Attenuated Function of Angiotensin-(1-7) in Placental Artery During Preeclampsia not through MAS1 Receptor

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Abstract

**Background:** As a counter-regulatory component of the renin-angiotensin-system (RAS), the angiotensin-(1–7) [Ang-(1–7)] plays a protective role in cardiovascular diseases. However, the association between Ang-(1–7) and preeclampsia in humans is unclear.

**Methods:** Experiments were conducted in the placentas and peripheral blood collected from 32 women with normal pregnancy and 20 women with preeclampsia.

**Results:** The vasocontractions induced by Ang II were significantly inhibited by Ang-(1-7) in the normal placental artery (P<0.001) but not in the preeclampsia group. Treatment with A779 (MAS1R inhibitor) and PD (AT2R inhibitor) could not block the inhibitory effect of Ang-(1-7) on Ang II-induced vasoconstriction. Real-time quantitative PCR indicated that the mRNA levels of AT1R was significantly decreased (P<0.001) in preeclamptic group. In Hematoxylin-Eosin (HE) staining experiment, the placental artery in preeclamptic group were with a greater wall thickness and a smaller luminal area than those in normal group. In addition, as the consequence of the activation of β-arrestin pathway, the phosphorylation level of ERK protein was significantly reduced (P<0.01) in the nucleus and significantly increased (P<0.05) in the cytoplasm of placental vascular tissue in preeclamptic group.

**Discussion:** Together, these data indicated that Ang-(1-7) antagonizes the function of Ang II in placenta vessels of normal pregnant women, but this effect is obviously eliminated in preeclampsia. Treatment with A779 and PD indicated that Ang-(1-7) may not exert its effect through MAS1R and AT2R. Persistent activation of the β-arrestin pathway and the weaken function of Ang-(1-7) in placental artery might contribute to the pathogenesis of preeclampsia.

**Background**

Preeclampsia (PE) is the second leading cause of maternal mortality in the world, affecting 5–8% of all pregnancies and contributing significantly to stillbirths and neonatal morbidity and mortality. The revised International Society for the Study of Hypertension in Pregnancy (ISSHP) definition of preeclampsia in 2014 is hypertension developing after 20 weeks gestation and the coexistence of one or more of other new onset conditions, such as proteinuria, renal insufficiency (creatinine ≥ 90 umol/L), liver involvement, neurological complications, haematological complications, and uteroplacental dysfunction [1]. In addition to the short-term adverse effects of perinatal and postpartum, the Developmental Origins of Health and Disease (DOHaD) theory suggests that adverse complications during pregnancy (such as preeclampsia) are very likely to have a long-term impact on fetal health, and in short, it might increase the susceptibility of newborns to certain diseases, such as higher systolic blood pressure or higher diastolic blood pressure and cognitive dysfunction in late life [2–4]. It should be noted that preeclampsia also has a long-term impact on the health of the pregnant women themselves. Prospective cohort studies showed that hypertensive disorder of pregnancy was associated with a notably higher risk of cardiovascular disease death later in life, independent of other measured risk factors [5, 6]. It was found that
preeclampsia patients who continued to have high sensitivity to Ang after delivery might have a long-term adverse effect on the health [7, 8].

Thus, the prevention of preeclampsia would have a significant impact on maternal and neonatal outcome. Nevertheless, the pathophysiology of preeclampsia remains poorly understood. There are several hypotheses about the pathogenesis of preeclampsia, maternal endothelial dysfunction and hemodynamic changes, abnormal placental Renin-angiotensin-system (RAS), inflammation and immunologic alterations and genetic factors [9]. The only known cure is delivery of the placenta, demonstrating the important role of the placenta in the development of preeclampsia. In preeclampsia, abnormal trophoblast invasion leads to a reduction of blood flow into the placenta [10]. Then the placenta becomes hypoxic as gestation advances, leading to abnormal endocrine function of the placenta and eventually contribute to the occurrence of preeclampsia [11]. Previous study has reported that placental released factors may play a vital role in the development of preeclampsia, the pathogenesis of preeclampsia is currently unclear [12, 13].

