Original Paper

Inhibition of the JAK2/STAT3/SOSC1 Signaling Pathway Improves Secretion Function of Vascular Endothelial Cells in a Rat Model of Pregnancy-Induced Hypertension

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Key Words

JAK2/STAT3/SOSC1 signaling pathway • Pregnancy-induced hypertension • Vascular endothelial cells • Secretion function • Placenta • AG490

Abstract

Background/Aims: The present study aimed to investigate the effects of the JAK2/STAT3/SOSC1 signaling pathway on the secretion function of vascular endothelial cells (VECs) in a rat model of pregnancy-induced hypertension (PIH). **Methods:** A PIH rat model was established. Forty-eight pregnant Sprague-Dawley female rats were selected and assigned into four groups: the normal group (normal non-pregnant rats), the non-PIH group (pregnant rats without PIH), the PIH group (pregnant rats with PIH) and the AG490 group (pregnant rats with PIH treated with AG490). Systolic blood pressure (SBP) and urinary protein (UP) were measured. The expressions of JAK2/STAT3/SOSC1 signaling pathway-related proteins in placenta tissues were detected by Western blotting. Radioimmunoassay was applied to detect serum levels of nitric oxide (NO), super oxide dismutase (SOD), placental growth factor (PGF), thromboxane B2 (TXB2) and endothelin (ET). Enzyme-linked immunosorbent assay (ELISA) was used to determine serum levels of interleukin-6 (IL-6), interleukin-10 (IL-10) and tumor necrosis factor- α (TNF- α). **Results:** Compared with the normal and non-PIH groups, the PIH and AG490 groups had higher SBP and UP levels at 17th and 25th day of pregnancy. The expressions of p/t-JAK2, p/t-STAT3 and SOSC1 in the PIH and AG490 groups were higher than those in the non-PIH group, while the expressions of p/t-JAK2, p/t-STAT3 and SOSC1 in the AG490 group were lower than those in the PIH group. Compared with the non-PIH group, serum levels of ET, TXB2, IL-6 and TNF- α were increased in the PIH and AG490 groups, while serum levels of NO, SOD, 6-keto-PGF1 α and IL-10 levels were reduced. Furthermore, the AG490 had lower serum levels of ET, TXB2, IL-6 and TNF- α and higher serum levels of NO, SOD, 6-keto-PGF1 α and IL-

10 than those in the PIH group. **Conclusion:** Our study provides evidence that inhibition of the JAK2/STAT3/SOSC1 signaling pathway could improve the secretion function of VECs in PIH rats.

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Introduction

Pregnancy-induced hypertension (PIH) is a clinically important condition that compromises the normal process of pregnancy and is considered to be a major cause of perinatal and maternal mortality [1]. Surprisingly, this high-incidence disease of pregnant women can damage the brain, heart, kidney and other human organs [2]. PIH has been correlated with aberrant placentation, which leads to the release of anti-angiogenic factors into the mother's circulation, resulting in endothelial injury as well as maternal and fetal damage [3]. PIH induces a wide variety of disorders, such as gestational hypertension, preeclampsia and eclampsia [4]. Early diagnoses, antenatal surveillance, and timely intervention are important for PIH management [5], but currently, no cost-effective, reliable and reproducible screening test is available for PIH. Endothelial dysfunction may be a predisposing factor for obstetric complications, such as preeclampsia and PIH [3].

The JAK/STAT signaling pathway has a role in cell growth and differentiation and immune function [6, 7]. To date, four members of the JAK family have been identified, namely JAK1, 2, 3, and TYK2 [8], and the STAT protein family is composed of seven members, STAT 1, 2, 3, 4, 5A, 5B and 6, which have been shown to be crucial for tumorigenesis and mammary cell survival [9]. SOCSs are endogenous inhibitors of cytokine bioactivities transduced through the JAK/STAT signaling pathway [10]. SOCS family members consists of eight members, SOCS1-7 as well as cytokine-inducible SH2-containing protein [11], which are critically involved in a negative feedback loop of JAK/STAT signaling pathway [12]. AG490, a JAK2 specific inhibitor, is able to effectively block the activation of JAK2/STAT3 signaling pathway and decrease the expression of SOCS1 [13, 14]. Recent studies on vascular endothelial function have generally examined signaling pathways [15-18]. According to Huang et al., PAR-1/NADPH oxidase/ROS signaling pathway may be a novel therapeutic target for the prevention and treatment of preeclampsia [19]. However, JAK2/STAT3/SOSC1 signaling pathway is rarely studied. Thus, our study aimed to explore the inhibition of the JAK2/STAT3/SOSC1 signaling pathway by AG490 on vascular endothelial cell secretion function in PIH syndrome, which may identify a novel target for the treatment of PIH.

