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Low-molecular-weight heparin protects kidney through an anti-apoptotic mechanism in a rat pre-eclamptic model

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ABSTRACT

Objective: To investigate the effect of low-molecular-weight heparin (LMWH) for the treatment of pre-eclampsia (PE) and the underlying mechanism.
Study design: PE was established in a rat model using L-NG-nitroarginine methyl ester (L-NAME). The effect of LMWH was examined by measuring various physiological parameters known to be associated with PE.
Results: Blood pressure, urinary protein, blood urea nitrogen and serum creatinine were higher in L-NAME-treated rats compared with control rats. In addition, the number of fetuses, and the weight of fetuses and placentas was lower in L-NAME-treated rats, indicating the establishment of PE conditions. Apoptosis was found in kidney tissue in L-NAME-treated rats. LMWH treatment restored the PE-associated disorders, and inhibited apoptosis in kidney tissue in these rats.
Conclusions: LMWH can control PE conditions, protect renal function and improve fetal health. The mechanism of renal protection is likely to be related to the inhibitory effect of LMWH on apoptosis in kidney tissue. This study provides evidence that LMWH can potentially be used as a safe and effective anti-PE drug to improve PE conditions and protect renal function by inhibiting PE-induced apoptosis.

Introduction

Pre-eclampsia (PE) is a serious disorder that typically occurs after 20 weeks of pregnancy. It is usually associated with high blood pressure and urinary protein. Without treatment, PE can develop into life-threatening eclampsia. The incidence rate of PE is ~2% to 7% in developed countries, and three times higher in developing countries [1,2]. Worldwide, it is estimated that approximately 76,000 maternal and 500,000 infant deaths are caused by PE and other hypertension-related disorders in pregnancy each year. PE is a primary cause of maternal and perinatal mortality globally [3–5]. Unfortunately, it is difficult to control the occurrence of PE due to a lack of understanding of the underlying mechanism. Various clinical treatments have been applied in different cases. Usually, the duration of pregnancy is prolonged as much as possible to increase the neonatal survival rate and decrease the incidence of various complications in premature infants. However, if PE is severe and becomes life-threatening over time, the pregnancy has to be terminated in order to protect the mother. Due to the life-threatening risk of PE and the lack of effective treatment, there is an urgent need to understand the mechanism of PE and develop effective treatment to protect both the mother and baby.

Low-molecular-weight heparin (LMWH) is a safe and effective drug that has been used to treat various obstetric diseases. For example, LMWH has been suggested to treat recurrent miscarriage caused by obstetric thrombophilia [6], and to control and treat pregnancy thromboembolism [7]. In China, LMWH has been used empirically to prevent PE in pregnant women at 20 weeks of pregnancy or even earlier. Once PE is established, LMWH treatment can also help to control blood pressure, reduce urinary protein and improve neonatal outcomes. Such evidence has been observed in China; however, the results have not been evaluated systematically in clinical studies. Recent studies have found that the formation of microthrombus caused by the damage of vascular endothelial cells may be related to PE, leading to the hypothesis that LMWH may improve the prognosis in cases of PE [8].

In order to use LMWH in cases of PE, the relationship between LMWH and PE and the underlying mechanism need to be understood. This study established a rat PE model using L-NG-nitroarginine methyl ester (L-NAME), and investigated the effects of LMWH on PE [9–12]. As the kidney is the organ to experience

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greatest damage in cases of PE, the mechanism of PE-induced kidney malfunction was examined, and the effect of LMWH on renal protection was evaluated. LMWH was found to control PE, protect renal function and improve fetal health. The mechanism of renal protection is likely to be related to the inhibitory effect of LMWH on apoptosis in kidney tissue.

Materials and methods

Model establishment

Wistar rats were used in this study. All rats were purchased from the Animal Centre of Southern Medical University, Guangzhou, China and handled in accordance with the guidelines of the Institutional Animal Care and Use Committee. Female rats were 8–10 weeks old and weighed 200–230 g, and male rats were 9–11 weeks old and weighed 330–340 g.

Rats were mated using a 1:1 ratio. Female rats were examined for the presence of a vaginal plug on the second day of mating at 6:00 am. Rats with a plug were considered to be pregnant, and this was defined as Gestational Day 1. L-NAME 200 mg/kg/day (Cayman Chemical Co., Ann Arbor, MI, USA) was loaded continuously for 5 days from Gestational Day 13. Blood pressure was monitored in female rats prior to pregnancy, on Gestational Day 15 (third day of L-NAME treatment) and on Gestation Day 21 (after LMWH treatment). The PE model was considered to be successful if blood pressure increased to $\geq$30 mmHg after L-NAME treatment.