RAS plays an important role in regulating water and salt metabolism, and plays a critical role in the pathogenesis of preeclampsia. Normal pregnancy will also be accompanied by changes in the levels of RAS components. Chesley et al. reported that normal pregnant women can be resistant to the pressor effects of Ang II [14], and they remain normotensive despite a 2-fold increase in Ang II. However, pregnant women with preeclampsia will respond to the changes of RAS components in a different way. Studies have shown that the occurrence of preeclampsia is closely related to the sensitivity change of maternal blood vessels to Ang II [15]. Gant et al. found that pregnant women with preeclampsia are highly sensitive to Ang injection, while normal pregnant women are resistant to Ang injection, and usually need a larger dose of Ang injection to produce the same blood pressure rise response as pregnant women with preeclampsia [16]. How this system may be altered in women with preeclampsia is less understood. A previous study has shown that Ang II-induced vasoconstrictions were significantly reduced in preeclamptic placentas compared to the placental artery from normal pregnancy, and the reduced All-mediated placental vasoconstrictions may cause compensatory responses, resulting in an increase in Ang II and Ang I Converting Enzyme (ACE) in the maternal-placental circulation that may induce hypertension in preeclampsia [17].

In addition, apart from classical ACE-Ang II-AT1R axis, non-classical RAS system members ACE2-Ang-(1-7)-MAS1R are receiving more and more attention. Ang-(1-7) is a bioactive component of the RAS, which has depressor, vasodilatory, and antihypertensive actions. Many studies have shown that Ang-(1-7) may be a physiological antagonist of Ang II and mainly involves vascular tension, cell proliferation, oxidative stress, fibrosis, and seems to play a protective role in cardiovascular diseases [18–23]. However, the interaction of Ang-(1-7) with Ang II in the placental artery has not yet been investigated. Therefore, how Ang-(1-7) functions and its role in the pathogenesis of preeclampsia deserves further study.

Materials And Methods
Human samples

The experimental group consisted of 20 women with preeclampsia (no other perinatal complications except preeclampsia) who were on their (36.79 ± 0.49) week, whereas the control group consisted of 32 healthy pregnant women at term (38.87 ± 0.09 week). Preeclampsia diagnosis was made based on the American College of Obstetricians and Gynecologists (ACOG) recommendations: in pregnant women who did not have hypertension in the previous weeks, after the 20th week, proteinuria (0.3 g or more in 24h urine) criteria were taken as the reference, if the systolic blood pressure was ≥140 mmHg, or if the diastolic blood pressure was ≥90 mmHg [24].

The inclusion and exclusion criteria of patients refer to previously published literature [25]. In brief, patients with with renal insufficiency, impaired liver function, pulmonary edema development, cerebral and visual symptoms, and eclamptic seizure, were not included in the study. Healthy pregnant women at term were included in the control group. Healthy pregnant participants were defined as blood pressure < 120/90 mmHg and no clinically significant complications. Those who have multiple pregnancies, diabetes, chronic hypertensive disorder, chronic liver and kidney disease, hypo- or hyperthyroidism, active bacterial or viral infection, chorioamnionitis, macroscopic anomalies in the placenta, fetal chromosomal aneuploidy or malformation, and stillbirth were not included in the study.

Placentas of normal pregnancy (N=32) or preeclampsia (N=20) were obtained from the local hospitals, Suzhou, China. Informed consent regarding all experiments was obtained from participants, in accordance with the Declaration of Helsinki (2013) of the World Medical Association. The study was approved by Ethics Committee of the First Affiliated Hospital of Soochow University. All the placentas in this study were macroscopically normal. The clinical characteristics of all participants were detailed in Table 1. The placenta was taken immediately after delivery, put into the oxygen enriched precooled 4 °C HEPES-PSS buffer, and transferred to the laboratory (within 30 minutes). The placental blood vessels with appropriate size were separated under the microscope and placed in the HEPES-PSS buffer for subsequent tests.

