Effects of S1PR2 antagonist on blood pressure and angiogenesis imbalance in preeclampsia rats

TENGFEI ZHANG[1], DANJIE GUO[1], WEIPING ZHENG[2] and QUNYUN DAI[3]

[1]Department of Gynaecology and Obstetrics, Zhuji Affiliated Hospital of Shaoxing University, Zhuji, Zhejiang 311800; [2]Department of Gynecology, Shaoxing People's Hospital and Shaoxing Hospital, Zhejiang University School of Medicine, Shaoxing, Zhejiang 312000; [3]Obstetrics Department, Jinhua Maternal and Child Health Hospital, Jinhua, Zhejiang 321000, P.R. China

Received August 21, 2020; Accepted March 3, 2021

DOI: 10.3892/mmr.2021.12095

Abstract. Preeclampsia (PE), a hypertensive multisystem disorder, can lead to increased maternal and fetal mortality and morbidity. Sphingosine-1-phosphate (S1P) plays various roles, depending on the cell type, by binding to S1P receptors (S1PR). The present study evaluated the changes of S1PRs and investigated the potential role of S1PRs in pregnancy-induced hypertension. PE rats were established by reduced uterine perfusion pressure. The involvement of S1PR2 was evaluated using JTE-013, a specific S1PR2 antagonist, in PE rats. After the treatment, inflammatory cytokines were evaluated using enzyme linked immunosorbent assay, and the expression of vascular endothelial growth factor (VEGF), inducible nitric oxide synthase (iNOS) activation and endothelial nitric oxide synthase (eNOS) were evaluated by reverse transcription-quantitative PCR and western blotting. Results showed that S1PR2, but not S1PR1 and S1PR3, was significantly increased in the serum and placenta tissues of PE rats. Notably, JTE-013 significantly decreased blood pressure, attenuated infiltration of inflammatory cells and decreased inflammation, as indicated by the decreased expression of inflammatory cytokines, including tumor necrosis factor-α, interleukin-1β (IL-1β) and IL-6, in placental tissues. Mechanistic studies demonstrated that JTE-013 significantly increased the expression of VEGF and decreased the expression of fms-like tyrosine kinase 1 in placental tissue. Furthermore, JTE-013 prevented iNOS activation and increased eNOS in placental tissue. In summary, the present study demonstrated that S1PR2 contributed to hypertension and angiogenesis imbalance in PE rats.

Correspondence to: Dr Qunyun Dai, Obstetrics Department, Jinhua Maternal and Child Health Hospital, 266 Houshan Road, Jinhua, Zhejiang 321000, P.R. China
E-mail: daiqunyunun@126.com

Key words: pregnancy, hypertension, sphingosine-1-phosphate receptor 2 antagonist, preeclampsia

Introduction

Preeclampsia (PE) is the presence of new-onset hypertension and proteinuria or other end organ damage that occurs after 20 weeks of pregnancy (1). In the clinical setting, this disease is characterized by systolic blood pressure (SBP) ≥140 mmHg or diastolic BP ≥90 mmHg, as well as proteinuria (≥0.3 g/24 h) (2). In humans, it is a hypertensive multisystem disorder, which can lead to maternal and fetal mortality and morbidity (3), and substantially contributes to prematurity of the fetus and long-term cardiovascular disease in the mother (4).

It has been proposed that the development of PE is closely associated with placenta formation, particularly in early pregnancy stages (5). Previous studies have reported that there are several alterations that contribute to the development of PE, including vascular dysfunction, oxidative stress and metabolic abnormalities (6,7). In particular, it has been demonstrated that placental vascular development is considerably altered (8-10), and this vascular dysfunction in placenta tissues plays a critical role in BP increase and PE (11,12). However, the molecular mechanism underlying PE remains largely unknown.

Sphingosine-1-phosphate receptors (S1PR) are a class of G-protein-coupled receptors, which participate in different cellular responses, such as proliferation and apoptosis (13,14). The S1PRs, S1PR1, S1PR2 and S1PR3 are widely expressed in various tissues, while S1P4 is only found in lymphoid and hematopoietic tissues, and S1P5 is mainly expressed in the central nervous system (15). Increasing evidence suggests that S1P is involved in BP, such as pulmonary arterial hypertension (PAH) (16). For example, Chen *et al* (16) reported that S1P levels were upregulated in the lungs of patients with PAH, and that the pharmacological inhibition of sphingosine kinase 1 and S1PR2 prevents the development of hypoxia-mediated pulmonary hypertension in rats. Thus, it was speculated that S1P may regulate portal pressure. Ikeda *et al* (17) demonstrated that S1P increases portal pressure in isolated rat perfused liver, and this effect is mediated by an increase in the activity of Rho via a S1PR2-dependent mechanism.

Based on the critical role of S1P in BP regulation, it was hypothesized that S1PRs may play a role in the development of PE. Thus, the present study aimed to assess changes in the levels of S1P, S1PR1, S1PR2 and S1PR3 in placenta tissues,

and determine whether S1P plays a role in BP and angiogenesis imbalance using a PE rat model.

Materials and methods

Ethics statement. The present study was approved by the Institutional Animal Care and Use Committee (IACUC) of Shaoxing People's Hospital (Jinhua, China; approval no. 20190713). The animal experiments were conducted according to the IACUC Care and Use of Laboratory Animals guidelines (18).

Animals. A total of 60 Sprague Dawley (SD) rats (30 males and 30 females; weight, 250-300 g; age, 7-9 weeks) were obtained from Shanghai SLAC Laboratory Animal Co., Ltd. The rats were housed at 22±2˚C with a 12-h light-dark cycle and free access to food and water. The ratio of male to female was 1:1. Appearance of the sperm plug or sperms detected in vaginal smear was regarded as gestational day (GD) 0.