Animal groupings

From 50 female rats, 10 were selected at random for the non-pregnant group, and injected with 0.5 ml saline. The remaining 40 female rats were mated with male rats using a 1:1 ratio. After mating, 10 pregnant rats were selected at random for the control group, and injected with 0.5 ml saline on Gestational Days 13 and 20. The remaining 30 pregnant rats were used to establish the PE model. The model was established successfully in 23 rats, 20 of which were selected at random and divided into two groups of 10 rats: the PE group and the LMWH group. Rats in the PE group were injected with 0.5 ml saline from Gestational Day 15 to Gestational Day 20, and rats in the LMWH group were injected with 40 μl/kg LMWH (40 μl LMWH has 380 IU of anti-factor Xa activity) (Fraxiparine GSK, Tianjin, China).

Blood pressure measurement

Systolic blood pressure of the rats was measured before pregnancy and on Gestational Days 15 and 21 using the BP-6 animal non-invasive blood pressure measuring system (Taimeng, Chengdu, China).

24-h urinary protein determination

Rats were placed in metabolism cages at 8:00 am on Gestational Days 1 and 20, and urine was collected for 24 h. Urine samples were analysed on the second day to quantify 24-h urinary protein.

Renal function analysis

Rats were anesthetized with 3% pentobarbital sodium on Gestational Day 21. Blood samples (2–3 ml) were obtained from the femoral artery and stored in a clean, dry biochemistry tube. Blood urea nitrogen (BUN) and serum creatinine (SCr) were determined using an automatic biochemistry analyser. The kidneys were removed rapidly and placed on sterile gauze. Part of each kidney was stored in liquid nitrogen and the remainder was fixed with 100% formalin for analysis.

Detection of apoptotic index

The extent of apoptosis in kidney tissue was detected using the terminal deoxynucleotidyl transferase dUTP nick end-labeling (TUNEL) detection kit (Merck, Munich, Germany). Nuclei of apoptotic cells had a brownish–yellow color. The numbers of apoptotic cells and total cells were counted. The number of apoptotic cells per 100 total cells was used to calculate the apoptotic index.

Measurement of mRNA levels by quantitative polymerase chain reaction

The total RNA of the kidney tissue was extracted using a RNAprep pure animal total RNA extraction kit (Tiangen, Beijing, China). RNA quality was assessed by agarose gel electrophoresis, and purity was determined using a spectrophotometer. cDNA was synthesized using a Quantscript RT kit (Tiangen). mRNA levels of caspase-3, Bcl-2 and Bcl-2-associated X protein (Bax) were measured using a RealMaster Mix kit (Tiangen) for quantitative polymerase chain reaction (PCR). For each sample, ΔCt was calculated by subtracting Ct(β-actin) from Ct(sample). The mRNA level was then calculated using $2^{-\Delta Ct}$. The mRNA level in each group was normalized to the non-pregnant group. The primers for rat caspase-3, Bcl-2 and Bax were designed using Primer 5.0 PCR software and synthesized by Shanghai Invitrogen Co. (Shanghai, China).

Statistical analysis

Statistical Package for the Social Sciences Version13.0 (IBM Corp., Armonk, NY, USA) was used for statistical analyses. All results were expressed as mean ± standard deviation. Error bars were calculated based on $n = 10$ in each group. Multiple comparisons among various groups were carried out by one-way analysis of variance. Least-square difference was used when the data were normally distributed; otherwise, Dunnett's T3 method was used after the data were calibrated using Welch's method [13]. $p < 0.05$ was considered to indicate significance.

Results

LMWH reduced blood pressure in PE model

Blood pressure was measured at three time points: pre-pregnancy, Gestational Day 15 and Gestational Day 21 (Fig. 1). For the control group, blood pressure remained constant throughout the study. There was no difference in blood pressure between the control group and the non-pregnant group, suggesting that pregnancy did not induce hypertension in the study. No differences were found in pre-pregnancy blood pressure between all groups. However, on Gestational Day 15, blood pressure was significantly higher in the PE and LMWH groups compared with the control group, indicating successful establishment of the PE model. On Gestational Day 21, blood pressure was significantly lower in the LMWH group compared with the PE group. However, blood pressure remained higher in the LMWH group compared with the control group.

LMWH reduced urinary protein in PE model

Urinary protein is usually associated with PE, indicating renal malfunction. Urinary protein was measured in this study (Fig. 2). On Gestational Day 1, all groups had a similar level of urinary