The potential mechanism of transient receptor potential vanilloid type 1 combined with ATP-sensitive potassium channel in severe preeclampsia

Xianyi Zhou[1], Wei Li [1], Hairui Lin[1], Yingjun Tan[1], and Xiaodong Fu[1]

[1]The Affiliated Hospital of Southwest Medical University

April 05, 2024

Abstract

Objective To investigate the potential mechanism of transient receptor potential vanilloid type 1 (TRPV1) and ATP-sensitive potassium channel (KATP) in severe preeclampsia. Design Basic research. Setting The pathogenesis of severe preeclampsia. Samples Human placental arterioles and HUVECs. Methods (1) Tissue level: The samples were divided into the normotensive pregnancy (NP) group and severe preeclampsia (SP) group. Hematoxylin-eosin staining, quantitative PCR, Western blotting and immunohistochemistry were performed. (2) Cellular level: The samples were divided into the control group, TRPV1 agonist group (capsaicin), and TRPV1 inhibitor group (capsazepine), quantitative PCR and Western blotting were performed. Main Outcome Measures The relative expression of TRPV1, KATP subtype Kir6.1/SUR2B, nitric oxide synthase (eNOS) in placental arterioles ; the relative expression of TRPV1, Kir6.1/SUR2B, and eNOS in the cell groups. Results (1) The endothelial cell layer in the SP group was obviously damaged. The relative gene and protein expression of TRPV1, Kir6.1, SUR2B and eNOS was significantly lower than that in the NP group ($P<0.01$). (2) The relative gene and protein expression of TRPV1, Kir6.1, SUR2B and eNOS in the control group were significantly lower than those in the capsaicin group ($P<0.01$) and were significantly higher than those in the capsazepine group ($P<0.01$). Conclusion TRPV1 plus the downregulation of KATP downregulates the expression of eNOS, reducing vasodilation, which may be critical in the pathogenesis of severe preeclampsia. Funding Luzhou Science and Technology Bureau (2020-SYF-27). Key words: Transient receptor potential vanilloid type 1; ATP-sensitive potassium channel; Endothelial nitric oxide synthase; Nitric oxide; Severe preeclampsia

Title Page

The potential mechanism of transient receptor potential vanilloid type 1 combined with ATP-sensitive potassium channelin severe preeclampsia

Running head:mechanism of TRPV1 combined with KATP in SP

Zhou Xianyi[a], Li Wei[b], Lin Hairui[a], Tan Yingyun[a], Fu Xiaodong[a]*

[a]Department of Obstetrics, The Affiliated Hospital of Southwest Medical University, Luzhou, 646000, Sichuan, China

[b]Division of Neonatology, Department of Pediatrics, The Affiliated Hospital of Southwest Medical University, Luzhou, 646000, Sichuan, China.

XiaoDong Fu[a] *- Corresponding author:

Address: Department of Obstetrics, the Affiliated Hospital of Southwest Medical University, No. 8, Section 2, Kangcheng Road, Luzhou, Sichuan, 646000, China

Phone and fax numbers: +8618090182528, 0830-3161658

E-mail:dongerfu@163.com

Abstract

Objective

To investigate the potential mechanism of transient receptor potential vanilloid type 1 (TRPV1) and ATP-sensitive potassium channel (KATP) in severe preeclampsia.

Design

Basic research.

Setting

The pathogenesis of severe preeclampsia.

Samples

Human placental arterioles and HUVECs.

Methods

(1) Tissue level: The samples were divided into the normotensive pregnancy (NP) group and severe preeclampsia (SP) group. Hematoxylin-eosin staining, quantitative PCR, Western blotting and immunohistochemistry were performed.

(2) Cellular level: The samples were divided into the control group, TRPV1 agonist group (capsaicin), and TRPV1 inhibitor group (capsazepine), quantitative PCR and Western blotting were performed.

Main Outcome Measures

The relative expression of TRPV1, KATP subtype Kir6.1/SUR2B, nitric oxide synthase (eNOS) in placental arterioles ; the relative expression of TRPV1, Kir6.1/SUR2B, and eNOS in the cell groups.

Results

(1) The endothelial cell layer in the SP group was obviously damaged. The relative gene and protein expression of TRPV1, Kir6.1, SUR2B and eNOS was significantly lower than that in the NP group ($P<0.01$).

(2) The relative gene and protein expression of TRPV1, Kir6.1, SUR2B and eNOS in the control group were significantly lower than those in the capsaicin group ($P<0.01$) and were significantly higher than those in the capsazepine group ($P<0.01$).

Conclusion

TRPV1 plus the downregulation of KATP downregulates the expression of eNOS, reducing vasodilation, which may be critical in the pathogenesis of severe preeclampsia.

Funding

Luzhou Science and Technology Bureau (2020-SYF-27).

Key words: Transient receptor potential vanilloid type 1; ATP-sensitive potassium channel; Endothelial nitric oxide synthase; Nitric oxide; Severe preeclampsia

Tweetable abstract

By measuring the relative expression of TRPV1, KATP subtype SUR2B/Kir6.1 and nitric oxide synthase (eNOS) in the placental arteriosa in normal pregnancy and severe preeclampsia and by culturing human umbilical vein endothelial cells and measuring the relative expression of TRPV1, SUR2B/Kir6.1 and eNOS after the addition of TRPV1 agonists and inhibitors, the results showed that TRPV1 combined with the

downregulation of KATP expression downregulates the expression of eNOS, reducing the synthesis and release of NO and reducing vasodilation, which may be critical in the pathogenesis of severe preeclampsia.