Material and Methods

Experimental animals

The Sprague-Dawley male rats (230-300 g) and female rats (210-280 g) (SLAC Laboratory Animal Co. Ltd., Shanghai, China) of clean grade, aged 8-10 weeks, were kept in a quiet animal room at 22-25°C housed with freely accessible food and water at a ratio of 2:1. The next day, vaginal smears were performed to examine the sperm. Many sperm cells were observed when successful intercourse occurred. The day that the sperm was found was counted as day 0 of pregnancy. A total of 48 pregnant rats were selected for the experiment, and 12 non-pregnant rats functioned as the controls. The experiment was approved by the Animal Ethics Committee of Subei People's Hospital of Jiangsu Province, Clinical Medical College, Yangzhou University. All experimental procedures were in strict accordance with the animal protection principles of the International Association for the Study of Pain [20].

Establishment of a PIH rat model and animal grouping

Forty-eight pregnant Sprague-Dawley female rats were selected and assigned into four groups: the normal group (normal non-pregnant rats), the non-PIH group (pregnant rats without PIH), the PIH group (pregnant rats with PIH) and the AG490 group (pregnant rats with PIH treated with AG490). Treatment regimens of each group were listed in Table 1. Starting on the 14th day of pregnancy, the non-PIH rats underwent tail vein injection every day with 0.8 mL normal saline for 4 consecutive days, while the PIH rats were injected each day with 7 mg/kg NG-nitro-L-arginine methyl ester (L-NAME, Sigma, St. Louis, USA), an

Table 1. Treatment regimens for the rats in each group. PIH, pregnancy-induced hypertension; AG490, alpha cyano-(3-hydroxy) N-styrene benzyl amine

Group	Treatment regimen
The normal group	Normal Sprague-Dawley female rats without any treatment
The non-PIH group	Pregnant Sprague-Dawley female rats treated with 0.8 mL normal saline every day from the 14 th day of pregnancy (4 days of continuous injection)
The PIH group	Pregnant Sprague-Dawley female rats treated 7 mg/kg L-NAME every day from the 14 th day of pregnancy (4 days of continuous injection) and treated with 0.8 mL normal saline every day from the 18 th day of pregnancy (7 days of continuous injection)
The AG490 group	Pregnant Sprague-Dawley female rats treated 7 mg/kg L-NAME every day from the 14 th day after pregnancy (4 days of continuous injection) and treated with 1 mg AG490 every day from the 18 th day of pregnancy (7 days of continuous injection)

inhibitor of nitric oxide synthase that induced hypertension and proteinuria until the delivery as well as other PIH syndromes, such as delayed development in fetal rats. The injections were performed for 4 days. At the 14th and 17th day of pregnancy, the systolic blood pressure was measured in each group. Observation of elevated pressure of 30 mmHg demonstrated successful establishment of PIH model rats, which were selected on the 18th day for intervention treatment. The experiment used alpha cyano-(3-hydroxy) N-styrene benzyl amine (AG490), a common JAK2 kinase inhibitor, to simulate the inhibition effect of SOCS1 on JAK2-STAT3 in the signaling intervention. Each rat was injected every day with 1 mg AG490 for 7 consecutive days. The rats in the PIH group were injected with normal saline at the same volume.