Histological examination of placental artery

Isolated placental artery near the umbilical cord were fixed in 10 % formalin neutral buffer solution overnight at 4 °C, and then the placental artery were sent to the Pathology Department of the First Affiliated Hospital, Soochow University, for hematoxylin and eosin (HE) staining. HE-stained sections were observed and photographed under light microscopy, and representative histological images were recorded at ×100 and ×200 magnification.

Vascular ring experiment
Within 30 minutes after delivery, placenta were collected and placed in chilled (4 °C) HEPES-PSS solution (mM: NaCl 141.85, KCl 4.7, MgSO\textsubscript{4} 1.7, EDTA 0.51, CaCl\textsubscript{2}.2H\textsubscript{2}O 2.79, KH\textsubscript{2}PO\textsubscript{4} 1.17, Glucose 5.0, and HEPES 10.0, pH 7.4). Human placental artery were gently isolated from connective tissue under microscope and placed in chilled HEPES-PSS solution.

All collected placental artery were cut into rings about 2 mm in length and mounted in a M4 myograph system (Radnoti Glass Technology, Inc., USA) filled with HEPES-PSS solution gassed with 95% O\textsubscript{2}, 5% CO\textsubscript{2} at 37 °C. Isometric tension was recorded continuously. Vascular rings were allowed to equilibrate for 60 minutes, and potassium chloride (KCl, 0.12 M) was added to achieve an optimal resting tension which was considered as a reference. 30 minutes later after KCl was washed out, all vascular rings were incubated for 30 minutes with Ang II antagonist (Ang-(1-7), 10\textsuperscript{-5}M) alone or together with MAS1R antagonist (A779, 10\textsuperscript{-5}M), AT2R antagonist (PD, 10\textsuperscript{-5}M). Then accumulative concentrations of Ang II (10\textsuperscript{-11}-10\textsuperscript{-5}M) and 5-HT (5-hydroxytryptamine, 10\textsuperscript{-9}-10\textsuperscript{-4}M) were added to test the vessel function. All drugs were purchased from Sigma-Aldrich and prepared freshly.

**Quantitative Real-Time Polymerase Chain Reaction and Western Blot Analysis**

Total RNA was extracted from placental artery using TRIZOL reagent (Invitrogen Life Technologies). Then the purified total RNA (about 1μg) was reverse-transcribed using the RevertAid First Strand cDNA Synthesis Kit (K1622, Thermo Scientific) according to the manufacturer’s instructions. The expression of messenger RNA (mRNA) was determined by Real-time fluorescence quantitative PCR using a Bio-Rad CFX96 PCR System. All measurements were performed in triplicate, and the relative levels of mRNA were normalized for each sample with the expression levels of the reference gene (ACTB) mRNA using the 2\textsuperscript{-ΔΔCT} method. Information about the gene-specific primer sequences is given in Table 2.

**Western blot analysis**

Placental vessels were lysed in RIPA buffer with protease inhibitors, then put on ice and cracked for 30 minutes. After centrifugation at 13,800g for 30 min at 4 °C, extract part of the supernatant and measure the protein concentration according to the instructions of Detergent Compatible Bradford Protein Assay Kit (P0006C-2, Beyotime). The rest of the supernatant was denatured at 96 °C for 10 minutes and subjected to Western blot analysis. Protein (20μg) were loaded to 12% SDS-PAGE gels and transferred to PVDF membrane. The membranes were incubated with the antibodies against MAS1 (1:2500, sc-390453, Santa Cruz), ERK (1:2500, 4695t, CST), p-ERK (1:2500, 4370t, CST) and β-actin (1:2000, a1978-200ul, Sigma) overnight at 4 °C. After washing with Tris-buffered saline with Tween (TBS-T), the membranes were incubated with secondary antibodies (1:10000, goat anti-mouse for MAS1 and β-actin, goat anti-rabbit for ERK and p-ERK) for an hour. The protein bands were visualized using an enhanced chemiluminescence (ECL) detection system (GE Healthcare, Piscataway, NJ, USA). Results were
quantified using a UVP Bio-imaging system EC3 apparatus (UVP, Upland, CA, USA). MAS1 protein abundance was assessed by normalized to β-actin.