Text

Introduction

Severe preeclampsia develops as a result of persistent elevated blood pressure and/or urinary protein levels, the progression of maternal organ function or placental-fetal complications based on preeclampsia[1]. The pathogenesis of this disease has not been fully elucidated. The basic pathophysiological changes are systemic vasospasm and endothelial injury, but the pathogenesis of severe preeclampsia caused by vascular endothelial dysfunction is still unclear[2] . Transient receptor potential vanilloid type 1 (TRPV1) has attracted increasing attention in the study of the cardiovascular system in recent years[3-5] . A recent study showed that dietary capsaicin could activate TRPV1 on endothelial cells and promote the eNOS/NO pathway, which could enhance endothelial production of nitric oxide (NO) and reduce blood pressure in rats with spontaneous hypertension [6] . ATP-sensitive potassium channel (KATP), a specific type of voltage-dependent potassium ion channel, is composed of inwardly rectifying potassium channel (Kir) and sulfonylurea receptor (SUR), and the SUR2B/Kir6.1 subtype is also known as the vascular type [7] . Studies have shown that the KATP channel-specific agonist etakarin not only directly relaxes vascular smooth muscle but also acts on endothelial cells to increase eNOS expression and promote the synthesis and release of NO[8, 9] . At present, correlations between transient receptor potential channels and potassium ion channels have been reported [4, 10, 11] . A study confirmed the involvement of large-conductance K^+(BKca) in coronary endothelium-dependent relaxation mediated by TRPV1[4]; however, there have been few reports on TRPV1 and the KATP subtype SUR2B/Kir6.1 in severe preeclampsia. Since the placenta is an important tissue connecting the fetus and the mother and this disease can be rapidly alleviated after delivery of the placenta, the placenta may play an important role in the development of this disease. As one of the vasoactive substances regulating blood flow homeostasis in the body, NO must also be one of the important factors regulating blood flow perfusion in placental arterioles. Therefore, this study aimed to confirm whether severe preeclampsia is caused by impaired expression of TRPV1 and KATP in placental arterioles, leading to impaired eNOS/NO pathway activity, resulting in endothelial dysfunction and decreased diastolic function, and promoting the development of severe preeclampsia.

Methods

Management of human placental arterioles

This study was approved by the Clinical Trial Ethics Committee of the Affiliated Hospital of Southwest Medical University (registration number: KY2019039). All work was undertaken according the provisions of the Declaration of Helsinki. All patients signed informed consent for specimen collection. Ten single pregnant women diagnosed with severe preeclampsia were selected as the experimental group (SP), and 10 single pregnant women with normotensive pregnancy were chosen as the control group (NP). All patients were hospitalized and delivered by either vaginal delivery or cesarean section in the Department of Obstetrics in our hospital from May 2020 to May 2021. Inclusion criteria for the SP group were based on the American College of Obstetricians and Gynecologists (ACOG) Guidelines[12]. All subjects were excluded from the study if they had chronic hypertension, kidney disease, cardio-cerebrovascular disease, severe liver and kidney function impairment, other basic diseases, systemic diseases or other pregnancy complications. Patients who had histories of smoking, alcohol abuse, syphilis, hepatitis virus and human immunodeficiency virus were also excluded. We collected patient demographic and clinical characteristics, such as maternal age, gestational age and blood pressure (Table 1). After the placenta was delivered, the fetal membrane was immediately stripped under sterile conditions, and 3-5 pieces of placental tissue with small placental arterioles near the edge of the placenta, approximately 4*1*1 cm^3 in size, were extracted and placed in the prepared specimen box, which was immediately transferred to the laboratory under low temperature. Then, the perivascular connective tissue was rapidly and gently removed under a microscope, taking care to minimize the damage

to the blood vessels, and vessels with diameters of approximately 0.1-0.2 cm and lengths of approximately 2-3 cm were separated. Some of the isolated blood vessels were frozen in liquid nitrogen and then quickly transferred to a freezer at -80 °C for cryopreservation, and these samples were used for subsequent PCR and Western blot analyses. The rest were fixed in 4% paraformaldehyde, and the specimens were used for subsequent HE staining and immunohistochemical analysis.

Hematoxylin-eosin staining of placental arterioles

After fixation, the placental arterioles were dehydrated by an ethanol gradient and cleared with xylene. The transparent tissue blocks were embedded in melted paraffin, and the tissue blocks were then sliced and baked after being cooled and solidified. The dried slices were dewaxed by xylene, dehydrated by gradient ethanol, stained by hematoxylin and eosin and finally dehydrated, made transparent, sealed and imaged.

Human umbilical vein endothelial cell culture

After resuscitation, HUVECs (Fuxiang Biotechnology Co., LTD, Shanghai, CN) were cultured in high-glucose DMEM containing 10% fetal bovine serum and 1% penicillin and streptomycin at 5% CO_2 and 37 °C in a humidified chamber. HUVECs at the logarithmic growth stage were subcultured and inoculated in six-well plates. The cells were divided into the control group, capsaicin group and capsazepine (MCE, USA) group. After approximately 80% of the cells were fused, high-glucose DMEM containing capsaicin and capsazepine, which were prepared in DMSO at a final concentration of 1 μmol/L, was administered, and ordinary high-sugar DMEM containing an equal volume of DMSO was administered to the control group. After 24 hours of incubation, total RNA and protein were extracted from the cells.

Quantitative PCR

Total RNA was extracted from tissues and cells with TRIzol reagent (Tiangen Biotech, Beijing, CN), the absorbance of the extracted RNA was measured with a spectrophotometer (Bio–Rad Laboratories, West Berkeley, California, USA), and the integrity was examined by gel electrophoresis (Bio–Rad Laboratories, West Berkeley, California, USA). RNA with an A260/A280 ratio between 1.8-2.0 and three complete bands of 28S, 18S and 5S after 2% agarose gel electrophoresis was chosen for reverse transcription (Fig. 1A and B). Taq PCR Mastermix (Tiangen Biotech, Beijing, CN) was used for reverse transcription, and SYBR Green chemistry (Toyobo Co., LTD, Japan) was used for real-time PCR. Using the GAPDH gene as an internal reference, the reaction system and parameters were set according to the instructions. The internal reference and target gene primers were designed and synthesized by Shanghai Sangon Biological Co, Ltd (Table 2). Finally, the relative expression of each target gene was calculated by the following formula: 2^{-t} (Ct value is the circulating domain value). The experiment was repeated three times.

