Journal of Hainan Medical University

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Effect of adjuvant low−molecular−weight heparin therapy on placental hypoxia and cell apoptosis in puerperae with severe preeclampsia

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ARTICLE INFO

Article history:
Received 13 Apr 2017
Received in revised form 18 Apr 2017
Accepted 19 Apr 2017
Available online 24 May 2017

Keywords:
Severe preeclampsia
Low-molecular-weight heparin
Oxidative stress
Apoptosis

ABSTRACT

Objective: To study the effect of adjuvant low-molecular-weight heparin therapy on placental hypoxia and cell apoptosis in puerperae with severe preeclampsia. **Methods:** A total of 94 puerperae with severe preeclampsia who received treatment and safely gave birth in our hospital between May 2014 and May 2016 were selected as the research subjects and randomly divided into the LMWH group who received low-molecular-weight heparin combined with conventional symptomatic treatment and the control group who received conventional symptomatic treatment. Before and after treatment, serum was collected respectively to determine the levels of placental hypoxia-related cytokines, and after delivery, the placentas were collected to detect oxidative stress indexes and cell apoptosis indexes. **Results:** After treatment, serum PLGF and PAPP-A levels of both groups were significantly higher than those before treatment while sFlt-1 and sEng levels were significantly lower than those before treatment, and after treatment, serum PLGF and PAPP-A levels of LMWH group were significantly higher than those of control group while sFlt-1 and sEng levels were significantly lower than those of control group; ROS and RNS levels as well as Fas, FasL, caspase-3 and caspase-8 protein expression in placenta tissue of LMWH group were significantly lower than those of control group while GPx-1, SOD-1 and Trx levels as well as Survivin, XIAP and Bcl-2 protein expression were significantly higher than those of control group. **Conclusion:** Adjuvant low-molecular-weight heparin therapy can relieve the placental hypoxia, improve oxidative stress reaction and inhibit cell apoptosis in puerperae with severe preeclampsia.

1. Introduction

Preeclampsia is a common complication during pregnancy, severe preeclampsia is in a critical condition and causes great harm to both pregnant women and fetus, the incidence of preterm birth and neonatal mortality significantly increase, and the clinical treatment is tough[1,2]. In recent years, the study about the pathogenesis of preeclampsia believes that endothelial function injury-induced small vasospasm and anticoagulant-procoagulant imbalance are the key pathological links that cause high blood pressure and kidney damage in puerperae with preeclampsia, and spasmolysis, anticoagulation and improving microcirculation are the common method for the treatment of severe preeclampsia. Magnesium sulfate is the most common drug for clinical treatment of preeclampsia, has spasmolysis and vasodilative effect, can effectively control the condition of puerperae with mild preeclampsia, but has limited effects on controlling the condition of puerperae with severe preeclampsia. Low-molecular-weight heparin (LMWH) is the anticoagulant drug that can influence both endogenous and exogenous coagulation pathways, and it has been used in the treatment of severe preeclampsia in recent years[3,4]. In the following study, the effect of adjuvant low-molecular-weight heparin therapy on placental hypoxia and cell apoptosis in puerperae with severe preeclampsia was analyzed.

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Fund Project: Research Project of Sichuan Provincial Health Department No: 150549.

2. Subjects and methods

2.1 Research subjects

A total of 94 puerperae with severe preeclampsia who received treatment and safely gave birth in our hospital between May 2014 and May 2016 were selected as the research subjects, and all included subjects were in accordance with the diagnostic criteria for severe preeclampsia, without gestational hypertension, and without requirement of immediate termination. After signing the informed consent, the puerperae were randomly divided into the LMWH group (n=47) who received low-molecular-weight heparin combined with conventional symptomatic treatment and the control group (n=47) who received conventional symptomatic treatment. LMWH group were 28-34 years old, the BMI was (24.9±3.6) kg/m^2 and the gestational age at delivery was (37.6±4.2) weeks; control group were 25-33 years old, the BMI was (25.4±3.9) kg/m^2 and the gestational age at delivery was (38.7±4.7) weeks. The two groups of puerperae were not significantly different in general data (P>0.05).