In the first set of experiments, 20 pregnant SD rats were randomly divided into four groups (n=5), as follows: i) Control group; ii) model group; iii) S1P group; and iv) model + S1P group. In the model group, on GD 14, pregnant rats were anesthetized (~3% isoflurane in 2 l/min O_2), the uterus was exteriorized and silver clips were used to clamp the abdominal aorta (above the kidneys) and branches of the ovarian arteries to construct the reduced uterine perfusion pressure (RUPP) rat model, as previously described (19). Whereas, pregnant rats that underwent a sham procedure were used as the control on GD 14. A 1 mM stock of S1P (Sigma-Aldrich; Merck KGaA) was prepared in 10 mM NaOH and diluted to desired S1P concentration in saline with 0.1% BSA (pH 7.8-8.0; Beyotime Institute of Biotechnology). In the S1P group, pregnant rats were only administered with S1P (0.1 mg/kg body weight) by intravenous injection in the tail vein for 3 consecutive days after GD 14 (20). In the model + S1P group, RUPP rats were administered with S1P (0.1 mg/kg body weight) by intravenous injection in the tail vein for 3 consecutive days after GD 14.

After 7 days (GD 21), the rats were euthanized with CO_2 (CO_2 displacement rate was 25% vol/min) 3 weeks after the last injection, followed by cervical dislocation. Caesarean sections were performed on the rats and the placental tissues were stored at -80˚C for further assessment. The fetal weights, placental weights, litter size and/or number of resorptions/pregnancy losses were not obviously changed in this RUPP model.

In the second set of experiments, twenty pregnant SD rats were randomly divided into four groups (n=5) on GD 14, as follows: i) Control group; ii) model group; iii) model + S1PR2 antagonist low (JTE-013, 2.5 mg/kg body weight) group; and iv) model + S1PR2 antagonist high (JTE-013, 5 mg/kg body weight) group. The control and model groups consisted of the same mice as those used for these groups in the first set of experiments. JTE-013 was intraperitoneally administrated at the indicated concentration once every other day, until GD 21. When the aforementioned animal experiments were finished, animals were euthanized by 25% CO_2.

BP measurement. SBP was measured using the BP-2000 Series II non-invasive tail-artery pressure measuring instrument (Visitech Systems) every day from days 15-21 of the pregnancy. Briefly, rats were fixed on the pre-heated plate at 37˚C, and the pressure-cuff was attached to the rat tail. Rat tails were connected to the pressure sensor of the BP-2000 Series II instrument. SBP of the tail artery was measured in each pregnant rat at least three times, and the average value of each measurement was calculated.

Hematoxylin and eosin (H&E) staining. Harvested placental tissues were immersed in 10% (v/v) buffered formalin overnight at room temperature and subsequently embedded in paraffin. Paraffin-embedded tissue samples were cut into 5-μm thick sections and stained with H&E for 15 min at room temperature, according to standard protocols. Stained sections were observed using a light microscope. The number of immune cells was quantified using ImageJ software (version 1.8.0; National Institutes of Health).

Enzyme-linked immunosorbent assay (ELISA). ELISA was performed to detect serum S1P, inducible nitric oxide synthase (iNOS), tumor necrosis factor-α (TNF-α), interleukin (IL)-1β and IL-6 levels. The kits for S1P (cat. no. ML-Elisa-0470) and iNOS (cat. no. CS-E01909) were respectively purchased from Shanghai enzyme-linked Biotechnology Co., Ltd. and Shanghai Ulva Biotechnology Co., Ltd. The kits for TNF-α (cat. no. MTA00B), IL-1β (cat. no. MLB00C) and IL-6 (cat. no. D6050) were purchased from R&D Systems, Inc.

Measurement of NO. Serum NO was measured using the NO assay kit (cat. no. EMSNO; Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's instructions. Total NOx (nitrite + nitrate) was used as an indicator of NO synthesis in serum (20).

Reverse transcription-quantitative (RT-q)PCR. Following treatment, placental tissues were collected from pregnant rats and total RNA was extracted using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.). Total RNA was reverse transcribed into cDNA using the SYBR PrimeScript™ RT-PCR kit (cat. no. RR066A; Takara Bio, Inc.). qPCR was subsequently performed for the S1PR1, S1PR2, S1PR3, vascular endothelial growth factor (VEGF) and fms-like tyrosine kinase 1 (Flt-1) genes using the Applied Biosystems 7500 Standard system (Thermo Fisher Scientific, Inc.). cDNA equivalent to 100 ng total RNA was used for qPCR, using the TaqMan Universal PCR Master Mix (Applied Biosystems; Thermo Fisher Scientific, Inc.). PCR thermocycling conditions were as follows: 4 min at 95˚C, followed by 30 sec at 95˚C, 20 sec at 65˚C and 30 sec at 72˚C, for 35 cycles. The primer sequences were as follows: S1PR1 forward, 5'-CAGCAAATC GGACAATTCCT-3' and reverse, 5'-GCCAGCGACCAAGTA AAGAG-3'; S1PR2 forward, 5'-TGTATGGCAGCGACAAGA GC-3' and reverse, 5'-ACCGAGGACCAGCGAGATG-3'; S1PR3 forward, 5'-GCCACCCGCCAGTCTTG-3' and reverse, 5'-GCCAGCTTCCCCACGTAAT-3'; VEGF forward, 5'-ACC ATGAACTTTCTGCTC-3' and reverse, 5'-GGACGGCTT GAAGATATA-3'; Flt-1 forward, 5'-TTTGCATAGCTTCCA ATAAAGTTG-3' and reverse, 5'-CATGACAGTCTAAAG TGGTGGAAC-3'; and GAPDH forward, 5'-CACCACCAT GGAGAAGGC-3' and reverse, 5'-CCATCCACAGTCTTC