Western blotting

The total proteins were extracted from the tissue and cell groups with RIPA buffer (Biyuntian Biotechnology Co., LTD, Shanghai, CN) containing the protease inhibitor phenylmethanesulfonyl fluoride (PMFF, Biyuntian Biotechnology Co., Ltd, Shanghai, CN). After the protein concentration was measured by a BCA protein assay kit (Biyuntian Biotechnology Co., LTD, Shanghai, CN) and the samples were denatured, 50 μg of protein per sample were separated by electrophoresis. Then, the proteins were transferred to a polyvinylidene difluoride (PVDF) membrane, and the membrane was sealed in 5% skim milk powder dissolved in 0.5% Tween-TBS for 2 h at room temperature. After the membrane was washed, GAPDH (1:10000), TRPV1 (1:1000), SUR2B (1:500), Kir6.1 (1:1000) and eNOS (1:1000) primary antibodies were added and incubated overnight at 4 on a shaker. The next day, after the membrane was washed, horseradish peroxidase-labeled secondary antibodies (1:10000) were added and incubated at 37 for 2 h. Finally, the proteins were detected by chemiluminescence assays, GAPDH was used as an internal reference to analyze the optical density values of the target bands, and the ratio of the gray value of each target protein to GAPDH was used to calculate the relative expression level of the target protein. The experiment was repeated three times.

Immunohistochemistry

After being baked at 60 °C for 2 hours, the paraffin sections were dewaxed and hydrated in turn, washed with PBS and incubated with 3% hydrogen peroxide for 5-10 minutes (at room temperature) to eliminate the activity of endogenous peroxidase. After antigen repair, goat serum was added and incubated at room temperature for 15 minutes. The primary antibody working solution containing TRPV1 (1:200), Kir6.1 (1:200), SUR2B (1:200), and eNOS (1:200) was added to cover the tissue, and the tissue was incubated at 4 °C overnight. After being rinsed, the secondary antibody was diluted with PBS, added and incubated at room temperature for 2 hours. Then, DBA reagent was used for color development, the samples were stained again with hematoxylin and subjected hydrochloric acid alcohol differentiation, dehydration, transparency, and sealing with neutral gum. Then, the images were observed and photographed under a microscope. The expression of various proteins in endothelial cells was mainly observed. Brown–yellow staining indicated positive expression, and the relative expression level is represented by the optical density value. The images were analyzed by Q-IMAGING software.

Statistical analysis

All data were analyzed with SPSS 20.0 and GraphPad Prism 5.0, and normally distributed data are presented as the mean ± SD. Independent sample t tests were used to compare the NP and SP groups, one-way ANOVA was used to compare cell groups, and a P value <0.05 was considered statistically significant.

Results

Impaired endothelial layers in severe preeclampsia

The results are shown in Figure 2. Endothelial cells in the NP group were uniformly and regularly arranged, and the nucleus was oblong, bluish and protruded slightly from the official cavity (Fig. 2A). In the SP group, the walls of placental arterioles were thickened, collagen fibers were increased, the arterioles were hyalinized, the endothelial cells were disorderly arranged, and the endodermis was obviously damaged (Fig. 2B).

Reduced TRPV1, Kir6.1/SUR2B, and eNOS expression in severe preeclampsia

The localization of the TRPV1, Kir6.1/SUR2B, and eNOS channels was evaluated in placental arterioles by immunohistochemistry (Fig. 3), and the results showed that each channel was mainly distributed in the vascular endothelial layer. It was noted that the counterstained nuclei in the endothelial cell layer was significantly decreased, as was membrane staining of each channel in the SP group compared with the NP group. The optical densities of TRPV1, Kir6.1/SUR2B, eNOS in the SP group were 11660.0±1721.0, 2975.0±505.5, 10236.0±1355.0, 14663.0±2320.0, respectively, which were lower than those in the NP group (24917.0±2044.0, 4495.0±775.0, 14663.0±2320.0, 20988.0±2289.0, $P<0.01$) (Fig. 3). We performed quantitative PCR to examine the relative expression of the four channel genes. The relative expression of TRPV1, Kir6.1, SUR2B and eNOS in the SP group was 0.559±0.609, 0.419±0.281, 0.166±0.087 and 0.383±0.110, respectively, which was significantly lower than that in the NP group (1.098±0.341, 1.024±0.085, 1.243±0.335 and 1.219±0.247, $P<0.01$) (Fig. 4). Since gene expression does not fully reflect protein expression, we performed Western blotting to quantitatively examine whether this difference was also present at the protein level. The relative quantitative analysis results (Fig. 5) showed that the ratio of the gray value of each target protein to GAPDH in the SP group was 0.282±0.058, 0.058±0.005, 0.132±0.007, 0.059±0.023, respectively, which was lower than that in the NP group (0.688±0.145, 0.196±0.010, 0.514±0.018, 0.327±0.063, $P<0.01$), suggesting that the relative protein expression of TRPV1, KATP subtype Kir6.1/SUR2B and eNOS in the SP group was also significantly downregulated.