2.2 Therapy

Control group received magnesium sulfate therapy, which was as follows: intravenous drip (within 30 min) of 5g 25% magnesium sulfate in 10% glucose injection, then intravenous drip (1 g/h) of 15 g 25% magnesium sulfate in 500 mL 5% glucose injection, then intramuscular injection of 2.5 g 25% magnesium sulfate in the morning and evening according to the situation of blood pressure control, and the total dose of magnesium sulfate no more than 30 g. On the basis of symptomatic magnesium sulfate treatment, LMWH group received low-molecular-weight heparin therapy, which was as follows: subcutaneous injection of low-molecular-weight heparin calcium (Fraxiparine) 4100 IU, 2 times/d, for 5 d in a row, as a course of treatment; one course was repeated every week until 24 h before delivery.

2.3 Serum sample collection and index detection methods

Before treatment and after treatment (before delivery), 5 mL of peripheral blood was extracted from the two groups and centrifuged to separate and get serum and store it in a -80 ℃ refrigerator; for test, the serum specimens were taken out and thawed at 4 ℃, and then enzyme-linked immunosorbent assay kits were used to determine PLGF, PAPP-A, sFlt-1 and sEng levels.

2.4 Placenta sample collection and index detection methods

Within 30 min after delivery, moderate amount of placental tissue near the maternal side was collected and washed with saline repeatedly, then filter paper was used to absorb all moisture, then the placenta tissue was transferred into the freezing tubes and shortly frozen in liquid nitrogen, and then the freezing tubes were taken out and stored in a -80 ℃ refrigerator. For detection, the placenta tissue was taken out and added in protein lysis buffer to extract the protein samples, then BCA kits were used to determine total protein content, radioimmunoprecipitation kits were used to detect ROS, RNS, GPx-1, SOD-1, Trx levels, and enzyme-linked immunosorbent assay kits were use to detect Fas, FasL, caspase-3, caspase-8, Survivin, XIAP and Bcl-2 levels. The total protein content was used to calculate the ROS, RNS, GPx-1, SOD-1, Trx, Fas, FasL, caspase-3, caspase-8, Survivin, XIAP and Bcl-2 levels.

2.5 Statistical methods

SPSS 20.0 software was used to input and statistically analyze the experimental data, measurement data comparison between two groups was analyzed by t test and $P<0.05$ indicated statistical significance in differences.

3. Results

3.1 Placenta hypoxia–related cytokine levels in serum

Before and after treatment, analysis of serum PLGF (ng/mL), PAPP-A (μg/mL), sFlt-1 (pg/mL) and sEng (ng/mL) levels between two groups of puerperae was as follows: before treatment, differences in serum PLGF, PAPP-A, sFlt-1 and sEng levels were not statistically significant between two groups of puerperae (P>0.05); after treatment, serum PLGF and PAPP-A levels of both groups were significantly higher than those before treatment while sFlt-1 and sEng levels were significantly lower than those before treatment, and differences in serum PLGF, PAPP-A, sFlt-1 and sEng levels were statistically significant within group before and after treatment ($^*P$<0.05); serum PLGF and PAPP-A levels of LMWH group were significantly higher than those of control group while sFlt-1 and sEng levels were significantly lower than those of control group, and differences in serum PLGF, PAPP-A, sFlt-1 and sEng levels were statistically significant between groups after treatment ($^\&P$<0.05).

Table 1.
Comparison of serum placental hypoxia-related cytokines between two groups of puerperae.

Groups	n	Treatment	PLGF	PAPP-A	sFlt-1	sEng
LMWH group	47	Before treatment	42.59±6.83	1.85±0.22	221.32±35.82	39.57±5.58
		After treatment	97.59±10.42$^{*\&}$	3.42±0.41$^{*\&}$	104.51±13.45$^{*\&}$	15.25±1.86$^{*\&}$
Control group	47	Before treatment	43.21±6.28	1.91±0.23	225.29±33.12	40.22±5.82
		After treatment	64.72±8.12*	2.39±0.32*	156.76±20.15*	28.59±3.47*

*: comparison within group before and after treatment, $P<0.05$; $^\&$: comparison between groups after treatment, $P<0.05$.