The detection of p-ERK in Nucleus and cytoplasm

Cytoplasmic and nuclear proteins were extracted from the placental artery using the Minute™ Cytoplasmic and Nuclear Extraction KIT (SC-003, Invent Biotechnologies) according to the manufacturer's instructions. In brief, appropriate amount of tissues were added into cytoplasmic extraction buffer and vortexed for 15 seconds. After incubating on ice for 5 minutes, the samples were centrifuged at 4 °C for 5 minutes (16,200g). The supernatant (cytoplasmic protein) was collected and stored in a new microcentrifuge tube. The rest of the sediment was resuspended with precooled phosphate buffer saline (PBS) and centrifuged at 4 °C for 5 minutes (6,200g). After the supernatant was removed, the sediment was resuspended in the nucleus extraction buffer, vortexed 15 seconds, and incubated on ice for 1 minute. Repeat vortexing and incubation steps 4 more times. Finally, the samples were centrifuged at 4 °C for 30 seconds (16,200g), the supernatant (nuclear protein) was transferred and saved to a new microcentrifuge tube.

Enzyme-linked immunosorbent assay

The levels of ACE2 and Ang-(1-7) in maternal blood and placenta from normal and preeclamptic pregnancies were measured via enzyme-linked immunosorbent assay (ELISA) using commercially available assay kits by Haling Biochemical Corporation (Shanghai, China). All experiments were processed and analyzed in a blind manner.

Statistical analysis

Concentration-response curves of vasoconstrictions were analyzed with GraphPad Prism 8.0 (GraphPad Software, Inc., San Diego, CA, USA). Statistical significance was calculated by unpaired two-tailed Student’s t-test, one-way ANOVA with Tukey’s post hoc tests or two-way ANOVA with Tukey’s post hoc tests. P < 0.05 was considered significant. Data were expressed as the mean ± SEM. *p < 0.05; **p < 0.01; ***p < 0.001, ****p < 0.0001. Sample size was chosen on the basis of similar previous studies, and not on statistical methods to predetermine sample size.

Results

Clinical features of two groups

As shown in Fig.1A and Fig.1B, the preeclamptic subjects had significant hypertension as shown by increases in systolic BP (159.5 ± 2.02 versus 118.1 ± 1.53 mm Hg; P<0.0001), diastolic BP (106.2 ± 1.95
versus 72.25 ± 0.92 mm Hg; P<0.0001). The average weeks of gestational in preeclampsia is shorter than those in normal pregnancy (36.79 ± 0.49 versus 38.87 ± 0.09 weeks; P<0.0001, Fig. 1C). The birth weight of the preeclamptic subjects was significantly lower than that of the normal pregnant subjects (2528 ± 175.2 versus 3297 ± 66.82 g, P<0.0001, Fig. 1D), indicating that preeclampsia could have an effect on fetal growth and result in lower birth weight. In addition, Ang II and Ang-(1-7) are closely related to the proliferation and apoptosis of smooth muscle cells. Therefore, the dysfunction of RAS system in placenta might be preliminarily detected by observing the morphological changes of vascular smooth muscle cells of placenta with histological staining. As shown in Fig. 1, compared with the placental artery of the normal group (Fig. 1E, 1F), the placental artery in preeclamptic group had a greater wall thickness and a smaller luminal area (Fig. 1G, 1H).