Measurement of systolic blood pressure (SBP) and urinary protein (UP) levels

The systolic blood pressure (SBP) was measured by using a sphygmomanometer (MK-1030; Muromachi Kikai Co., Ltd., Tokyo, Japan). Data analysis was performed with a MedLab data acquisition system (Nanjing Medease Co., Ltd., Nanjing, China). Consistent with the sphygmomanometer instructions, the rats were preheated at 40°C for 5 min and fixed in a comfortable place. The pressure cuff was bound to the tail and inflated to increase the pressure to over 30 mmHg (the pulse disappeared when the pressure was at systolic pressure level). Then, the cuff was slowly decompressed. The pressure transducer and amplifier were connected to the Medlab system to record the blood pressure. The SBP of each rat was measured 3 times with interval of 60 s, and the median was obtained for analysis. The rats were assessed on days 14, 17 and 25 after pregnancy. The non-pregnant rats were treated identically.

From the 14th of the pregnancy, the rats were numbered and placed in standard metabolic cages. The urine at 24 h was collected on days 14, 17 and 25 days after pregnancy, and 1 mL urine samples were used for measurement. The urine of non-pregnant rats was collected at the same time. The UP level was detected by Coomassie blue staining. Coomassie brilliant blue G-250 (Qcbio Science & Technologies Co., Ltd., Shanghai, China; 100 mg) was dissolved in 50 mL 95% ethanol. Then, 100 mL concentrated phosphoric acid was added, and distilled water was used to dilute the solution to 200 mL. A total of 8 centrifuge tubes were numbered, and standard protein solutions of varying concentrations and 0.5 mL Coomassie brilliant blue were added. Distilled water was added to 1 mL for a standard curve. Clean test tubes were obtained, and 0.1 mL liquid sample and 0.5 mL Coomassie brilliant blue solution were added. After mixing, the solution was maintained at room temperature for 5 min. One tube was used as a blank control, and the optical density values of the standard solution and urine samples were measured at 595 nm by the colorimetric method. A standard curve was generated with the protein content as the horizontal coordinate and the absorption value as the vertical coordinate. The UP level was calculated based on the standard curve.

Sample collection

On the 25th day of the pregnancy, abdominal anesthesia with 40 mg/kg 1% pentobarbital sodium was applied to the rats, and a cesarean section was immediately performed. Cytokine secretion was detected by enzyme linked immunosorbent assay (ELISA) after 1.5 mL venous blood sampling. The length and weight of the fetal rats were determined with an electronic balance (BSA223S-CW, Mettler-Toledo International Inc., Shanghai, China). The placentas were weighted (electronic balance of BSA223S-CW, Mettler-Toledo International Inc., Shanghai, China), and the renal tissues were also collected for future experiments.

Ultrastructure observation of renal tissues

The renal tissues were fixed with 2.5% glutaraldehyde for 2 h at 4°C. Then, the tissues were fixed again with 1% osmic acid (Junrui Biotechnology Co., Ltd., Shanghai, China) for 2 h. After dehydration with

a gradient of alcohol, propylene oxide and Epon812 resin (Beijing HedeBio Technology Ltd., China) were applied for saturation, embedding, and polymerization. Subsequently, the renal tissues were sliced into ultrathin section (less than 100 nm thick). After double staining by 3% uranyl acetate and lead citrate (E Micron Technologies Limited, Shanghai, China), the structures were observed under a transmission electron microscope (JEM100CX-II, Japan).

Western blotting

The total protein was extracted from the placental tissues, and its concentration was determined using a bicinchoninic acid (BCA) protein assay kit (Wuhan Boster Bioengineering Co., Ltd., China). The extracted protein sample was mixed with loading buffer and boiled at 95°C for 10 min. Then, 40 µg of sample was added to each well. The proteins were separated using a 10% polyacrylamide gel (Wuhan Boster Bioengineering Co., Ltd., China) at an initial voltage of 80 V and separation voltage of 120 V. The polyvinylidene fluoride (PVDF) membrane transfer was performed with a wet transmission at a constant voltage of 100 mV for 90-120 min, after which the membrane was sealed at room temperature for 1 h in 5% bovine serum albumin (BSA). After adding the primary antibody against SOCS1, p-JAK2, JAK2, p-STAT3, STAT3 or β-actin (dilution at 1:1000, all purchased from Abcam Co., Cambridge, MA, USA), the samples were incubated overnight at 4°C. Then, the samples were washed with Tris-buffered saline and Tween-20 (TBST) 3 times, each for 5 min. The secondary antibodies (Abcam Co., Cambridge, MA, USA) then were added for incubation at room temperature for 1 h. TBST was applied to wash 3 times, each for 5 min. Electro-Chemiluminescence (ECL) reagent (Hoffmann-La Roche Ltd., Germany) was applied. The β-actin was used as an internal reference gene. Blots were analyzed by densitometry using ImageJ software.