The roles ofeNOS and Kir6.**1/SUR2B channels in capsaicin/capsazepine-induced relaxation in human umbilical vein endothelial cells**

To explore the interaction of the four channels, we carried out further cytological experiments. The relative gene expression of TRPV1, Kir6.1, SUR2B and eNOS in the control group was 0.986±0.129, 1.439±0.358, 1.479±0.403 and 1.162±0.090, respectively; in the capsaicin group, it was 4.568±0.810, 4.014±0.781, 5.505±0.287 and 2.821±0.377, respectively; and in the capsazepine group, it was 0.077±0.010, 0.036±0.014, 0.046±0.010 and 0.083±0.005, respectively, and the differences were statistically significant ($P < 0.01$) (Fig.

6). The Western blot results showed that the relative protein expression of TRPV1, Kir6.1, SUR2B and eNOS in the control group was 0.266±0.026, 0.226±0.014, 0.166±0.013 and 0.277±0.025, respectively; expression in the capsaicin group was 0.763±0.044, 0.687±0.046, 0.493±0.035 and 0.498±0.022, respectively; and expression in the capsazepine group was 0.157±0.040, 0.057±0.005, 0.065±0.005 and 0.058±0.008, respectively ($P<0.01$), and these differences were statistically significant ($P<0.01$) (Fig. 7).

Discussion

The vascular endothelium is located throughout the body and acts as not only a mechanical barrier between blood and smooth muscle cells but also as the largest endocrine organ of the body, which can synthesize and release various vasoactive substances through various mechanisms to regulate vascular tension[13-15]. It is generally accepted that endothelial dysfunction promotes the occurrence of cardiovascular diseases such as hypertension and that the degree of endothelial injury is directly related to the severity of hypertension. In patients with hypertension, endothelial cells are damaged due to elevated blood pressure, which reduces the secretion of NO and other vasoactive substances, thus leading to endothelial-dependent diastolic dysfunction, which in turn becomes an initiating factor of various complications of hypertension, forming a vicious cycle[16]. Severe preeclampsia is a specific kind of hypertensive disease, and many studies have shown that NO levels in the serum of patients with preeclampsia are significantly downregulated, indicating that there are functional changes after vascular endothelial injury in preeclampsia [17-20]. Boeldt et al.[21] showed that maternal peripheral endothelial dysfunction is central to the disease stage of preeclampsia. A review of endothelial nitric oxide signaling in preeclampsia also concluded that preeclampsia was a complex obstetric syndrome associated with maternal vascular dysfunction in which the NO signaling pathway is a key driver of disease progression and severity[22]. Our study showed that the vascular walls of placental arterioles in the SP were thickened, collagen fibers were increased, arteriole hyaline was changed, and the endothelial cell layer was obviously damaged. In addition, other results showed that the relative expression of eNOS in the SP group was significantly downregulated, indirectly indicating that NO synthesis by endothelial cells was significantly decreased compared with that in women with normal term pregnancy. We hypothesize that the decreased expression of eNOS in the placental arteriole results in a decrease in NO synthesis, leading to endothelial diastolic dysfunction and vasospasm, both of which form a vicious cycle and participate in the changes of uterine placental ischemia and hypoxia and the release of a variety of placental factors, which enter maternal blood circulation, activate systemic inflammatory reaction, damage vascular endothelial cells, and eventually lead to hypertension and various complications, which is consistent with the classical theory of preeclampsia due to poor placentation**[23].** Since circulating NO reflects the total activity of all three nitric oxide synthase subtypes (not just that in endothelial cells) and NO levels are influenced by dietary intake, different studies have different results, and so we did not measure blood levels of NO in the two groups, which is where this experiment was limited.

TRPV1 was originally discovered by researchers to be specifically activated by capsaicin, which is why it is known as the capsaicin receptor [24]. As the most studied member of the TRPV subfamily, TRPV1 was originally identified in the nervous system and is present in the vagus nerve, trigeminal ganglion and dorsal ganglion neurons[25]. In recent years, an increasing number of studies have shown that TRPV1 also plays an important role in the regulation of cardiovascular disease and is mainly expressed in cardiomyocytes, smooth muscle cells, vascular endothelial cells, inflammatory cells and peripheral vascular adipose tissue[26-29]. TRPV1 is a nonselective cationic channel that can mediate the transmembrane flow of cations dominated by Ca^{2+} when ligands bind to the receptor, which can change intracellular Ca^{2+} concentrations, activate a series of intracellular signals and participate in a variety of intracellular physiological and pathological processes[26, 30]. The synthesis of NO is closely related to the increase in intracellular Ca^{2+}. Ca^{2+} binding to calmodulin in endothelial cells can activate eNOS and promote the synthesis and release of NO, thereby dilating blood vessels and reducing vascular resistance[31-33]. TRPV1 may play an important role in the physiological and pathological status of endothelial cells to maintain normal vascular function and the pathological process of vascular lesions. It has been confirmed in animal experiments that TRPV1 can mediate coronary artery relaxation in an endothelium-dependent manner[4], and TRPV1 can stimulate NO synthesis through different signaling pathways[6, 34]. It is well known that structural dysfunction of potassium