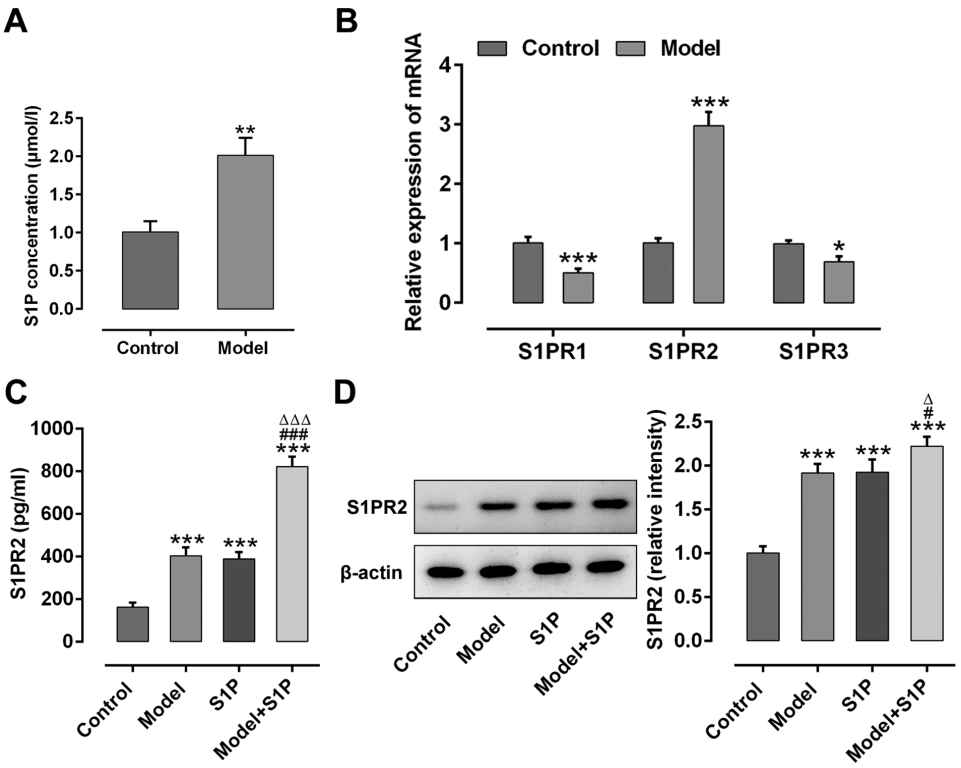

Figure 1. S1PR2 is increased in the serum and placental tissue of PE rats. (A) Measurement of S1P in serum via ELISA. (B) The expression of S1PR1, S1PR2 and S1PR3 in serum of control and PE rats was analyzed by reverse transcription-quantitative PCR analysis. (C) The effect of S1P on the expression of S1PR2 in the plasma of PE rats was analyzed via ELISA. (D) The effect of S1P on the expression of S1PR2 in the placental tissues of PE rats was analyzed by western blotting. n=3. *P<0.05, **P<0.01 and ***P<0.001 vs. Control group; $^{\#}$P<0.05 and $^{\#\#\#}$P<0.001 vs. Model group; $^{\Delta}$P<0.05 and $^{\Delta\Delta\Delta}$P<0.001 vs. S1P group. S1P, sphingosine-1-phosphate; S1PR2, sphingosine-1-phosphate receptor 2; PE, preeclampsia; ELISA, enzyme-linked immunosorbent assay.

TGA-3'. The relative expression levels of mRNAs were normalized to GAPDH, and were calculated with $2^{-\Delta\Delta Cq}$ method (21).

Western blotting. Total protein was extracted from placenta tissues using RIPA lysis buffer (Beyotime Institute of Biotechnology), according to the manufacturer's instructions. Total protein was quantified using the BCA Protein Assay kit (cat. no. P0012S; Beyotime Institute of Biotechnology) and 10 μg protein/lane was separated via SDS-PAGE on a 8% gel. The separated proteins were subsequently transferred onto nitrocellulose membranes (EMD Millipore) and blocked with 0.5% non-fat milk for 1 h at room temperature. The membranes were incubated with primary antibodies against S1PR2 (cat. no. 21180-1-AP; 1:1,000; ProteinTech Group, Inc.), VEGF (cat. no. 66828-1-AP; 1:1,000; ProteinTech Group, Inc.), Flt-1 (cat. no. 13687-1-AP; 1:1,000; ProteinTech Group, Inc.), endothelial (e)NOS (cat. no. ab76198; 1:1,000; Abcam) and β-actin (cat. no. ab8226; 1:1,000; Abcam) overnight at 4°C. Following the primary incubation, membranes were incubated with anti-rabbit (cat. no. SA00001-2; 1:1,000; ProteinTech Group, Inc.) or anti-mouse (cat. no. SA00001-1; 1:1,000; ProteinTech Group, Inc.) HRP-conjugated secondary antibodies for 2 h at room temperature. Protein bands were visualized using the enhanced chemiluminescence kit (cat. no. GERPN2105; Millipore Sigma; Merck KGaA) and semi-quantified using ImageJ 1.8.0 software (National Institutes of Health).

