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Expression of soluble vascular endothelial growth factor receptor-1 and placental growth factor in fetal growth restriction cases and intervention effect of tetramethylpyrazine

Shu-Wei Li, Yi Ling, Song Jin*, Ye-Fei Lin, Ze-Jun Chen, Chun-Xia Hu, Ming-Hua Wang, Mao-Zhong Yao

Department of Obstetrics and Gynecology, Affiliated Hospital of Hainan Medical University, Haikou 570201, China

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ABSTRACT

Objective: To investigate the expression of soluble vascular endothelial growth factor receptor-1 (sFlt-1) and placental growth factor (PLGF) in the fetal growth restriction (FGR) cases and the intervention mechanism of tetramethylpyrazine. **Methods:** A total of 60 fetal growth restriction cases that admitted to our hospital were randomly divided into ligustrazine intervention group (group A) and nutritional support group (group B). A total of 50 healthy pregnant women were also enrolled as control group (group C). Expression level of maternal serum sFlt1, PLGF and fetal growth parameters including HC, AC, FL, BPD, EFW as well as placenta PLGF, sFlt-1 mRNA expression were recorded and compared among the three groups. A total of 15 SD rats were selected and were divided into three groups, TMP group, alcohol and tobacco group and blank control group. Three groups of rats were dissected on the twentieth day of gestation. **Result:** Expression level of sFlt-1 and PLGF in group A was not significantly different from that of group C ($P>0.05$); but significant difference in SFlt1 and PLGF expression level was observed between group C and group B ($P<0.05$). Before treatment, HC, AC, FL, BPD and EFW of group A and group B were significant lower than those of group C, but after treatment, those parameters in group A were significantly improved ($P<0.05$). In the animal experiment there was no significant difference in sFlt-1 between treatment group and FGR group without treatment or control group ($P>0.05$). There was significant difference in PLGF between FGR group with treatment and FGR group without treatment or control group ($P<0.01$). **Conclusions:** PLGF level is decreased and sFlt-1 increased in patients suffered from fetal growth restriction, and FGR rats show increased sFlt-1 and decreased PLGF, thus they can be indicator of the fetal growth restriction. Ligustrazine can effectively improve sFlt-1,PLGF expression level in fetal growth restriction cases, which can be used as treatment for FGR.

1. Introduction

Fetal growth restriction (FGR) is one of the important subjects of modern obstetrics due to the close relation between birth weight and the morbidity and mortality rate of the perinatal infants. And because of complex mechanisms of FGR, high perinatal mortality and lack of effective treatment, FGR is well known as refractory disease in the field of fetal medicine. The present study aimed to study the expression change of soluble vascular endothelial growth factor receptor-1 (soluble VEGF receptor 1, sFlt-1) and placental growth factor (PIGF) in the placentas and peripheral blood of FGR models of rats and pregnant women, to study the intervention effect of ligustrazine on the above mentioned indicators in fetal rats and pregnant women, and to explore the role and mechanisms of sFlt-1 and PLGF in the pathogenesis of FGR, so as to provide some ideal methods for clinical treatment of FGR and possible monitoring indicators.

*Corresponding author: Song Jin, Department of Obstetrics and Gynecology, Affiliated Hospital of Hainan Medical University, Haikou 570201, China.
Tel: 13322052996
E-mail: mdjkyl@tom.com.cn
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2. Materials and methods

2.1. Animal experiments

To investigate the expression changes of sFlt-1 and PLGF in the fetals of FGR rats and the treatment effect of tetramethylpyrazine (TMP) injection on FGR, 15 SD rats were used and randomly divided into TMP group (FGR treatment group), FGR non-treatment group and normal control group. The FGR treatment group was daily intragastrically administered with alcohol and exposed to tobacco smoking for three times (each time for 15 min). From the 13th day, after the treatment of alcohol and tobacco TMP were injected (*ip*.) at a dose of 8 mg/kg each day. Rats in the non-treatment group, from the 7th day of pregnancy, were daily administered with alcohol and exposed to tobacco smoking three times (each time for 15 min). The normal control rats were administered with normal saline at a dose of 9 mL/kg at 9 o′clock from the 7th day and kept in the smoking boxes without smoking for three times each day (each time for 15 min). All the rats in the three groups were dissected on Day 20 and RT-PCR was used to detect and compare the expression of PLGF and sFlt-1 in the placentas of the rats among the three groups.