channels can disrupt the balance of vasoconstriction and the diastole of blood vessels themselves, increasing vascular tension and eventually leading to the pathological state of hypertension[35, 36]. The KATP channel is a potassium ion channel. For a long time, reports on KATP have mainly focused on vascular smooth muscle cells, and scholars later showed that KATP in endothelial cells also participates in the regulation of vascular tone[37]. Studies have shown that the KATP agonist etacarin can increase Ca^{2+}levels in endothelial cells, which can increase the expression of eNOS, promote the synthesis and release of NO, and indirectly relax smooth muscle[8, 9]. Bratz et al.[4] found that endothelium-dependent dilation mediated by TRPV1 could be attenuated by iberiotoxin, a selective inhibitor of Ca^{2+}-activated K channels (BKca), suggesting that BKca is involved in capsaicin-induced relaxation. In addition, TEA, a nonspecific potassium channel blocker, also attenuates TRPV1-mediated endothelium-dependent relaxation, suggesting that multiple potassium channels are involved in TRPV1-mediated endothelium-dependent relaxation. Based on this research, our study showed that TRPV1 and Kir6.1/SUR2B mainly existed in the endothelial cell layer in human placental arteriolar cells, and the relative gene and protein expression of TRPV1 and Kir6.1/SUR2B in the SP group was significantly downregulated compared with that in the NP group. We hypothesized that there might be some correlation between TRPV1 and the KATP subtype SUR2B/Kir6.1. To further verify this hypothesis, cell experiments were carried out. We cultured human umbilical vein endothelial cells and found that in the agonist and blocker groups, the relative expression of SUR2B/Kir6.1 and eNOS was also significantly upregulated and downregulated, respectively, compared with that in the control group, indicating that activation or inhibition of TRPV1 could upregulate or downregulate the expression of Kir6.1/SUR2B and eNOS. Referring to the previous theory, we hypothesize that TRPV1 activation in endothelial cells activates Kir6.1/SUR2B by some unknown mechanism, causing hyperdifferentiation of the endothelial membrane and thereby increasing Ca^{2+}influx into cells. As a nonselective cation channel, TRPV1 can also mediate the transmembrane flow of cations dominated by Ca^{2+} when the ligand binds to the receptor. Through two possible approaches, Ca^{2+} is further increased in endothelial cells, which enhances the expression and activity of eNOS, thereby increasing the synthesis and release of NO and the endothelium-dependent vasodilation response. In patients with severe preeclampsia, the expression of TRPV1 and Kir6.1/SUR2B is reduced due to endothelial cell damage, and the function of TRPV1 plus KATP and NO is also impaired, leading to endothelial dysfunction. In addition, NO diffusion to vascular smooth muscle is correspondently reduced, and vascular smooth muscle cannot relax properly, causing arteriole spasm in the body and insufficient blood supply to the placenta, leading to a series of maternal and infant complications. However, due to the limitations of experimental conditions, we did not conduct vascular ring tension tests and additional electrophysiological experiments, which was a great limitation of this study.

Conclusion

As a specific type of hypertensive disease during pregnancy, severe preeclampsia may be characterized by multiple factors, mechanisms and pathways. Our study investigated changes in the expression of TRPV1 and the KATP subtype SUR2B/Kir6.1 in placental arterioles in severe preeclampsia and the possible mechanism. However, further electrophysiological experiments are still necessary to further understand the etiology and pathogenesis of this disease and provide a new theoretical basis for the prevention, diagnosis and treatment of this disease.

Acknowledgements : Thanks to the medical Experiment Center platform of the Affiliated Hospital of Southwest Medical University for providing experimental site and experimental guidance.

Disclosure of Interests : The authors declare that they have no conflicts of interest.

Contribution to Authorship :

Zhou Xianyi: Conception and design, Acquisition of data, Analysis and Interpretation of data, Drafting of the manuscript, Statistical analysis.

Li Wei: Acquisition of data, Analysis and Interpretation of data, Drafting of the manuscript, Statistical analysis.

Lin Hairui: Conception and design, Acquisition of data, Analysis and Interpretation of data, Critical revision of the manuscript for important intellectual content , Statistical analysis.

Tan Yingyun: Conception and design, Acquisition of data, Analysis and Interpretation of data, Critical revision of the manuscript for important intellectual content , Statistical analysis.

Fu Xiaodong: Conception and design, Critical revision of the manuscript for important intellectual content, Obtaining funding, Supervision.

Details of Ethics Approval : This experiment was approved by the Clinical Trial Ethics Committee of the Affiliated Hospital of Southwest Medical University (registration number: KY2019039).

Funding : Luzhou Science and Technology Bureau: Expression and influence of KV7 channel in placental chorionic artery smooth muscle cells of pregnant women with fetal growth restriction due to preeclampsia (No.2020-SYF-27).

References :

1. Agrawal, A. and N.K. Wenger, *Hypertension During Pregnancy.*Curr Hypertens Rep[J], 2020. **22** (9): p. 64.

2. *Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy.* Obstet Gynecol[J], 2013. **122** (5): p. 1122-31.

3. Zhu, Z., Z. Luo, S. Ma, et al., *TRP channels and their implications in metabolic diseases.* Pflugers Arch[J], 2011.**461** (2): p. 211-23.

4. Bratz, I.N., G.M. Dick, J.D. Tune, et al., *Impaired capsaicin-induced relaxation of coronary arteries in a porcine model of the metabolic syndrome.* Am J Physiol Heart Circ Physiol[J], 2008.**294** (6): p. H2489-96.

5. Marshall, N.J., L. Liang, J. Bodkin, et al., *A role for TRPV1 in influencing the onset of cardiovascular disease in obesity.*Hypertension[J], 2013. **61** (1): p. 246-52.

6. Yang, D., Z. Luo, S. Ma, et al., *Activation of TRPV1 by dietary capsaicin improves endothelium-dependent vasorelaxation and prevents hypertension.* Cell Metab[J], 2010. **12** (2): p. 130-41.

7. Yokoshiki, H., M. Sunagawa, T. Seki, et al., *ATP-sensitive K+ channels in pancreatic, cardiac, and vascular smooth muscle cells.* Am J Physiol[J], 1998. **274** (1): p. C25-37.

8. Lückhoff, A. and R. Busse, *Activators of potassium channels enhance calcium influx into endothelial cells as a consequence of potassium currents.* Naunyn Schmiedebergs Arch Pharmacol[J], 1990.**342** (1): p. 94-9.