The interaction between Ang-(1-7) and Ang II in placental artery and its mechanism

Treatment with Ang-(1-7) significantly inhibits Ang II (10^{-7}M, 10^{-6}M and 10^{-5}M) -induced contraction in normal placental artery (Fig. 2A, 2B, red line, P < 0.001, P< 0.01, P< 0.001, respectively), but not in preeclamptic group (Fig. 2C, 2D, red line). To be noticed, pretreatment with the MAS1 receptor antagonists A779 in placental artery from both groups failed to block the inhibitory effect of Ang-(1-7) on Ang II-induced dose-dependent vasoconstriction (Fig. 2A, 2B, 2C, 2D, blue line). Similarly, pretreatment with the AT2 receptor selective antagonist PD in normal placental artery also failed to block the inhibitory effect of Ang-(1-7) on Ang II-induced vasoconstriction (Fig. 2E, 2F).

To be noticed, in order to exclude the effects caused by differences in gestational weeks, we analyzed the responses of placental artery from preterm and term pregnant women to Ang II in the preeclamptic group. The result showed that there was no significant difference in the response of placental artery to Ang II in different gestational weeks (Fig. S1).

Interaction of Ang-(1-7) with 5-HT in placental artery

In addition to Ang II, 5-HT can also cause vasoconstriction and regulate vascular tone. In order to test whether Ang-(1-7) only exerts a specific antagonistic effect on Ang II, we detected the effect of Ang-(1-7) on 5-HT in placental artery. After treatment of normal placental artery with Ang-(1-7), there was no significant inhibitory effect on the 5-HT-induced vasoconstriction (Fig. S2A, S2B).

Changes of Ang-(1-7) specific receptor MAS1

MAS1 is a receptor for Ang-(1-7), which is derived from Ang II by the action of ACE2. Hence, to better understand the functional changes in vessels, the mRNA and protein levels of MAS1 receptor were tested and analyzed. As shown in Fig. 3, mRNA expression of MAS1 receptor was significantly down-regulated
in the preeclamptic group (Fig. 3A). However, there was no significant difference in MAS1 receptor protein levels between the two groups (Fig. 3B, 3C). Yet, this result and the previous functional experiment results (Fig. 2B, 2D) suggest that Ang-(1-7) does not exert an inhibitory effect on Ang II through MAS1 receptor, and the specific mechanism still needs further study.

Detection of other components of RAS by PCR and ELISA

In order to further explore the possible mechanism of Ang-(1-7) functional difference between the two groups, other components of RAS were tested. As shown in Fig. 4, the mRNA expression of AT1R in preeclamptic group was significantly down-regulated (Fig. 4A, 1.086 ± 0.123 vs. 0.488 ± 0.098, p <0.001); while the mRNA expression of MRGPRD, AT2R and ACE2 was not statistically different between the two groups (Fig. 4B, 4C, 4D). In order to verify whether the levels of Ang-(1-7) and ACE2 related to RAS changed during preeclampsia, the peripheral blood and placental vascular tissues collected from pregnant women were tested by ELISA. Compared with normal pregnant women, Ang-(1-7) levels in the plasma and placental vascular tissues of pregnant women in preeclampsia did not change significantly (Fig. 4E, 4F). Similarly, there was no significant difference in ACE2 levels between the two groups (Fig. 4G, 4H).

β-arrestin pathway in placental vascular tissue

The results above indicate that Ang-(1-7) does not exert its effect through MAS1R and AT2R, previous studies suggest an important role for AT1R in the placental artery, so whether Ang-(1-7) acts as an endogenous β-arrestin-biased ligand for AT1R is worthy of further study. Not as expected, real time quantitative PCR results showed that the mRNA levels of ARRB1 and ARRB2 were not different between the two groups (Fig. S3). Hence, whether the activation of β-arrestin pathway is altered needs further study. To be noticed, β-arrestins bind to G protein-coupled receptor kinase (GRK)-phosphorylated seven transmembrane receptors, and the activated β-arrestin pathway resulted in a significant increase in the cytosolic pool of phospho-ERK1/2 and a corresponding decrease in the nuclear pool of phospho-ERK1/2. Therefore, we separated the nucleus and cytoplasmic protein of placental vascular tissue, and the phosphorylation level of ERK protein was detected. The results showed that the phosphorylation level of ERK protein in the nucleus of placental vascular tissue in the preeclamptic group was significantly reduced (Figure. 5A, 5C, P< 0.01), while the phosphorylation level of ERK protein in the cytoplasm of placental vascular tissue in preeclamptic group was significantly increased (Figure. 5B, 5C, P< 0.05).