2.2. Clinical experiments

2.2.1. FGR diagnostic criteria

Patients with the following conditions at the same time were included in the study: 1) with a clear gestational age and without endogenous FGR, like fetal malformation and chromosome abnormality; 2) fetal weights by ultrasound examination were estimated to below the average weight of gestational age or lower than the average weight by two standard deviations. Ultrasound diagnosis and monitoring were operated by designated persons. During the monitoring, ultrasonic Doppler examination of uterine artery and umbilical cord blood flow were done at the same time.

2.2.2. Methods

A total of 60 FGR cases that admitted to our hospital were selected and 50 healthy pregnant women were also enrolled as control group (group C). Urine and serum samples (2 mL) were collected from all the pregnant women and kept at −80 ℃ in the refrigerator for further use.

The 60 FGR cases were randomly divided into two treatment groups, ie. ligustrazine intervention group (group A) and nutritional support group (group B) with 30 cases in each group. Group B were given low molecular weight dextran, vitamin C and intravenous drip of amino acid once a day for 7 days. For group A, in addition to the treatment for group B, patients were also given intravenous drip of ligustrazine (120 mg) once a day for 7 days. The urine and serum samples of all the patients were collected before and after the treatments.

Follow-up visits were done after the birth of fetal to record their growth and developement parameters, including fetal head circumference (HC), abdomen circumference (AC), femur length (FL), fetal biparietal diameter (BPD), estimated fetal weight (EFW). Placenta specimens of the pregnant women were taken after childbirth. A sample of 1 cm×1 cm×1 cm placenta tissue near the root of the umbilical cord were taken and kept at − 80 ℃ in a refrigerator. The mRNA levels of sFlt-1 and PIGF were detected using RT-PCR method.

Peripheral blood, sFlt-1 and PLGF of placenta and growth parameters of fetals by ultrasound were recorded. The results were analyzed and the differences of detected indicators were compared among all the groups.

3. Results

3.1. Expression of sFlt-1 and PLGF in the serum of FGR cases

The PLGF levels in the experimental and control groups were (1 462.39±364.51) and (2 685.05±322.61) pg/mL, respectively. And the sFlt-1 levels of the two groups were (9 509.67±1 406.91) and (4 103.42±661.82) pg/mL, respectively. The PLGF and sFlt-1 levels in both groups were statistically significant ($P<0.05$). The level of sFlt-1 was elevated and PLGF decreased in the serum of FGR pregnant women. Hence, sFlt-1 and PLGF can be considered as screening indicators of FGR.

3.2. Expression of sFlt-1 and PLGF in the placenta of FGR cases

mRNA of PLGF and sFlt-1 was expressed in the placentas of both groups. The expression of PLGF in FGR group was significantly lower than that in the control group, with 20.57 ±2.10 and 25.95±3.68, respectively ($P<0.05$). The expression of sFlt-1 mRNA in FGR group was significantly higher than that of control group with 32.36±3.13 and 27.25±3.34, respectively ($P<0.05$).

The expression of PLGF mRNA in patients with FGR decreased in placental tissue, while sFlt-1 mRNA expression increased, which may have certain effect to the pathogenesis of FGR.

3.3. Effect of ligustrazine on fetal growth parameters

After treatment, BPD, HC, AC, FL, EFW in group A increased significantly, which were statistically significant compared with those in group B (P<0.05). The resistance to umbilical cord blood flow and blood rheological parameters in Group A pregnant woman were significantly lower than that in group B (P<0. 05). The cure rate and total effective rate in group A was significantly higher (P<0.05) (Table 1).

3.4. Effect of ligustrazine intervention on the placental sFlt-1 and PLGF

Application of ligustrazine for the treatment of FGR can significantly improve the levels of sFlt -1 and PLGF in placenta, ameliorate blood rheological status of the FGR pregnant women, reduce the circulation resistance of uterine placenta, improve the placental villus microcirculation, promote in utero fetal growth and development and increase the neonatal birth weight.

3.5. Effect of ligustrazine intervention on the placental sFlt-1 and PLGF

The expression levels of sFlt-1 and PLGF in group A showed no significant difference compared with group C (P>0.05). Expression of sFlt-1 and PLGF in Group B was significant different from that of group C (P<0.05) (Table 2).