Detection of biochemical parameters

Endothelin (ET): the ET kit was provided by the Institute of East Asian Immune Technology, People's Liberation Army General Hospital. The rat blood from each group was obtained (1 ml), and 15 µL 10% edetate disodium (Na₂EDTA) and 20 µL aprotinin were added to the samples. After mixing, the solution was centrifuged at 4°C for 3000 r/min for 10 min to separate the plasma. The reaction solution was made according to kit instructions. After the solution was mixed and incubated at room temperature for 15 min, it was centrifuged at 3500 rpm for 20 min. The supernatant was aspirated. ¹²⁵I-ET competed with the standard solution and sample solution to combine with the ET antibody to assess ET content based on the standard curve from the radioactive content in the sample solution.

Nitric oxide (NO): The supernatant to be tested (1 mL) was combined with 0.5 mL 0.02% 4-hydroxycoumarin (dissolved in dimethylformamide: 2 mol/L HCl = 1:1, volume ratio, Sigma, St. Louis, USA). After mixing, the solution was incubated on ice for 5 min and 50 µL 8% sodium hyposulfite was added (Na₂S₂O₃, Sigma, St. Louis, USA). After 10 min, 0.5 mL 1.5 mol/L sodium hydroxide (NaOH, Sigma, St. Louis, USA) was added. A fluorescence spectrophotometer (Thermo Fisher Scientific Inc., California, USA) was used to detect relative fluorescence intensity after 10 min. Sodium nitrite (NaNO₂) was used as the reference standard for the standard curve, which was used for the calculation of NO relative concentration.

Super oxide dismutase (SOD): the SOD kit was provided by the Nanjin Jiancheng Bioengineering Institute. The reactive solution was prepared according to the kit instructions. After mixing, the solution was incubated at room temperature for 10 min. The optical density (OD) was measured with a colorimetric methods using a 722 spectrophotometer (Thermo Fisher Scientific Inc., California, USA) at a wavelength of 550 nm. SOD activity (U/L) = (OD of the control tube - OD of the sample tube) / OD of the control tube / 50% * dilution multiple.

Thromboxane B2 (TXB2) and 6-keto-prostaglandin F1 α (6-keto-PGF1α): metabolic stable form of thromboxane A2 (TXA2) and prostaglandin 2 (PGI2), respectively. The contents of TXB2 and 6-keto-PGF1α were indicative of TXA2 and PGI2 levels, respectively. Radioimmunoassays were performed using a radioimmunoassay kit (Beijing North Institute of Bioengineering and Technology). The standard curve was generated with the standards, and the contents of TXB2 and 6-keto-PGF1α were analyzed.

Interleukin-6 (IL-6), interleukin-10 (IL-10) and tumor necrosis factor-α (TNF-α): The levels of IL-6, IL-10 and TNF-α were determined by an enzyme-linked immunosorbent assay (ELISA) kit which was purchased from Abcam (USA). Rat tail vein blood (3 mL) was collected into anticoagulative tubes containing ethylenediamine tetra-acetic acid (EDTA). After centrifugation at 4°C 3,000 r/min for 10 min, supernatant was collected and measurement was conducted strictly according to kit instructions.

Statistical analysis

Data were analyzed by the statistical package for the social sciences (SPSS) version 18.0 (SPSS Inc.; Chicago, IL, USA). Continuous data are shown as the mean $\pm$ standard deviation. The differences between two groups with a normal distribution were compared with a *t* test, and the differences among multiple groups were analyzed by one-way analysis of variance (ANOVA). A *P* level of less than 0.05 was considered statistically significant.