Discussion

Principal findings

In this study we found the function change of Ang-(1–7) on Ang II in the placenta vessels from preeclamptic women as compared with healthy pregnant women. By vascular tone detection, we
demonstrated that Ang-(1–7) lost its effect on Ang II in preeclamptic group, but not in normal group. After treatment with A779 and PD in two groups, we found that these two small molecule compounds had no influence on the interaction between Ang-(1–7) and Ang II. These findings indicated that Ang-(1–7) may not exert its effect on Ang II through MAS1R and AT2R. Subsequently, we detected the level of p-ERK in Nucleus and cytoplasm, data showed that the phosphorylation level of ERK protein in the nucleus of placental vascular tissue in the preeclamptic group was significantly reduced, while significantly increased in the cytoplasm as compared to the normal group. Thus, the preeclampsia group in our study is characterized with weakened function of Ang-(1–7) and persistent activation of the β-arrestin pathway.

**Weakened effect of Ang-(1–7) on Ang II**

Studies have shown that abnormal RAS are closely related to the onset of preeclampsia and maternal clinical manifestations [26, 27]. In addition to the RAS in the kidney, there is a tissue-based RAS in the uteroplacental unit. Local tissue-based RAS in the uteroplacental unit may regulate the regional maternal intervillous blood flow. Disturbances of the uteroplacental RAS may lead to dysfunctional bleeding and to reduced uteroplacental blood flow in pregnancies complicated by preeclampsia and intrauterine growth retardation [28]. In preeclampsia, the response of maternal blood vessels to angiotensin peptides changes, and as a result, the abnormal activation of RAS in the kidney causes hypertension and kidney damage, affects maternal fluid balance and electrolyte balance, and leads to a series of pathophysiological changes in preeclampsia [29]. Many of the previous investigations of RAS have shown that normal pregnancy is associated with decreased vascular responsiveness to Ang II [30], and preeclampsia is associated with increased sensitivity to Ang II that may develop before the clinical manifestations of the disease [16]. In addition, a decrease in Ang-(1-7), the vasodilatory arm of RAS in the maternal peripheral plasma, has been reported in preeclampsia [31]. The reduction of Ang-(1-7) levels in the serum of pregnant women and the down-regulation of RAS-related receptors such as MAS1 receptor in the kidney may contribute to the direct podocyte injury and ultimately lead to proteinuria in preeclampsia [32]. Many previous reports indicate that Ang-(1-7) mainly exerts its effect by antagonizing Ang II. Studies have shown that ACE2 balances the function of Angiotensin I Converting Enzyme (ACE), and the balance between these two proteases determines the local and systemic levels of RAS-related peptides (such as Ang  and Ang-(1-7). The loss of balance between Ang-(1-7) and Ang II may lead to the inflammation and vascular dysfunction during the onset of diabetes, and eventually lead to hypertension [33].