So it can be concluded that the application of ligustrazine for the treatment of FGR can significantly recover sFlt-1 and PLGF levels in placentas, which is safe and cost-efficient.

3.6. sFlt - 1, PLGF expression in placentas of FGR rats and effect of ligustrazine intervention

Compared with the control group, the value of sFlt-1 in FGR non-treatment group significantly increased (P<0.01), while no significant difference was observed between FGR treatment group and FGR non treatment group (P>0.05).

Compared with control group, the value of PLGF in FGR non treatment group decreased (P<0.01) and this value in decreased in FGR treatment group compared with FGR non treatment group (P<0.01) (Table 3).

The level of sFlt-1 increased and PLGF decreased in the placentas of FGR rats, so these two parameters may be considered as the screening indicators of FGR. Treatment with ligustrazine did not change sFlt-1 in the placentas of rats significantly, although PLGF elevated. Hence, ligustrazine can be considered as treatment drug for FGR.

It could be concluded from the present study that PLGF mRNA expression in placental tissue of patients with FGR reduced and sFlt-1 mRNA expression elevated. sFlt-1 in the pregnancy serum of FGR elevated and PLGF decreased. sFlt-1 increased and PLGF decreased in the placentas of FGR rats. After the treatment of ligustrazine, the sFlt-1 in the placentas of rats did not show any significant change but

Table 1
Growth and development parameters in the fetal of three groups (mean±sd).

Group		No. of cases	HC (cm)	AC (cm)	FL (cm)	BPD (cm)	EFW (kg)
Group A	Before treatment	30	26.96±1.51*	25.02±2.15*	5.52±0.53*	7.51±0.62*	1.41±0.28*
	After treatment		28.41±1.38#	27.06±2.66#	5.86±0.48#	7.86±0.55#	1.72±0.04#
Group B	Before treatment	30	27.13±1.62*	25.01±2.41*	5.41±0.62*	7.50±0.46*	1.43±0.41*
	After treatment		28.31±1.53#	26.32±2.82#	5.63±0.53#	7.67±0.58#	1.61±0.33#
Group C		50	28.43±1.82	27.11±2.53	5.88±0.63	7.92±0.52	1.71±0.31

*P<0.05 compared with group C; #P<0.05 compared with parameters before treatment within a group.

Table 2
Comparison of sFlt-1 and PLGF expression in the three groups (mean±sd).

Group	n	sFlt-1	PLGF
A	30	28.17±3.0	24.89±3.15
B	30	32.36±3.135*#	20.57±2.1*#
C	50	27.25±3.34	25.95±3.68

*P<0.05 compared with Group C; #P<0.05 compared with Group A.

Table 3
Comparison of PLGF and sFlt-1 expression in placentas of rats in three groups.

Group	n	PLGF	sFlt-1
FGR treatment (A)	10	2.75±0.22#	1.39±0.35
FGR non-treatment(B)	10	0.28±0.07*	1.69±0.55*
Normal control (C)	10	1.36±1.37	1.13±0.09

*P<0.01 when compared with control group; #P<0.01 when compared with FGR non-treatment group.

PLGF increased. The mutual antagonism between sFlt-1 and PLGF was likely to have certain effect on the pathogenesis of FGR, which was one of the most potential detection indexes in FGR study. Both animal experiments and clinical study have shown that application of ligustrazine treatment in FGR cases can siginificantly recover the sFlt-1 and PLGF levels. Ligustrazine can improve the rheological property of blood, decrease circulation resistance of placenta, improve the improve the placental villus microcirculation, promote in utero fetal growth and development and increase the neonatal birth weight.