9. Lückhoff, A. and R. Busse, *Calcium influx into endothelial cells and formation of endothelium-derived relaxing factor is controlled by the membrane potential.* Pflugers Arch[J], 1990. **416** (3): p. 305-11.

10. Nelson, M.T., H. Cheng, M. Rubart, et al., *Relaxation of arterial smooth muscle by calcium sparks.* Science[J], 1995.**270** (5236): p. 633-7.

11. Guarini, G., V.A. Ohanyan, J.G. Kmetz, et al., *Disruption of TRPV1-mediated coupling of coronary blood flow to cardiac metabolism in diabetic mice: role of nitric oxide and BK channels.* Am J Physiol Heart Circ Physiol[J], 2012. **303** (2): p. H216-23.

12. Espinoza, J., A. Vidaeff, C.M. Pettker, et al., *Gestational Hypertension and Preeclampsia.* Obstetrics and Gynecology[J], 2020.**135** (6): p. E237-E260.

13. Moncada, S., R.M. Palmer, and E.A. Higgs, *The discovery of nitric oxide as the endogenous nitrovasodilator.* Hypertension[J], 1988. **12** (4): p. 365-72.

14. Sumpio, B.E., J.T. Riley, and A. Dardik, *Cells in focus: endothelial cell.* Int J Biochem Cell Biol[J], 2002.**34** (12): p. 1508-12.

15. Konukoglu, D. and H. Uzun, *Endothelial Dysfunction and Hypertension.* Adv Exp Med Biol[J], 2017. **956** : p. 511-540.

16. Al-Magableh, M.R., B.K. Kemp-Harper, and J.L. Hart, *Hydrogen sulfide treatment reduces blood pressure and oxidative stress in angiotensin II-induced hypertensive mice.* Hypertens Res[J], 2015.**38** (1): p. 13-20.

17. Ducat, A., L. Doridot, R. Calicchio, et al., *Endothelial cell dysfunction and cardiac hypertrophy in the STOX1 model of preeclampsia.*Sci Rep[J], 2016. **6** : p. 19196.

18. Pimentel, A.M.L., N.R. Pereira, C.A. Costa, et al.,*L-arginine-nitric oxide pathway and oxidative stress in plasma and platelets of patients with pre-eclampsia.* Hypertension Research[J], 2013. **36** (9): p. 783-788.

19. Eleuterio, N.M., A.C.T. Palei, J.S.R. Machado, et al.,*Relationship between adiponectin and nitrite in healthy and preeclampsia pregnancies.* Clinica Chimica Acta[J], 2013.**423** : p. 112-115.

20. Sandrim, V.C., A.C.T. Palei, I.F. Metzger, et al., *Nitric oxide formation is inversely related to serum levels of antiangiogenic factors soluble fms-like tyrosine kinase-1 and soluble endogline in preeclampsia.* Hypertension[J], 2008. **52** (2): p. 402-407.

21. Boeldt, D.S. and I.M. Bird, *Vascular adaptation in pregnancy and endothelial dysfunction in preeclampsia.* Journal of Endocrinology[J], 2017. **232** (1): p. R27-R44.

22. Osol, G., N.L. Ko, and M. Mandalà, *Altered Endothelial Nitric Oxide Signaling as a Paradigm for Maternal Vascular Maladaptation in Preeclampsia.* Curr Hypertens Rep[J], 2017. **19** (10): p. 82.

23. Brosens, I., *A STUDY OF THE SPIRAL ARTERIES OF THE DECIDUA BASALIS IN NORMOTENSIVE AND HYPERTENSIVE PREGNANCIES.* The Journal of obstetrics and gynaecology of the British Commonwealth[J], 1964.**71** : p. 222-30.

24. Caterina, M.J., M.A. Schumacher, M. Tominaga, et al., *The capsaicin receptor: a heat-activated ion channel in the pain pathway.*Nature[J], 1997. **389** (6653): p. 816-24.

25. Caterina, M.J., *Vanilloid receptors take a TRP beyond the sensory afferent.* Pain[J], 2003. **105** (1-2): p. 5-9.

26. Yang, X.R., M.J. Lin, L.S. McIntosh, et al., *Functional expression of transient receptor potential melastatin- and vanilloid-related channels in pulmonary arterial and aortic smooth muscle.* Am J Physiol Lung Cell Mol Physiol[J], 2006.**290** (6): p. L1267-76.

27. Zsombok, A., *Vanilloid receptors–do they have a role in whole body metabolism? Evidence from TRPV1.* J Diabetes Complications[J], 2013. **27** (3): p. 287-92.

28. Gunthorpe, M.J. and A. Szallasi, *Peripheral TRPV1 receptors as targets for drug development: new molecules and mechanisms.* Curr Pharm Des[J], 2008. **14** (1): p. 32-41.

29. Xin, H., H. Tanaka, M. Yamaguchi, et al., *Vanilloid receptor expressed in the sarcoplasmic reticulum of rat skeletal muscle.* Biochem Biophys Res Commun[J], 2005. **332** (3): p. 756-62.

30. Song, M.Y. and J.X. Yuan, *Introduction to TRP channels: structure, function, and regulation.* Adv Exp Med Biol[J], 2010.**661** : p. 99-108.

31. Cai, H., M.E. Davis, G.R. Drummond, et al., *Induction of endothelial NO synthase by hydrogen peroxide via a Ca(2+)/calmodulin-dependent protein kinase II/janus kinase 2-dependent pathway.* Arterioscler Thromb Vasc Biol[J], 2001. **21** (10): p. 1571-6.

32. Zhang, M. and H.J. Vogel, *Characterization of the calmodulin-binding domain of rat cerebellar nitric oxide synthase.* J Biol Chem[J], 1994. **269** (2): p. 981-5.