**Abnormal activation of β-arrestin pathway**

Increasing evidence suggests that Ang-(1-7) mediates at least part of its cardioprotective effects by acting as an endogenous β-arrestin-biased agonist at the AT1R [34, 35]. Like many other G protein coupled receptors (GRCPs), AT1R is rapidly phosphorylated following stimulation by both second messenger-activated kinases and specific GPCR kinases (GRKs) and then bound by cytosolic proteins
termed β-arrestins that prevent interaction of receptors with G proteins and terminate signaling, a phenomenon referred to as desensitization [36, 37]. Receptor-bound β-arrestins interact with clathrin, ERK, MEK and Raf-1, thereby promoting GPCR internalization [38]. GPCRs are classified into categories A and B according to their selectivity for β-arrestin 1 and β-arrestin 2. Class A receptors preferentially recruit β-arrestin 2, represented by β2-adrenergic receptors; class B receptors, such as AT1R, have a high affinity for both β-arrestin. Moreover, when class B receptor interacts with β-arrestin, it will form a stable complex and internalize into endoplasmic body. However, the interaction between β-arrestin and class A receptor is transient, and after the receptor is transferred to endoplasmic body, β-arrestin will return to cytoplasm in free state [39]. In the process of AT1R activation and internalization, β-arrestin 2 cooperates with AT1R to form a multiprotein complex containing ERK-MAPK and its upstream activating factor, including AT1R, β-arrestin 2 and ERK cascade kinase, cRaf1, MEK1, etc. At this time, the activated ERK will be stabilized in the cytoplasm, resulting in the decrease of the activated ERK entering the nucleus [40, 41]. It was found that the fourth tyrosine (Tyr4) and eighth phenylalanine (Phe8) of Ang II peptide are necessary to activate the AT1R-G protein signal, if the fourth or eighth amino acid changes, it will specifically activate AT1R-(β-arrestin) signal [42]. Cell experiments showed that Ang-(1-7) is an endogenous β-arrestin biased-ligand of AT1R and may have protective effect on cardiovascular system [34, 35]. In this study, the p-ERK level in the nucleus of placental vascular tissue in preeclamptic group was significantly reduced, while the p-ERK level in the cytoplasm significantly increased. These results suggest that the β-arrestin signaling pathway has changed during the onset of preeclampsia, and may explain the function difference of Ang-(1-7) between two groups.

**Conclusion**

In conclusion, due to the heterogeneity of preeclampsia, clarifying its pathogenesis is necessary to explore new prevention and treatment strategies. Our research further shows the important role of placenta and RAS in preeclampsia. The study of RAS component Ang-(1-7) will help to improve the understanding of the pathophysiology of preeclampsia. As an endogenous antagonist of Ang II, Ang-(1-7) may be used as a target for clinical intervention and treatment of preeclampsia, and is expected to improve the short-term and long-term outcomes of preeclampsia.

**Abbreviations**

RAS: renin angiotensin system; Ang-(1–7): angiotensin-(1–7); MAS1R: Mas-Related G Protein-Coupled Receptor A; AGTR1: Angiotensin II Receptor Type 1; AGTR2: Angiotensin II Receptor Type 2; MRGPRD: MAS Related GPR Family Member D; HE: Hematoxylin-Eosin; ACE2: Angiotensin I Converting Enzyme 2; ELISA: enzyme linked immunosorbent assay; A779: ERK: Mitogen-Activated Protein Kinase 1; Ang II: Angiotensin II; PE: Preeclampsia; ISSHP: International Society for the Study of Hypertension in Pregnancy; DOHaD: Developmental Origins of Health and Disease; ACE: Ang I Converting Enzyme; ACOG: American College of Obstetricians and Gynecologists; KCl: Potassium chloride; ACTB: Actin Beta; TBS-T: Tris-buffered saline with Tween; PBS: phosphate buffer saline; GRCPs: G protein coupled receptors; GRKs:
GPCR kinases; MEK: Mitogen-Activated Protein Kinase Kinase 1; Raf-1: RAF Proto-Oncogene Serine/Threonine-Protein Kinase.

Declarations

Authors’ contributions

MS, ML and QH conceived and designed the study and drafted the manuscript. LL, YS, and JL carried out the data analysis. LL and WL conducted the Vascular ring experiment. TZ conducted the hematoxylin and eosin (HE) staining. LL and DZ conducted the Quantitative Real-Time Polymerase Chain Reaction and Western Blot Analysis. ZX and QG helped for the problems encountered in the experiment. HG, BH, YC and FX collected and analyzed the clinical information. All authors revised the article and approved the final version to be published.

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Availability of data and materials

The datasets used and analyzed in this study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study was approved by Ethics Committee of the First Affiliated Hospital of Soochow University. Written informed consent was obtained from women before they participated in the study.

Consent for publication

Not applicable
Competing interests

The authors declare that they have no competing interests.