3.2 Oxidative stress injury molecule levels in placenta

After delivery, analysis of oxidative stress products ROS (μmol/mg total protein) and RNS (μmol/mg total protein) as well as antioxidant enzymes GPx-1 (U/mg total protein), SOD-1 (U/mg total protein) and Trx (U/mg total protein) levels in placenta tissue between two groups of puerperae was as follows: oxidative stress products ROS and RNS levels in placenta tissue of LMWH group were significantly lower than those of control group while antioxidant enzymes GPx-1, SOD-1 and Trx levels were significantly higher than those of control group. Differences in ROS, RNS, GPx-1, SOD-1 and Trx levels in placenta tissue were statistically significant between two groups of puerperae (P<0.05).

3.3 Apoptosis gene expression in placenta

After delivery, analysis of pro-apoptosis molecules Fas (ng/mg total protein), FasL(ng/mg total protein), caspase-3 (pg/mg total protein) and caspase-8 (pg/mg total protein) in placenta tissue between two groups of puerperae was shown in Table 3: Fas, FasL, caspase-3 and caspase-8 protein expression in placenta tissue of LMWH group were significantly lower than those of control group; analysis of anti-apoptosis molecules Survivin, XIAP and Bcl-2 in placenta tissue between two groups of puerperae was shown in Table 4: Survivin, XIAP and Bcl-2 protein expression in placenta tissue of LMWH group were significantly higher than those of control group. Differences in Fas, FasL, caspase-3, caspase-8, Survivin, XIAP and Bcl-2 protein expression in placenta tissue were statistically significant between two groups of puerperae (P<0.05).

4. Discussion

Severe preeclampsia damage to the maternal and infant is great, the pathological changes of systemic arteriolar spasm, endothelial dysfunction and insufficient placental blood perfusion are more significant than those of mild preeclampsia, and the effect of conventional magnesium sulfate spasmolysis is not ideal[5]. In recent years, the study about severe preeclampsia has shown that the persistent endothelial injury in patients can cause anticoagulant-procoagulant factor imbalance and cause hypercoagulable state, and micro-thrombosis in target organs will further aggravate the ischemic viscera injury and lead to significantly elevated blood pressure, massive proteinuria and other clinical symptoms. On the basis of the understanding about the correlation between hypercoagulable state and severe preeclampsia development, anticoagulant drug low-molecular-weight heparin (LMWH) is increasingly used in the treatment of diseases. LMWH can affect both endogenous and exogenous coagulation pathways, reduce the activity of the X factor and XIIa factor in blood coagulation pathway, combine with antithrombin, and restrain the thrombin function, and it can also activate the tPA and uPA and promote micro-thrombolysis[6,7]. Studies have shown that LMWH treatment of severe preeclampsia can improve the clinical symptoms of hypertension and proteinuria, and correct the abnormal hypercoagulable state in patients, too[8].

Placenta is the important accessory organ for the fetus during

Table 2.

Comparison of oxidative stress injury molecules in placenta between two groups of puerperae.

Groups	n	Oxidative stress products		Antioxidant enzymes		
		ROS	RNS	GPx-1	SOD-1	Trx
LMWH group	47	5.28±0.89	3.34±0.41	23.83±3.42	40.29±5.61	37.65±5.26
Control group	47	8.41±0.93	7.52±0.93	11.38±1.75	22.32±3.25	21.25±3.06
T		7.938	12.571	11.038	9.281	8.325
P		<0.05	<0.05	<0.05	<0.05	<0.05

Table 3.

Comparison of pro-apoptosis molecules in placenta between two groups of puerperae.

Groups	n	Fas	FasL	Caspase-3	Caspase-8
LMWH group	47	3.19±0.52	2.32±0.33	93.41±13.28	145.65±18.38
Control group	47	5.84±0.78	4.99±0.61	221.35±35.84	325.48±42.59
T		7.984	11.385	13.291	14.395
P		<0.05	<0.05	<0.05	<0.05

Table 4.

Comparison of anti-apoptosis molecules in placenta between two groups of puerperae (ng/mg total protein).