33. Zhang, M., T. Yuan, J.M. Aramini, et al., *Interaction of calmodulin with its binding domain of rat cerebellar nitric oxide synthase. A multinuclear NMR study.* J Biol Chem[J], 1995.**270** (36): p. 20901-7.

34. Torres-Narváez, J.C., I. Pérez-Torres, V. Castrejón-Téllez, et al., *The Role of the Activation of the TRPV1 Receptor and of Nitric Oxide in Changes in Endothelial and Cardiac Function and Biomarker Levels in Hypertensive Rats.* Int J Environ Res Public Health[J], 2019. **16** (19).

35. Nieves-Cintrón, M., A.U. Syed, M.A. Nystoriak, et al.,*Regulation of voltage-gated potassium channels in vascular smooth muscle during hypertension and metabolic disorders.*Microcirculation[J], 2018. **25** (1).

36. Jackson, W.F., *K(V) channels and the regulation of vascular smooth muscle tone.* Microcirculation[J], 2018. **25** (1).

37. Wu, Y., M.Y. He, J.K. Ye, et al., *Activation of ATP-sensitive potassium channels facilitates the function of human endothelial colony-forming cells via Ca(2+) /Akt/eNOS pathway.* J Cell Mol Med[J], 2017. **21** (3): p. 609-620.

1. Agrawal, A. and N.K. Wenger, *Hypertension During Pregnancy.*Curr Hypertens Rep, 2020. **22** (9): p. 64.

2. *Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy.* Obstet Gynecol, 2013. **122** (5): p. 1122-31.

3. Zhu, Z., Z. Luo, S. Ma, and D. Liu, *TRP channels and their implications in metabolic diseases.* Pflugers Arch, 2011.**461** (2): p. 211-23.

4. Bratz, I.N., G.M. Dick, J.D. Tune, J.M. Edwards, Z.P. Neeb, U.D. Dincer, and M. Sturek, *Impaired capsaicin-induced relaxation of coronary arteries in a porcine model of the metabolic syndrome.* Am J Physiol Heart Circ Physiol, 2008. **294** (6): p. H2489-96.

5. Marshall, N.J., L. Liang, J. Bodkin, C. Dessapt-Baradez, M. Nandi, S. Collot-Teixeira, . . . S.D. Brain, *A role for TRPV1 in influencing the onset of cardiovascular disease in obesity.* Hypertension, 2013.**61** (1): p. 246-52.

6. Yang, D., Z. Luo, S. Ma, W.T. Wong, L. Ma, J. Zhong, . . . Z. Zhu,*Activation of TRPV1 by dietary capsaicin improves endothelium-dependent vasorelaxation and prevents hypertension.* Cell Metab, 2010. **12** (2): p. 130-41.

7. Yokoshiki, H., M. Sunagawa, T. Seki, and N. Sperelakis,*ATP-sensitive K+ channels in pancreatic, cardiac, and vascular smooth muscle cells.* Am J Physiol, 1998. **274** (1): p. C25-37.

8. Lückhoff, A. and R. Busse, *Activators of potassium channels enhance calcium influx into endothelial cells as a consequence of potassium currents.* Naunyn Schmiedebergs Arch Pharmacol, 1990.**342** (1): p. 94-9.

9. Lückhoff, A. and R. Busse, *Calcium influx into endothelial cells and formation of endothelium-derived relaxing factor is controlled by the membrane potential.* Pflugers Arch, 1990. **416** (3): p. 305-11.

10. Nelson, M.T., H. Cheng, M. Rubart, L.F. Santana, A.D. Bonev, H.J. Knot, and W.J. Lederer, *Relaxation of arterial smooth muscle by calcium sparks.* Science, 1995. **270** (5236): p. 633-7.

11. Guarini, G., V.A. Ohanyan, J.G. Kmetz, D.J. DelloStritto, R.J. Thoppil, C.K. Thodeti, . . . I.N. Bratz, *Disruption of TRPV1-mediated coupling of coronary blood flow to cardiac metabolism in diabetic mice: role of nitric oxide and BK channels.* Am J Physiol Heart Circ Physiol, 2012. **303** (2): p. H216-23.

12. Espinoza, J., A. Vidaeff, C.M. Pettker, H. Simhan, and G. Amer Coll Obstet, *Gestational Hypertension and Preeclampsia.* Obstetrics and Gynecology, 2020. **135** (6): p. E237-E260.

13. Moncada, S., R.M. Palmer, and E.A. Higgs, *The discovery of nitric oxide as the endogenous nitrovasodilator.* Hypertension, 1988.**12** (4): p. 365-72.

14. Sumpio, B.E., J.T. Riley, and A. Dardik, *Cells in focus: endothelial cell.* Int J Biochem Cell Biol, 2002. **34** (12): p. 1508-12.

15. Konukoglu, D. and H. Uzun, *Endothelial Dysfunction and Hypertension.* Adv Exp Med Biol, 2017. **956** : p. 511-540.

16. Al-Magableh, M.R., B.K. Kemp-Harper, and J.L. Hart, *Hydrogen sulfide treatment reduces blood pressure and oxidative stress in angiotensin II-induced hypertensive mice.* Hypertens Res, 2015.**38** (1): p. 13-20.

17. Ducat, A., L. Doridot, R. Calicchio, C. Mehats, J.L. Vilotte, J. Castille, . . . D. Vaiman, *Endothelial cell dysfunction and cardiac hypertrophy in the STOX1 model of preeclampsia.* Sci Rep, 2016.**6** : p. 19196.

18. Pimentel, A.M.L., N.R. Pereira, C.A. Costa, G.E. Mann, V.S.C. Cordeiro, R.S. de Moura, . . . A.C. Resende, *L-arginine-nitric oxide pathway and oxidative stress in plasma and platelets of patients with pre-eclampsia.* Hypertension Research, 2013. **36** (9): p. 783-788.