Statistical analysis. Statistical analysis was performed using SPSS 21.0 software (IBM Corp.). Data are presented as the mean ± standard deviation. The Kolmogorov-Smirnov test was used to detect the normality of all data. Unpaired Student's t-test was used to compare differences between two groups. One-way analysis of variance and Tukey's post hoc test were used to compare differences between multiple groups. P<0.05 was considered to indicate a statistically significant difference.

Results

S1PR2 is increased in the serum and placental tissues of PE rats. The expression levels of S1P, S1PR1, S1PR2 and S1PR3 were detected in PE rats. As presented in Fig. 1A, S1P serum expression was significantly upregulated in PE rats compared with the control rats. As presented in Fig. 1B, S1PR1 and S1PR3 mRNA expression levels were significantly downregulated, whereas S1PR2 mRNA expression was significantly upregulated in the serum of PE rats compared with the control rats. Consistently, S1PR2 expression levels in the serum and placental tissues were significantly upregulated in PE rats compared with the control rats, and S1P significantly increased S1PR2 expression levels in PE rats (Fig. 1C and D).

Inhibition of S1PR2 with JTE-013 decreases BP in PE rats. The present study assessed whether S1PR2 activation was involved in increased BP in PE. As presented in Fig. 2A and B, BP significantly increased in PE rats compared with the control rats. However, BP significantly decreased in PE rats pretreated with JTE-013. High dose JTE-013 displayed an enhanced effect on decreasing BP compared with low dose.

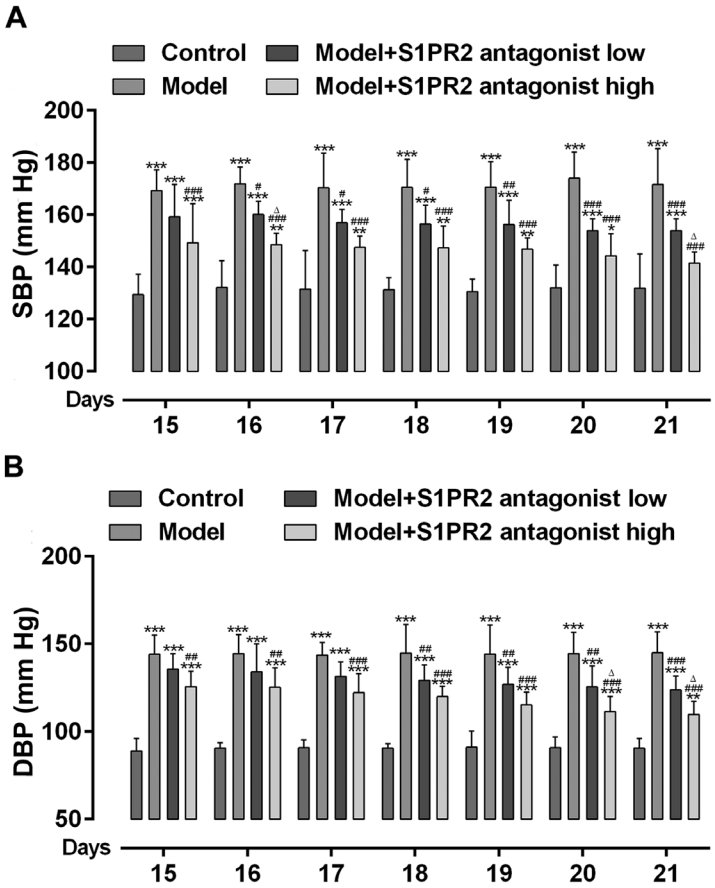

Figure 2. Inhibition of S1PR2 with JTE-013 decreases BP in PE rats. Effect of JTE-013 on (A) SBP and (B) DBP in PE rats in tail-cuff measurement. n=3. *P<0.05, **P<0.01 and ***P<0.001 vs. Control group; $^{\#}$P<0.05, $^{\#\#}$P<0.01 and $^{\#\#\#}$P<0.001 vs. Model group; $^{\Delta}$P<0.05 vs. Model + S1PR2 antagonist low group. S1PR2, sphingosine-1-phosphate receptor 2; PE, preeclampsia; BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Inhibition of S1PR2 with JTE-013 prevents iNOS activation and increases eNOS. The effect of S1PR2 inhibition on the NO (Fig. 3A), iNOS (Fig. 3B) and eNOS (Fig. 3C) signaling pathways was assessed. The serum NO and iNOS levels were significantly upregulated in PE rats. Conversely, eNOS expression in the placenta tissue was significantly downregulated in PE rats. Notably, these effects were reversed following treatment with JTE-013. High dose JTE-013 displayed an enhanced effect on decreasing serum NO and iNOS levels compared with low dose.

JTE-013 regulates VEGF and Flt-1 expression in placental tissues. The effect of S1PR2 inhibition on the changes in VEGF and Flt-1 expression levels in placental tissues was assessed. As presented in Fig. 4A and B, VEGF mRNA and protein expression levels were significantly downregulated in the placental tissues of PE rats. Conversely, Flt-1 mRNA and protein expression levels were significantly upregulated in the placental tissues of PE rats. Notably, these effects were reversed following treatment with JTE-013. High dose JTE-013 displayed an enhanced effect on increasing VEGF expression and decreasing Flt-1 expression compared with low dose.

JTE-013 attenuates pathological changes in placental tissues and increases the expression of inflammatory cytokines. The effect of S1PR2 inhibition on inflammation and pathological changes in placental tissues in PE was assessed. As presented in Fig. 5A, there was significant inflammation in the placental tissues of PE rats compared with the control rats, as indicated by the increased expression levels of inflammatory cytokines, including TNF-α, IL-1β and IL-6. As presented in Fig. 5B, there was significant infiltration of inflammatory cells in the placental tissues of PE rats, as demonstrated by H&E staining. Notably, these effects were reversed following treatment with JTE-013. High dose JTE-013 displayed an enhanced effect on suppressing inflammation compared with low dose.