Results

Comparisons of SBP and UP levels among four groups

The results revealed that there was no significant difference in the SBP level among the normal, non-PIH, PIH and AG490 groups at the 14th day of pregnancy (all *P* > 0.05). At the 17th day of pregnancy, compared with the normal and non-PIH rats, the SBP levels of the PIH and AG490 groups were significantly increased (all *P* < 0.05). At the 25th day of pregnancy, the PIH group showed higher SBP level than that of the non-PIH group (*P* < 0.05), while the SBP level of the AG490 group was lower than that of the PIH group but still higher than that of the non-PIH group (both *P* < 0.05) (Table 2). Therefore, the results indicated that AG490 may alleviate the PIH-induced elevation of SBP.

As shown in Table 3, there were also no significant differences in UP levels among four groups at the 14th day of pregnancy (all *P* > 0.05). At the 17th day of pregnancy, the UP levels of the PIH and the AG490 groups were significantly higher than those of the normal and non-PIH groups (all *P* < 0.05). At the 25th day of pregnancy, the UP levels of the PIH and AG490 groups were still higher than those of the normal and non-PIH groups (all *P* < 0.05), but the UP level of the AG490 group was lower than that of the PIH group (*P* < 0.05). Thus, these results indicated that AG490 could decrease the UP level caused by PIH.

Ultrastructure observation of renal tissue of rats among four groups

Compared with the rats in the normal group, there were no obvious changes in renal ultrastructure of rats in the non-PIH group. However, compared with the rats in the normal and non-PIH groups, the rats in the PIH group displayed injured glomeruli, focal necrosis and hyaline degeneration. In addition, the rats in the PIH group had a thickened basement membrane, fibrinoid necrosis, vacuoles in the endothelial cell cytoplasm and mild swelling

Table 2. Comparisons of systolic pressure of the rats in each group (mmHg). PIH, pregnancy-induced hypertension; AG490, alpha cyano-(3-hydroxy) N-styrene benzyl amine; *, compared with the non-PIH group, *P* < 0.05; #, compared with the PIH group, *P* < 0.05; Δ , compared with the 17th after pregnancy, *P* < 0.05

Group	The 14 th day of pregnancy	The 17 th day of pregnancy	The 25 th day of pregnancy
The normal group	102.60 $\pm$ 3.80	102.60 $\pm$ 3.60	103.51 $\pm$ 3.01
The non-PIH group	102.09 $\pm$ 3.69	102.51 $\pm$ 3.99	103.21 $\pm$ 3.89
The PIH group	101.80 $\pm$ 3.41	135.31 $\pm$ 3.89*	136.70 $\pm$ 3.60*
The AG490 group	102.30 $\pm$ 3.49	135.71 $\pm$ 3.87*	118.20 $\pm$ 3.30* Δ

Table 3. Comparisons of urinary protein concentration of the rats in each group (mg/L). PIH, pregnancy-induced hypertension; AG490, alpha cyano-(3-hydroxy) N-styrene benzyl amine; *, compared with the normal group, *P* < 0.05; #, compared with the PIH group, *P* < 0.05; Δ , compared with the 17th after pregnancy, *P* < 0.05

Group	The 14 th day of pregnancy	The 17 th day of pregnancy	The 25 th day of pregnancy
The normal group	167.32 $\pm$ 30.99	172.72 $\pm$ 32.07	171.18 $\pm$ 30.95
The non-PIH group	166.28 $\pm$ 35.04	170.19 $\pm$ 32.93	177.58 $\pm$ 35.95
The PIH group	168.11 $\pm$ 36.00	635.28 $\pm$ 66.93*	564.62 $\pm$ 61.05*
The AG490 group	168.98 $\pm$ 33.93	628.52 $\pm$ 61.05*	263.38 $\pm$ 41.95* Δ

Fig. 1. Ultrastructure observation of renal tissues of rats among four groups under a transmission electron microscope ($\times 12000$). (A) Renal tissues of rats in the normal group; (B) Renal tissues of rats in the non-PIH group; (C) Renal tissues of rats in the PIH group; (D) Renal tissues of rats in the AG490 group; PIH, pregnancy-induced hypertension; AG490, alpha cyano-(3-hydroxy) N-styrene benzyl amine.