19. Eleuterio, N.M., A.C.T. Palei, J.S.R. Machado, J.E. Tanus-Santos, R.C. Cavalli, and V.C. Sandrim, *Relationship between adiponectin and nitrite in healthy and preeclampsia pregnancies.* Clinica Chimica Acta, 2013. **423** : p. 112-115.

20. Sandrim, V.C., A.C.T. Palei, I.F. Metzger, V.A. Gomes, R.C. Cavalli, and J.E. Tanus-Santos, *Nitric oxide formation is inversely related to serum levels of antiangiogenic factors soluble fms-like tyrosine kinase-1 and soluble endogline in preeclampsia.* Hypertension, 2008.**52** (2): p. 402-407.

21. Boeldt, D.S. and I.M. Bird, *Vascular adaptation in pregnancy and endothelial dysfunction in preeclampsia.* Journal of Endocrinology, 2017. **232** (1): p. R27-R44.

22. Osol, G., N.L. Ko, and M. Mandalà, *Altered Endothelial Nitric Oxide Signaling as a Paradigm for Maternal Vascular Maladaptation in Preeclampsia.* Curr Hypertens Rep, 2017. **19** (10): p. 82.

23. Brosens, I., *A STUDY OF THE SPIRAL ARTERIES OF THE DECIDUA BASALIS IN NORMOTENSIVE AND HYPERTENSIVE PREGNANCIES.* The Journal of obstetrics and gynaecology of the British Commonwealth, 1964.**71** : p. 222-30.

24. Caterina, M.J., M.A. Schumacher, M. Tominaga, T.A. Rosen, J.D. Levine, and D. Julius, *The capsaicin receptor: a heat-activated ion channel in the pain pathway.* Nature, 1997. **389** (6653): p. 816-24.

25. Caterina, M.J., *Vanilloid receptors take a TRP beyond the sensory afferent.* Pain, 2003. **105** (1-2): p. 5-9.

26. Yang, X.R., M.J. Lin, L.S. McIntosh, and J.S. Sham, *Functional expression of transient receptor potential melastatin- and vanilloid-related channels in pulmonary arterial and aortic smooth muscle.* Am J Physiol Lung Cell Mol Physiol, 2006. **290** (6): p. L1267-76.

27. Zsombok, A., *Vanilloid receptors–do they have a role in whole body metabolism? Evidence from TRPV1.* J Diabetes Complications, 2013. **27** (3): p. 287-92.

28. Gunthorpe, M.J. and A. Szallasi, *Peripheral TRPV1 receptors as targets for drug development: new molecules and mechanisms.* Curr Pharm Des, 2008. **14** (1): p. 32-41.

29. Xin, H., H. Tanaka, M. Yamaguchi, S. Takemori, A. Nakamura, and K. Kohama, *Vanilloid receptor expressed in the sarcoplasmic reticulum of rat skeletal muscle.* Biochem Biophys Res Commun, 2005.**332** (3): p. 756-62.

30. Song, M.Y. and J.X. Yuan, *Introduction to TRP channels: structure, function, and regulation.* Adv Exp Med Biol, 2010.**661** : p. 99-108.

31. Cai, H., M.E. Davis, G.R. Drummond, and D.G. Harrison,*Induction of endothelial NO synthase by hydrogen peroxide via a Ca(2+)/calmodulin-dependent protein kinase II/janus kinase 2-dependent pathway.* Arterioscler Thromb Vasc Biol, 2001. **21** (10): p. 1571-6.

32. Zhang, M. and H.J. Vogel, *Characterization of the calmodulin-binding domain of rat cerebellar nitric oxide synthase.* J Biol Chem, 1994. **269** (2): p. 981-5.

33. Zhang, M., T. Yuan, J.M. Aramini, and H.J. Vogel, *Interaction of calmodulin with its binding domain of rat cerebellar nitric oxide synthase. A multinuclear NMR study.* J Biol Chem, 1995.**270** (36): p. 20901-7.

34. Torres-Narváez, J.C., I. Pérez-Torres, V. Castrejón-Téllez, E. Varela-López, V.H. Oidor-Chan, V. Guarner-Lans, . . . L. Del Valle-Mondragón, *The Role of the Activation of the TRPV1 Receptor and of Nitric Oxide in Changes in Endothelial and Cardiac Function and Biomarker Levels in Hypertensive Rats.* Int J Environ Res Public Health, 2019. **16** (19).

35. Nieves-Cintrón, M., A.U. Syed, M.A. Nystoriak, and M.F. Navedo,*Regulation of voltage-gated potassium channels in vascular smooth muscle during hypertension and metabolic disorders.* Microcirculation, 2018. **25** (1).

36. Jackson, W.F., *K(V) channels and the regulation of vascular smooth muscle tone.* Microcirculation, 2018. **25** (1).

37. Wu, Y., M.Y. He, J.K. Ye, S.Y. Ma, W. Huang, Y.Y. Wei, . . . W.P. Xie, *Activation of ATP-sensitive potassium channels facilitates the function of human endothelial colony-forming cells via Ca(2+) /Akt/eNOS pathway.* J Cell Mol Med, 2017. **21** (3): p. 609-620.

Hosted file

`picture.docx` available at https://authorea.com/users/737275/articles/712362-the-potential-mechanism-of-transient-receptor-potential-vanilloid-type-1-combined-with-atp-sensitive-potassium-channel-in-severe-preeclampsia

Hosted file

`Table.docx` available at https://authorea.com/users/737275/articles/712362-the-potential-mechanism-of-transient-receptor-potential-vanilloid-type-1-combined-with-atp-sensitive-potassium-channel-in-severe-preeclampsia