Discussion

The results of the present study demonstrated that S1PR2 expression was upregulated in placental tissues, and that the inhibition of S1PR2 with JTE-013 decreased BP, inflammation and the infiltration of inflammatory cells. These results are consistent with previous findings, suggesting that S1P can induce its receptor, S1PR2, to inhibit the migration of trophoblast cells (22). In addition, another study demonstrated that expression of the anti-angiogenic factor, S1PR2, in villi tissue was upregulated in patients with PE (23).

Previous studies have reported that VEGF plays a key role in vasculogenesis and angiogenesis, both of which are important in the development of the placenta (24,25). Increasing evidence suggests that VEGF expression is downregulated (26,27), and the placenta produces elevated levels of VEGF receptor (Flt-1),

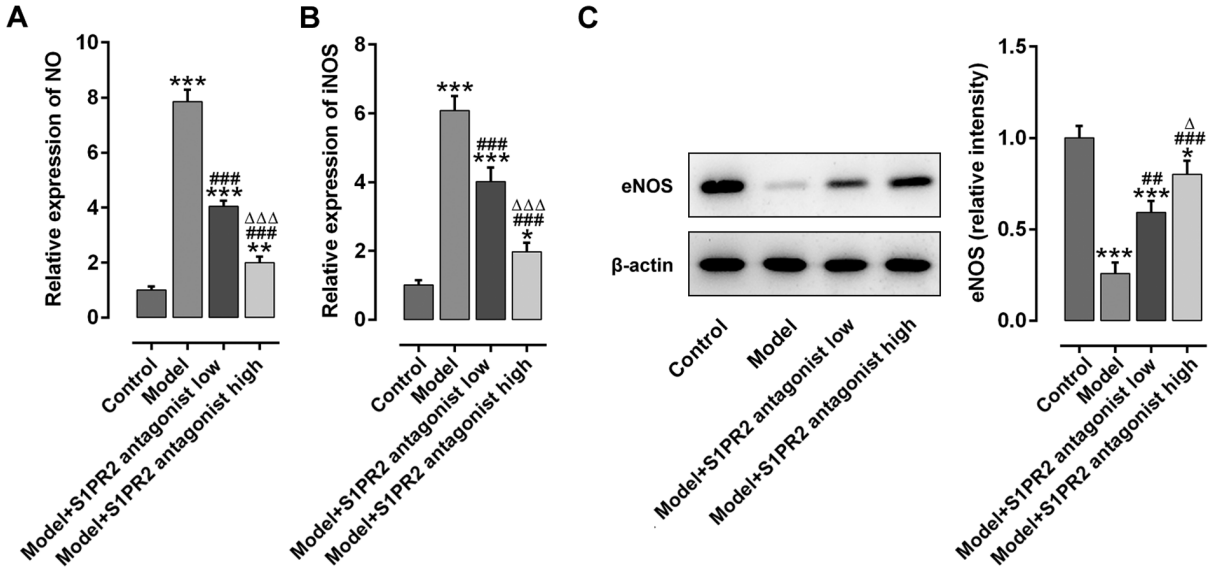

Figure 3. Effect of JTE-013 on serum NO and iNOS and the expression of eNOS in placental tissues. (A) Summarized data showing the inhibitory effect of JTE-013 on serum NO levels in PE rats. (B) Summarized data showing the inhibitory effect of JTE-013 on serum iNOS levels in PE rats. (C) Summarized data showing that JTE-013 prevented the decreased expression of eNOS in placental tissues of PE rats. n=3. *P<0.05, **P<0.01 and ***P<0.001 vs. Control group; $^{\#\#}$P<0.01 and $^{\#\#\#}$P<0.001 vs. Model group; $^{\Delta}$P<0.05 and $^{\Delta\Delta\Delta}$P<0.001 vs. Model + S1PR2 antagonist low group. S1PR2, sphingosine-1-phosphate receptor 2; PE, preeclampsia; iNOS, inducible nitric oxide synthase; eNOS, endothelial nitric oxide synthase; NO, nitric oxide.

