intracellular signaling decreased, as did plasma ET-1 concentration and placental ET-1 expression. Thus, it can be inferred that ET-1 production via intracellular PRR signaling in the placenta may be involved in PE development. Administration of HRP can reduce elevated maternal BP and prolong gestation after the placenta has been exposed to hypoxia. Once the effects of this treatment on the fetus, including any side effects, have been fully examined and safety established and/or circumnavigated, it should be put into practical and clinical use.

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Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.placenta.2023.05.002.

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