Table 4. Comparison of the features of fetal rats and placenta in each group. PIH, pregnancy-induced hypertension; AG490, alpha cyano-(3-hydroxy) N-styrene benzyl amine; *, compared with the non-PIH group, $P < 0.05$; #, compared with the PIH group, $P < 0.05$

Feature	The non-PIH group	The PIH group	The AG490 group
Weight of fetal rats (g)	5.32 ± 0.61	$4.16 \pm 0.38^*$	$4.58 \pm 0.52^\#$
Body length of fetal rats (cm)	4.42 ± 0.43	$3.21 \pm 0.31^*$	$3.57 \pm 0.38^\#$
Weight of placenta (g)	0.56 ± 0.13	$0.38 \pm 0.16^*$	$0.54 \pm 0.21^\#$
Stillbirth rate	0.00%	58.33%*	8.33%#
Deformity rate	0.00%	66.67%*	16.67%#

in the endothelial and epithelial cells. Moreover, the capillary lumen was narrowed, and swollen mitochondria were observed in the endothelium. The mesangial substrates increased, and mesangial cells proliferated. Renal tubular epithelial cells displayed vacuoles. The mitochondria swelled, and edema was found in the renal interstitium. Compared with the rats in the PIH group, the rats in the AG490 group had decreased focal necrosis and hyaline degeneration, and the thickened basement membrane was reduced. Additionally, the vascular endothelium was normal, and the cytoplasmic edema in renal tubular epithelial cells was alleviated (Fig. 1). The above results indicated that AG490 could ameliorate the renal injury induced by PIH.

Comparisons of the features of fetal rats and placenta among four groups

The weight and height of newborn rats in the non-PIH group were 5.32 ± 0.61 g and 4.42 ± 0.43 cm, respectively. The weight of placentas in the non-PIH group was 0.56 ± 0.13 g. No stillbirth and deformity were found. Compared with the non-PIH group, the PIH group exhibited significantly decreased in the weight and height of newborn rats and the placenta weight (all $P < 0.05$), but the stillbirth and deformity rates were increased in the PIH group in comparison to the non-PIH group (both $P < 0.05$). Compared with the PIH group, the AG490 group showed significantly increased in the weight and height of newborn rats and the placenta weight (all $P < 0.05$), while the stillbirth and the deformity rates decreased (both

Fig. 2. Expressions of the JAK2/STAT3/SOCS1 signaling pathway-related proteins detected by Western blotting. (A) Blots of SOCS1, p-JAK2, JAK2, p-STAT3 and STAT3 proteins; (B) Comparison of SOCS1 expression among three groups; (C) comparison of p/t-JAK2 expression among three groups; (D) Comparison of p/t-STAT3 expression among three groups; *, compared with the non-PIH group, $P < 0.05$; #, compared with the PIH group, $P < 0.05$.

Fig. 3. Changes of biochemical parameters (ET, NO, SOD, TXB2, 6-keto-PGF1α, IL-6, IL-10 and TNF-α) among the non-PIH, PIH and AG490 groups. *, compared with the non-PIH group, $P < 0.05$; #, compared with the PIH group, $P < 0.05$.

$P < 0.05$) (Table 4). These findings revealed that AG490 could improve the weight and height of newborn rats and the placenta weight and reduce the stillbirth and deformity rates.

Expressions of JAK2/STAT3/SOCS1 signaling pathway-related proteins among the non-PIH, PIH and AG490 groups

Western blotting analysis showed that the p/t-JAK2, p/t-STAT3 and SOCS1 expressions in the PIH group were higher than those in the non-PIH group (all $P < 0.05$), while the expressions of the above proteins in the AG490 group were significantly lower than those in the PIH group but still higher than those in the non-PIH group (all $P < 0.05$, Fig. 2). These

results demonstrated that AG490 could partly inhibit the activation of JAK2/STAT3/SOCS1 signaling induced by PIH.