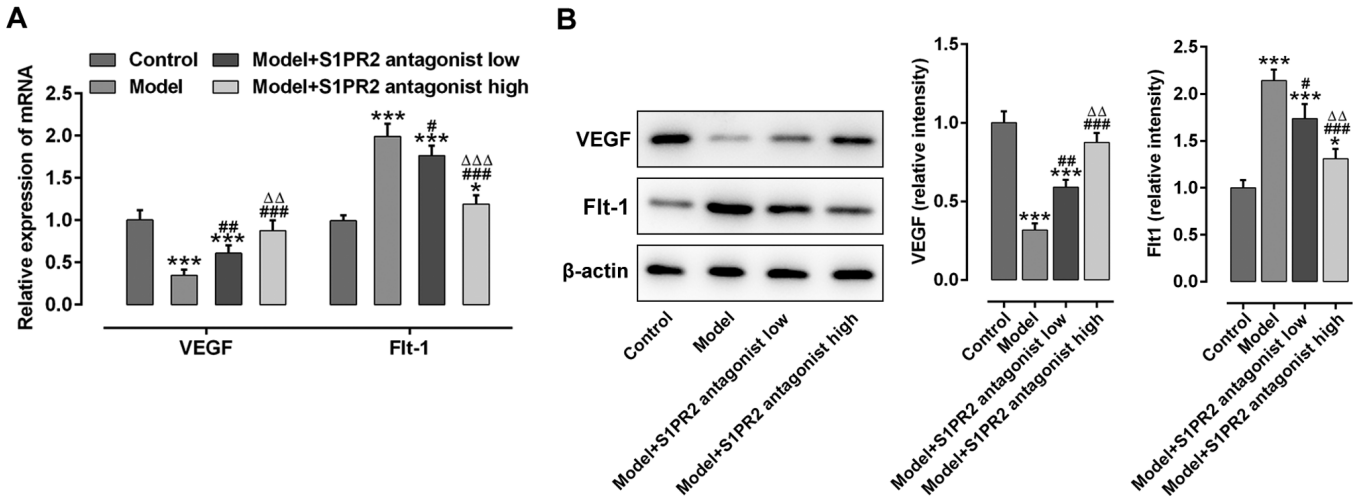

Figure 4. JTE-013 inhibits the PE model-induced expression levels of VEGF and Flt-1 receptor in PE rats. (A) Summarized data showing the inhibitory effect of JTE-013 on the PE model-induced mRNA levels of VEGF and Flt-1 in reverse transcription-quantitative PCR assay. (B) Representative western blotting images and summarized data showing the inhibitory effect of JTE-013 on the PE model-induced protein levels of VEGF and Flt-1 in the placental tissues of PE rats. n=3. *P<0.05 and ***P<0.001 vs. Control group; $^{\#}$P<0.05, $^{\#\#}$P<0.01 and $^{\#\#\#}$P<0.001 vs. Model group; $^{\Delta\Delta}$P<0.01 and $^{\Delta\Delta\Delta}$P<0.001 vs. Model + S1PR2 antagonist low group. S1PR2, sphingosine-1-phosphate receptor 2; PE, preeclampsia; VEGF, vascular endothelial growth factor; Flt-1, fms-like tyrosine kinase 1.

which captures free VEGF (28). It has also been reported that eNOS expression is downregulated in PE (29). These changes result in insufficient placental VEGF and eNOS expression, and endothelial dysfunction, thereby resulting in the initiation and development of PE (26-28). Amaral *et al* (30) demonstrated that iNOS expression is upregulated in PE rats, and that inhibition of iNOS significantly decreases RUPP-induced increase of plasma 8-isoprostane. Consistent with these findings, the results of the present study demonstrated that S1PR2 inhibition significantly increased VEGF and eNOS expression levels, and decreased the expression of iNOS and Flt-1 in placental tissues.

It has been reported in previous literature that oxidative stress is higher in women with PE, and at the same time, the relationship between PE and the inflammatory response has also attracted more attention (31). Notably, it has been reported that oxidative stress and inflammation increase in PE, and it may be a cause and consequence of the cellular pathology (32,33). PE is the excessive inflammatory response of women to pregnancy. Generally, there are different degrees of inflammatory responses in patients with PE and normal pregnancy, but the inflammatory reaction in PE is overactivated, and the level of inflammatory factors is significantly higher than that in women undergoing a normal