Groups	n	Survivin	XIAP	Bcl-2
LMWH group	47	45.29±6.98	19.23±2.45	68.61±8.23
Control group	47	21.38±3.38	10.65±1.35	29.52±4.18
T		11.482	9.285	16.72
P		<0.05	<0.05	<0.05

pregnancy, and the reduced placental perfusion and ischemia hypoxia are the important pathological links causing severe preeclampsia. At present, there is no clear report whether adjuvant LMWH therapy can improve the placental hypoxia state in the patients with severe preeclampsia. When the placenta is in the pathological conditions of ischemia hypoxia, the synthesis and secretion of a variety of cytokines will be in obvious disorder. PLGF is an important cytokine to promote the placenta cell proliferation and differentiation, and induce endothelial cell proliferation and migration, PAPP-A is the molecule synthesized and secreted by placental syncytiotrophoblast and can reflect the development and function of the placenta, and serum PLGF and PAPP-A contents significantly reduce in patients with severe preeclampsia[9,10]. SFlt-1 and sEng can be mutually combined with PLGF and VEGF and block their biological effects, then affect the placental angiogenesis, and cause endothelial function damage and placental function injury, and serum sFlt-1 and sEng levels significantly increase in patients with severe preeclampsia[11,12]. In the study, the analysis of the changes in the cytokine levels before and after the treatment showed that after treatment, serum PLGF and PAPP-A levels of both groups were significantly higher than those before treatment while sFlt-1 and sEng levels were significantly lower than those before treatment, and after treatment, serum PLGF and PAPP-A levels of LMWH group were significantly higher than those of control group while sFlt-1 and sEng levels were significantly lower than those of control group. This means that both conventional symptomatic treatment and adjuvant LMWH therapy can reduce placental hypoxia and regulate the secretion of cytokines in the placenta to a certain extent, and auxiliary LMWH treatment has much better reducing effect on placental hypoxia than conventional symptomatic treatment.

In the development and change of severe preeclampsia, ischemia hypoxia can not only cause the secretion disorder of a variety of cytokines, but can also activate oxidative stress and induce cell apoptosis. During the activation of oxidative stress reaction, the production of ROS and RNS in the local tissue increases, resulting in oxidative damage to a variety of biological structures in trophocytes and endothelial cells[13,14]. At the same time, the locally existing GPx-1, SOD-1, Trx and other antioxidant enzymes can remove ROS and RNS, but excessively generated ROS and RNS will exceed the compensatory range of the body's antioxidant capacity and massively consume GPx-1, SOD-1, Trx and other antioxidant enzymes, which will lead to the decreased antioxidant enzyme levels and the weakened antioxidant capacity in local tissue[15,16]. In the study, the analysis of the levels of above oxidative stress products and antioxidant enzymes in the placenta tissue showed that oxidative stress products ROS and RNS levels in placenta tissue of LMWH group were significantly lower than those of control group while antioxidant enzymes GPx-1, SOD-1 and Trx levels were significantly higher than those of control group. ROS and RNS can not only directly cause cellular structure and function damage, but can also induce apoptosis through the Fas/FasL way. Fas is a member of the tumor necrosis factor superfamily, and its combination with FasL can start Caspase-8 cascade activation pathway, finally result in Caspase-3 activation and mediate apoptosis[17]; at the same time, ROS and RNS can also inhibit the anti-apoptosis molecules Survivin, XIAP and Bcl-2 expression, Survivin and XIAP can directly antagonize caspase activity to inhibit apoptosis, and Bcl-2 can inhibit mitochondrial way to inhibit apoptosis[18]. In the study, analysis of the contents of above apoptosis-related molecular enzymes in the placenta tissue showed that Fas, FasL, caspase-3 and caspase-8 protein expression in placenta tissue of LMWH group were significantly lower than those of control group while Survivin, XIAP and Bcl-2 protein expression were significantly higher than those of control group. Analysis of above oxidative stress and cell apoptosis shows that LMWH can reduce placental oxidative stress injury and inhibit cell apoptosis.

Above all, adjuvant low-molecular-weight heparin therapy is of positive clinical value for severe preeclampsia, and can relieve the placental perfusion, reduce placental ischemia hypoxia, and inhibit oxidative stress response and cell apoptosis.

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