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**Tables**

Due to technical limitations, tables are only available as a download in the Supplemental Files section.

**Figures**

![Figure 1](image)

**Figure 1**

Clinical features of two groups and the HE staining of the placental blood vessels. Arrows indicate smooth muscle layer. A: systolic BP (mmHg) in two groups, B: diastolic BP (mmHg) in two groups, C: gestational week in two groups, D: birth weight between the two groups, E: normal placental artery (100 ×), F: normal placental artery (200 ×), G: preeclampsia placental artery (100 ×), H: preeclampsia placental artery (200 ×).
Figure 2

The interaction between Ang-(1-7) and Ang II in placental artery. A: representative images of Ang II, Ang-(1-7) + Ang II, A779 + Ang-(1-7) + Ang II-mediated vascular responses in the CON group, B: the dose-dependent response of the isolated vascular rings of human placenta to Ang II (10-11M-10-5M)-induced contraction after treatment with or without Ang-(1-7) (10-5M) and A779 (10-5M) under basal tension in CON group (CON-Ang II: N=32; CON- Ang-(1-7) + Ang II: N=19, CON-A779 + Ang-(1-7) + Ang II: N=20 ), C: representative images of Ang II, Ang-(1-7) + Ang II, A779 + Ang-(1-7) + Ang II-mediated vascular responses in the PE group, D: the dose-dependent response of the isolated vascular rings of human placenta to Ang II (10-11M-10-5M)-induced contraction after treatment with or without Ang-(1-7) (10-5M) and A779 (10-5M) under basal tension in PE group (PE-Ang II: N=20; PE- Ang-(1-7) + Ang II: N=15; PE-A779 + Ang-(1-7) + Ang II: N=11 ). **p<0.01***, p<0.001. E: representative images of Ang II, Ang-(1-7) + Ang II, PD + Ang-(1-7) + Ang II-mediated vascular responses in CON group, F: the dose-dependent response of the isolated vascular rings of human placenta to Ang II (10-11M-10-5M)-induced contraction after treatment with or without Ang-(1-7) (10-5M) and PD (10-5M) under basal tension in CON group (CON-Ang II: N=32; CON- Ang-(1-7) + Ang II: N=19, CON-PD + Ang-(1-7) + Ang II: N=8). **p<0.01***, p<0.001.
Figure 3

The expression of MAS1 mRNA and protein in placental artery. A: Expression of MAS1 mRNA in human placental vascular tissue (CON: normal pregnancy group, N=30; PE: preeclamptic group, N=20. **p<0.01). B-C: MAS1 protein levels in human placental vascular tissue (CON: normal pregnancy group, N=12; PE: preeclamptic group, N=12).

Figure 4
The level of RAS related gene in placental vascular tissue and maternal plasma. A-D: Detection of AT1R, MRGPRD, AT2R, ACE2 mRNA levels in human placental vascular tissue (CON: normal pregnancy group, N=30; PE: preeclampsia group, N=24), E: The Ang-(1-7) levels in maternal plasma detected by ELISA (CON: N=30; PE: N=30), F: The Ang-(1-7) levels in human placental tissue detected by ELISA (CON: N=30; PE: N=30), G: The ACE2 levels in maternal plasma detected by ELISA (CON: N=30; PE: N=30), H: The ACE2 levels in human placental tissue detected by ELISA (CON: N=30; PE: N=30). CON: normal pregnancy group, PE: preeclampsia group. ***, p<0.001.

Figure 5

The detection of p-ERK in Nucleus and cytoplasm. A: Phosphorylation level of ERK protein in the nuclei of placental vascular tissues in two groups (CON: N=12; PE: N=12), B: Phosphorylation level of ERK protein in the cytoplasm of placental vascular tissue in two groups (CON: N=12; PE: N=12). C: Analysis results of protein bands (analysis using AlphaEaseFC and GraphPad Prism 8.0). CON: normal pregnancy group, PE: preeclamptic group. *, p<0.05; **, p<0.01.

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