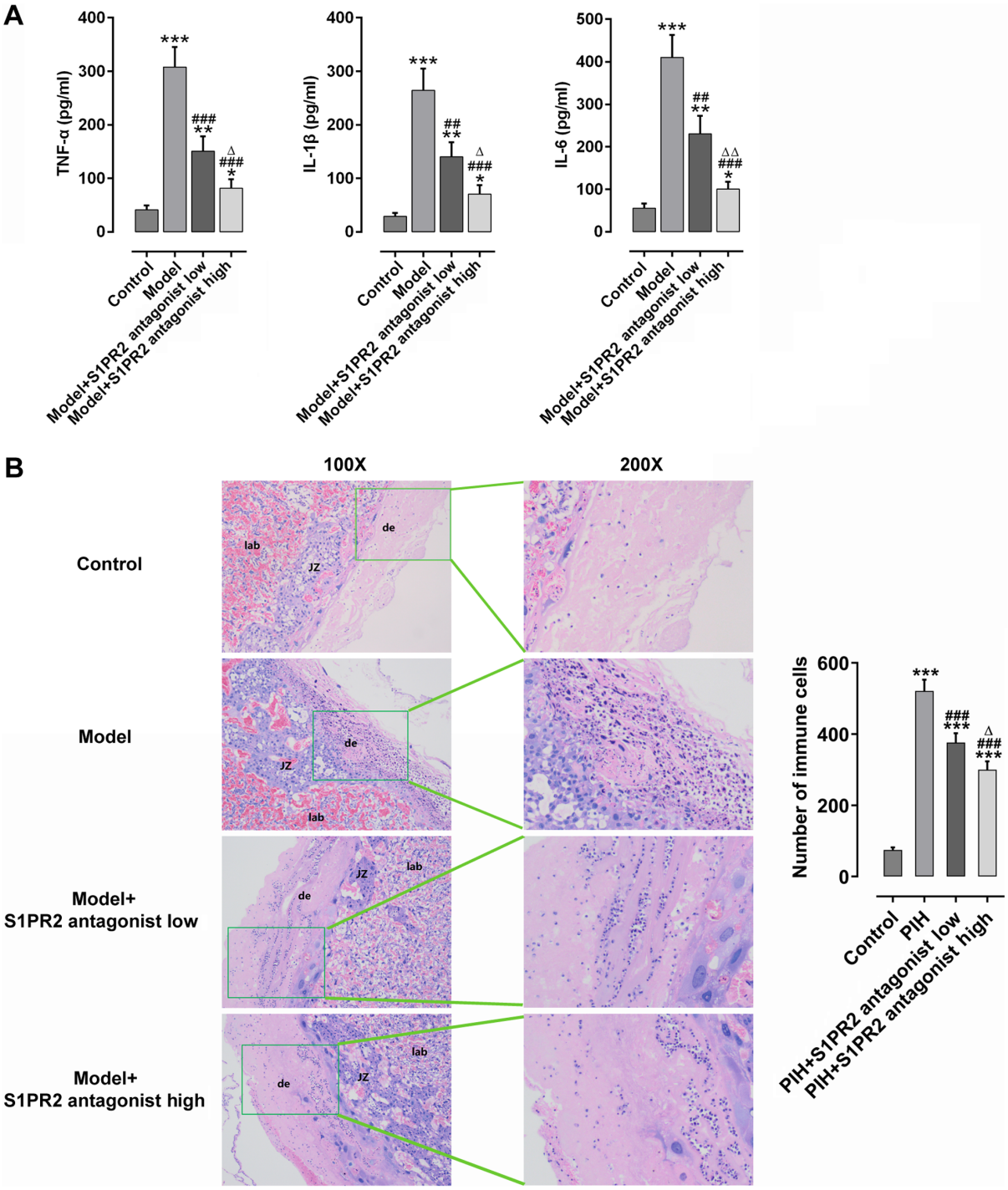

Figure 5. JTE-013 attenuates pathological changes in placental tissues and decreases inflammation in PE rats. (A) Summarized data showing the inhibitory effect of JTE-013 on the increased expression of serum TNF-α, IL-1β and IL-6, as determined by an enzyme-linked immunosorbent assay. (B) Summarized data showing JTE-013 attenuated the infiltration of inflammatory cells in placental tissues, as detected by hematoxylin and eosin staining (magnification, x100 or 200). n=3. *P<0.05, **P<0.01 and ***P<0.001 vs. Control group; $^{\#\#}$P<0.01 and $^{\#\#\#}$P<0.001 vs. Model group; $^{\Delta}$P<0.05 and $^{\Delta\Delta}$P<0.01 vs. Model + S1PR2 antagonist low group. PE, preeclampsia; TNF-α, tumor necrosis factor-α; IL-, interleukin; S1PR2, sphingosine-1-phosphate receptor 2; lab, labyrinth; JZ, junctional zone; de, decidua.

pregnancy (34). Serum levels of inflammatory mediators, such as IL-6, INF-γ, CRP and TNF-α, in patients with PE during early stages of pregnancy are significantly higher than those in healthy pregnant women (35). In the present study, it was also have found that the inflammatory cytokines (TNF-α, IL-1β and IL-6) were increased in PE rats, which was reversed by S1PR2 inhibition.

In conclusion, the results of the present study suggested that S1PR2 played a critical role in the initiation and development of PE by modulation of VEGF, eNOS and iNOS expression levels. Taken together, these results provide a novel pharmacological target for the prevention and treatment of PE. A key limitation of the present study is that Flt-1 expression was only detected in placental tissues, not in plasma.

The measured plasma Flt-1 level may further strengthen the present conclusion.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