Changes of biochemical parameters among the non-PIH, PIH and AG490 groups

Compared with the non-PIH group, serum levels of ET, TXB2, IL-6 and TNF- α were increased in the PIH group (all $P < 0.05$), while the NO, SOD, 6-keto-PGF1a and IL-10 levels were reduced (all $P < 0.05$). Serum levels of ET, TXB2, IL-6 and TNF- α in the AG490 group were lower than those in the PIH group but still higher than those in the non-PIH group (both $P < 0.05$). Serum levels of NO, SOD, 6-keto-PGF1a and IL-10 in the AG490 group were significantly higher than those in the PIH group but still lower than those in the non-PIH group (all $P < 0.05$) (Fig. 3).

Discussion

The present study aimed to investigate the effects of the JAK2/STAT3/SOCS1 signaling pathway on the secretion function of VECs in PIH rats. The findings in our study suggest that the PIH group exhibited elevated p/t-JAK2, p/t-STAT3, SOCS1, ET, TXB2, IL-6 and TNF- α levels, and reduced NO, SOD, 6-keto-PGF1a and IL-10 levels compared with the non-PIH group. After the AG490 injection, the expressions of p/t-JAK2, p/t-STAT3, SOCS1, ET, TXB2, IL-6 and TNF- α were down-regulated, while the levels of NO, SOD, 6-keto-PGF1a and IL-10 up-regulated. Collectively, the results indicated that inhibition of JAK2/STAT3/SOCS1 signaling improved the secretion function of the vascular endothelial cells, which provides new insight into PIH management.

First, our data revealed that the PIH rats exhibited increased SBP and UP levels, as well as injured glomeruli, focal necrosis, thickened basement membrane and vacuoles in the endothelial cell cytoplasm. Mukai et al. showed that the L-NAME treatment of pregnant rats caused a significant elevation in SBP and UP levels throughout pregnancy [21]. Compared with the PIH group, we observed that the AG490 group had lower SBP and UP levels. Wang and his colleagues reported that AG490 ameliorated portal hypertensive syndrome in partial portal hypertensive and liver cirrhosis rats via inhibition of JAK2/STAT3 signaling as well as its downstream cytokines [22]. Additionally, AG490 treatment can significantly improve renal function, reduce proteinuria and suppress histological lesions of the kidneys and salivary glands in lupus nephritis rats [23]. Acute renal injury was found in patients with pre-eclampsia and eclampsia [24]. Additionally, the fetal rats in the PIH group exhibited lower physiological indexes and increased stillbirth and deformity rates. They also observed fetal death in the uterus during later pregnancy and found that the weights of live fetuses and placentae were significantly reduced in the L-NAME-treated group [21].

In our study, the results of Western blotting showed that the relative expression levels of p/t-JAK2, p/t-STAT3 and SOCS1 in the PIH group increased significantly. The potential mechanism may be that in peripheral blood and placental tissues in PIH, abnormal cytokines levels can increase SOCS1 expression through activating the JAK/STAT pathway [25, 26]. Multiple cytokines can induce the expression of the SOCS gene through the JAK/STAT pathway, and SOCS protein finally inhibits tyrosine phosphorylation that negatively regulates the JAK/STAT pathway [27]. In the rat model of PIH constructed in our study, serum levels of IL-6, IL-10 and TNF- α were abnormal, and the abnormal expressions of IL-6, IL-10 and TNF- α may lead to the activation of the JAK/STAT pathway, and thereby inducing the high-expression of SOCS1. Increased SOCS1 content in the PIH model suggests its protective effect on the body. However, after the AG490 injection, p/t-JAK2, p/t-STAT3 and SOCS1 expressions were down-regulated. PIH causes the release of anti-angiogenic factors into the mother's circulation, resulting in endothelial injury [2, 3]. Previous studies demonstrated that the combined treatment of anti-placental growth factor (PLGF) and vascular endothelial growth factor A (VEGF-A) could reduce ocular hemangiomas growth and choroidal neovascularization [28, 29]. The activation of the SOCS/JAK/STAT signaling pathway is an essential pathogenic

mechanism leading to endothelial cell dysfunction [30-32]. AG490 is a common inhibitor of JAK2 kinase and inhibits the expression of SOCS1 [33]. SOCS family members can inhibit the activity of JAK/STAT signaling [34]. Thus, the p/t-JAK2 and p/t-STAT3 expressions were reduced by SOCS1 inhibition.