4. Discussion

The complicated mechanism of FGR leads to high perinatal mortality, and the lack of effective treatment makes it a refractory disease in the field of fetal medicine. The role of sFlt-1 and PIGF on the pathogenesis of FGR draws a lot of attention and is regarded as a new hot spot. PLGF is a kind of secretory dimers glycoprotein in the family of vascular endothelial growth factors. It can realize its biological effect by promoting the growth of blood vessels through binding with its receptors. The decrease of PLGF secretion from placenta trophocyte can cause angiogenesis obstacles in the placental villus. Transport of oxygen and nutrients would be blocked by underdeveloped placental vascular networks, resulting in the occurrence of FGR. The level of sFlt-1 expressed by placenta trophocyte may rise due to relatively anoxic environment induced during pregnancy. The combination of a large number of sFlt-1 and PLGF, VEGF can cause dysplastic placenta angiogenesis, leading to FGR. The mutual antagonism of sFlt-1 and PLGF makes it the most potential indicator in FGR study. The present study proved both in animal models and clinical tests, in mRNA and pregnancy serum that sFlt-1 increased and PLGF decreased in the placenta and blood of pregnant women when suffering from FGR. The mutual antagonism of sFlt-1 and PLGF has certain effect on the pathogenesis of FGR, which is one of the most potential detecting indicator in FGR study[1-4].

Appropriate treatment during gestation period can reduce the perinatal mortality when diagnosed with FGR. The chemical structure of ligustrazine used in the present study is tetramethylpyrazine which is extracted from the rhizome of Chinese medicinal plant *Ligusticum wallichii* (family Apiaceae). Ligustrazine possesses typical characteristics as a calcium antagonist. It can regulate the function of platelet, reduce blood viscosity, improve blood rheological property, expand capillaries and recover microcirculation. It also possesses anticoagulant properties. Ligustrazine can help to inhibit the fibrosis of placental villi and human embryo cells, enhance the level of HPL and E3. It can also expand blood vessels without affecting blood pressure at small doses and down-regulate the blood pressure at large doses. The equilibrium of the pro-oxidant/antioxidant system in the body can also be adjusted by ligustrazine through enhancing the activity of superoxide dismutase and radical scavenging effect of superoxide dismutase, reducing damages from oxygen free radicals on placental villi, preventing placenta fibrosis, improving the function of the placenta and promoting fetal growth and development. After ligustrazine treatment, sFlt-1 and PLGF levels in placenta was significantly improved. Hence, ligustrazine can be considered as a therapy for FGR. The selected subjects are all singleton pregnancy and FGR related researches on gemellary pregnancy are now being carried out. Due to its remarkable therapy effects, convenient accessibility, low cost, safe and economical efficiency, ligustrazine has a good clinical application prospect[5-7].

Both sFlt-1 and Flt 1 belong to vascular endothelial growth factor receptor (VEGFR). VEGFR competes with Flt 1 to combine with VEGF, completely inducing inactivation of VEGF, block of angiogenesis and affecting the permeability and integrity of vessel walls[8-12]. PLGF is one of the members of VEGF family. When PLGF combines with Flt 1 receptors, PLGF can promote the proliferation and infiltration of villi trophocyte. However, all these biology functions can be blocked by sFlt-1, as the strong antagonism effect of sFlt-1 against PLGF can regulate the function of PLGF[13,14]. The role of sFlt-1 in the occurrence and development process of preeclampsia may be related to the signal transduction pathway of phosphate ester acyl inositol 3 kinase/protein kinase B (PI3K/Akt). Combined determination of PLGF and sFlt-1 in the blood of pregnant women can provide a relatively high sensitivity and specificity in predicting the occurrence of preeclampsia[15-18].

With the development of research, it was found that the relationship of sFlt-1 and PLGF with the disease severity was linear, which was related to the non-synchronized development in twin pregnancy. At the same time, changes of sFlt-1 appeared earlier. When PLGF combines with Flt 1 receptors, it can promote the generation of angiogenesis and the proliferation and infiltration of villi trophocyte. It was suggested in the present animal sudy that ligustrazine showed a more apparent effect on placenta than sFlt-1 did. Recently, PLGF has been tried to be used and studied as an indicator of clinical, including the design of the kits for rapid detection[19-22]. PLGF detection is simple and efficient and could be used as early as 11-14 weeks in pregnancy, which provide a efficient way for the early detection, early prevention and early diagnosis.

In conclusion, sFlt-1 increased and PLGF decreased in FGR maternal serum and placenta, thus they can be used

as FGR detection indicators; ligustrazine therapy can significantly improve the blood rheological property and levels of PLGF and sFlt-1 in placenta in FGR pregnant women, thus ligustrazine can be used as an effective therapy for FGR with certain scientific significance and of favorable clinical application prospects.

Conflict of interest statement

We declare that we have no conflict of interest.

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