TZ, DG and WZ acquired the data, confirmed the authenticity of all the raw data and contributed to the analysis and interpretation of data. TZ and QD contributed to the design of the study. TZ drafted the manuscript, which was revised by QD. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Institutional Animal Care and Use Committee of Shaoxing People's Hospital (Jinhua, China; approval no. 20190713).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. Obstet Gynecol 122: 1122-1131, 2013.
2. Shu W, Li H, Gong H, Zhang M, Niu X, Ma Y, Zhang X, Cai W, Yang G, Wei M, *et al*: Evaluation of blood vessel injury, oxidative stress and circulating inflammatory factors in an L-NAME-induced preeclampsia-like rat model. Exp Ther Med 16: 585-594, 2018.
3. Kemse NG, Kale AA and Joshi SR: Supplementation of maternal omega-3 fatty acids to pregnancy induced hypertension Wistar rats improves IL10 and VEGF levels. Prostaglandins Leukot Essent Fatty Acids 104: 25-32, 2016.
4. Kuklina EV, Ayala C and Callaghan WM: Hypertensive disorders and severe obstetric morbidity in the United States. Obstet Gynecol 113: 1299-1306, 2009.
5. Lisonkova S and Joseph KS: Incidence of preeclampsia: Risk factors and outcomes associated with early-versus late-onset disease. Am J Obstet Gynecol 209: 544.e1-544 e12, 2013.
6. Hladunewich M, Karumanchi SA and Lafayette R: Pathophysiology of the clinical manifestations of preeclampsia. Clin J Am Soc Nephrol 2: 543-549, 2007.
7. Roberts JM and Gammill HS: Preeclampsia: Recent insights. Hypertension 46: 1243-1249, 2005.
8. Llurba E, Crispi F and Verlohren S: Update on the pathophysiological implications and clinical role of angiogenic factors in pregnancy. Fetal Diagn Ther 37: 81-92, 2015.
9. Verlohren S, Stepan H and Dechend R: Angiogenic growth factors in the diagnosis and prediction of pre-eclampsia. Clin Sci (Lond) 122: 43-52, 2012.
10. Maynard SE and Karumanchi SA: Angiogenic factors and preeclampsia. Semin Nephrol 31: 33-46, 2011.
11. Henao DE and Saleem MA: Proteinuria in preeclampsia from a podocyte injury perspective. Curr Hypertens Rep 15: 600-605, 2013.
12. Roberts JM and Escudero C: The placenta in preeclampsia. Pregnancy Hypertens 2: 72-83, 2012.
13. Chen W, Xiang H, Chen R, Yang J, Yang X, Zhou J, Liu H, Zhao S, Xiao J, Chen P, *et al*: S1PR2 antagonist ameliorate high glucose-induced fission and dysfunction of mitochondria in HRGECs via regulating ROCK1. BMC Nephrol 20: 135, 2019.
14. Cheng JC, Wang EY, Yi Y, Thakur A, Tsai SH and Hoodless PA: S1P stimulates proliferation by upregulating CTGF expression through S1PR2-Mediated YAP activation. Mol Cancer Res 16: 1543-1555, 2018.
15. Sanchez T and Hla T: Structural and functional characteristics of S1P receptors. J Cell Biochem 92: 913-922, 2004.
16. Chen J, Tang H, Sysol JR, Moreno-Vinasco L, Shioura KM, Chen T, Gorshkova I, Wang L, Huang LS, Usatyuk PV, *et al*: The sphingosine kinase 1/sphingosine-1-phosphate pathway in pulmonary arterial hypertension. Am J Respir Crit Care Med 190: 1032-1043, 2014.
17. Ikeda H, Nagashima K, Yanase M, Tomiya T, Arai M, Inoue Y, Tejima K, Nishikawa T, Watanabe N, Omata M and Fujiwara K: Sphingosine 1-phosphate enhances portal pressure in isolated perfused liver via S1P2 with Rho activation. Biochem Biophys Res Commun 320: 754-759, 2004.
18. Schapiro SA and Everitt JI: Preparation of animals for use in the laboratory: Issues and challenges for the Institutional Animal Care and Use Committee (IACUC). ILAR J 47: 370-375, 2006.
19. LaMarca BB, Bennett WA, Alexander BT, Cockrell K and Granger JP: Hypertension produced by reductions in uterine perfusion in the pregnant rat: Role of tumor necrosis factor-alpha. Hypertension 46: 1022-1025, 2005.
20. Chawla S, Rahar B and Saxena S: S1P prophylaxis mitigates acute hypobaric hypoxia-induced molecular, biochemical, and metabolic disturbances: A preclinical report. IUBMB Life 68: 365-375, 2016.
21. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
22. Westwood M, Al-Saghir K, Finn-Sell S, Tan C, Cowley E, Berneau S, Adlam D and Johnstone ED: Vitamin D attenuates sphingosine-1-phosphate (S1P)-mediated inhibition of extravillous trophoblast migration. Placenta 60: 1-8, 2017.
23. Dobierzewska A, Palominos M, Sanchez M, Dyhr M, Helgert K, Venegas-Araneda P, Tong S and Illanes SE: Impairment of Angiogenic Sphingosine Kinase-1/Sphingosine-1-Phosphate receptors pathway in preeclampsia. PLoS One 11: e0157221, 2016.
24. Pavlov N, Frendo JL, Guibourdenche J, Degrelle SA, Evain-Brion D and Badet J: Angiogenin expression during early human placental development; association with blood vessel formation. Biomed Res Int 2014: 781632, 2014.
25. Santos TC, Oliveira MF, Papa PC, Dantzer V and Miglino MA: VEGF system expression by immunohistochemistry and real-time RT-PCR study on collared peccary placenta. Theriogenology 82: 834-843, 2014.
26. Xu Y, Su Z, Li J, Wang Q, Meng G, Zhang Y, Yang W, Zhang J and Gao P: Role of RNA-binding protein 5 in the diagnosis and chemotherapeutic response of lung cancer. Oncol Lett 17: 2013-2019, 2019.
27. Adu-Bonsaffoh K, Antwi DA, Gyan B and Obed SA: Endothelial dysfunction in the pathogenesis of pre-eclampsia in Ghanaian women. BMC Physiol 17: 5, 2017.
28. Luttun A and Carmeliet P: Soluble VEGF receptor Flt1: The elusive preeclampsia factor discovered? J Clin Invest 111: 600-602, 2003.
29. Motta-Mejia C, Kandzija N, Zhang W, Mhlomi V, Cerdeira AS, Burdujan A, Tannetta D, Dragovic R, Sargent IL, Redman CW, *et al*: Placental vesicles carry active endothelial nitric oxide synthase and their activity is reduced in preeclampsia. Hypertension 70: 372-381, 2017.
30. Amaral LM, Pinheiro LC, Guimaraes DA, Palei AC, Sertório JT, Portella RL and Tanus-Santos JE: Antihypertensive effects of inducible nitric oxide synthase inhibition in experimental pre-eclampsia. J Cell Mol Med 17: 1300-1307, 2013.

31. Ramos JGL, Sass N and Costa SHM: Preeclampsia. Rev Bras Ginecol Obstet 39: 496-512, 2017.
32. Terlecky SR, Terlecky LJ and Giordano CR: Peroxisomes, oxidative stress, and inflammation. World J Biol Chem 3: 93-97, 2012.
33. Roy S, Dhobale M, Dangat K, Mehendale S, Lalwani S and Joshi S: Differential oxidative stress levels in mothers with preeclampsia delivering male and female babies. J Matern Fetal Neonatal Med 28: 1973-1980, 2015.
34. Raio L, Bersinger NA, Malek A, Schneider H, Messerli FH, Hürter H, Rimoldi SF and Baumann MU: Ultra-high sensitive C-reactive protein during normal pregnancy and in preeclampsia: A pilot study. J Hypertens 37: 1012-1017, 2019.
35. Black KD and Horowitz JA: Inflammatory markers and preeclampsia: A systematic review. Nurs Res 67: 242-251, 2018.