Several previous studies have demonstrated that the phosphorylation of JAK2 and STAT3 could be detected in human placenta and trophoblast cells [35, 36]. In our study, we detected the expressions of JAK/STAT signaling pathway-related proteins in placental tissues after the tail vein injection of AG490 in a rat model of PIH by Western blotting. The results demonstrated that in placental tissues, the expressions of p/t-JAK2 and p/t-STAT3 in the AG490 group were lower than those in the PIH group, which suggested that AG490 could also inhibit the activation of the JAK/STAT signaling pathway in placenta tissues. Additionally, our study found that serum levels of NO, SOD and 6-keto-PGF1a were lower, and ET and the TXB2 contents were increased in the PIH group. NO, PGI2, ET, TXA2 are vasoactive substances secreted by vascular endothelial cells and are involved in the regulation of vascular tone and proliferation of vascular smooth muscle cells [37]. Notably, these factors are important factors in the development of hypertension and prostanoid imbalance [38]. Xu et al. found that the NO and 6-keto-PGF1a levels were decreased and endothelia and TXB2 levels were increased in spontaneously hypertensive rats, which is consistent with our results [39]. NO plays an important role in regulating vasodilatation and vascular reactivity during pregnancy and its deficiency is associated with pathogenesis and maintenance of hypertension [21]. Pimentel et al. found that the activity of SOD was reduced in the patients with preeclampsia [40], which indirectly confirms our finding. Intriguingly, the SOCS-JAK-STAT pathway plays an important role in vascular smooth muscle cell proliferation [30], which is closely associated with endothelial cytokines. Vascular endothelial function could be achieved by regulation of NO release, anti-apoptotic and antioxidant actions and activation of cell survival signaling, which might improve the pregnancy-related disorders [41]. In our study, corresponding changes occurred in the cytokines of ET, NO, TXB2, SOD and 6-keto-PGF1a after AG490 intervention treatment. Thus, we hypothesized that AG490 intervention is able to alleviate the PIH syndromes of pregnant rats and improve physiological indexes and secretion function of cytokines by mediating JAK2/STAT3/SOSC1 signaling.

Although some researches have demonstrated that the activation of the JAK/STAT signaling pathway plays an important role in preeclampsia, this study also confirmed the role of the activation of the JAK/STAT signaling pathway in PIH. In rat placental tissues, the expression levels of JAK/STAT signaling pathway related proteins in the non-PIH group were lower as compared with the PIH group, and the expression levels of JAK/STAT signaling pathway related proteins in the AG490 group (injected with the inhibitor of JAK/STAT signaling pathway) were also lower compared with the PIH group. In order to further study the role of the JAK/STAT signaling pathway in PIH, our study focused on studying the effect of the JAK/STAT signaling pathway on the secretory function of vascular endothelial cells, revealing that serum levels of ET and TXB2 which reflected the secretory function of vascular endothelial cells in the PIH group increased while serum levels of NO, SOD and 6-keto-PGF1a decreased as compared with the non-PIH group; after the inhibition of the JAK/STAT signaling pathway, serum levels of ET and TXB2 decreased while serum levels of NO, SOD and 6-keto-PGF1a increased as compared with the PIH group. These findings suggested that the inhibition of the JAK2/STAT3/SOSC1 signaling pathway can improve the secretory function of vascular endothelial cells in rats with PIH, which is different from previous results and shows certain novelty.

To conclude, our study indicates that PIH is accompanied by increased SBP and UP, as well as renal dysfunction and delayed development in the placenta and abnormal VEC secretion. When JAK2/STAT3/SOSC1 signaling was inhibited, PIH syndrome was alleviated, and the secretion function of cytokines was normalized. Therefore, we demonstrated that the suppression of JAK2/STAT3/SOSC1 signaling can improve the secretion function of the vascular endothelial cells and alleviate PIH syndrome, which represents a novel therapeutic strategy for PIH treatment.

Acknowledgments

We would like to acknowledge the helpful comments received from our reviewers on this paper.

Disclosure Statement

None.

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