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Original Research Article

Aspirin protects against preeclampsia via p38MAPK signaling pathway

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Abstract

Purpose: *To investigate the protective effect of aspirin against preeclampsia and the involvement of p38MAPK signaling pathway in the process.*
Methods: *Sixty pregnant women who underwent antenatal care and delivery at Chancheng Central Hospital from September 2020 to September 2022 were selected and equally assigned to control group (CG) and experimental group (EG). From the 12th week of gestation, EG was administered 100 mg of aspirin and 1000 mg of calcium carbonate daily, while CG was given only 1000 mg of calcium carbonate daily. Both groups were treated up to the 35th week of gestation. Thereafter, blood samples were taken for measurement of serum levels of p38MAPK. In addition, the blood pressure of the women was measured. The incidence of preeclampsia and maternal-infant outcomes were assessed.*
Results: *EG had a lower p38MAPK level at week 35 of pregnancy, and lower blood pressure levels at the 27th and 35th weeks of gestation, than CG ($p < 0.05$). There were 5 cases of preeclampsia (16.7 %) in EG, and 13 cases (43.3 %) of preeclampsia in CG, with a lower incidence of preeclampsia in EG than in CG ($\chi^2 = 5.079$, $p < 0.05$). The numbers of newborns through premature delivery and cesarean section, as well as Apgar score ≤ 7 were lower in EG than in CG ($p < 0.05$).*
Conclusion: *Aspirin exerts a protective effect against preeclampsia through via p38MAPK signaling pathway. Therefore, aspirin treatment may be useful in reducing the incidence of preeclampsia and improving maternal-infant outcomes. However, further clinical trials are recommended prior to application in clinical practice.*

Keywords: *Aspirin, p38MAPK, Preeclampsia, Protective effect*

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INTRODUCTION

Preeclampsia often leads to adverse pregnancy outcomes such as restriction of fetal growth, placental abruption, fetal distress, and in severe cases, death of pregnant women, which make it a serious threat to mothers and infants [1]. At present, the incidence of preeclampsia has reached 8.0 % in China [2]. Due to the unclear pathogenesis of preeclampsia, only symptomatic treatment is used in the clinic, but its effects on the prevention of preeclampsia remain unsatisfactory.

Research has revealed that p38MAPK is crucial in the occurrence and progression of

preeclampsia [3]. Indeed, p38MAPK signal transduction pathways mediate cell injury, inflammation, and oxidative stress, and p38MAPK is significantly activated in the placenta of patients with preeclampsia [4]. Therefore, it may be speculated that the abnormal infiltration of extravillous trophoblasts caused by abnormal expression of p38MAPK is important in the pathogenesis of preeclampsia. Aspirin is a common drug for the prevention of preeclampsia [5].

A study has found that aspirin increases the release of extravillous trophoblast cytokines and improves the function of trophoblasts [6]. Therefore, the effect of aspirin on inflammation, vascular endothelial injury, and trophoblasts may be closely associated with the p38MAPK signal route. Presently, there are limited relevant reports in China and elsewhere about the influence of aspirin on this signal route. Therefore, this research was focused on the determination of the mitigating influence of aspirin on preeclampsia in early pregnancy.

METHODS

Study plan

This research was carried out in Chancheng Central Hospital from February 2020 to February 2021, to investigate the protective influence of aspirin against preeclampsia and the involvement of the p38MAPK signal route in the process. The study was double-blinded, and neither the subjects nor the researchers knew about the grouping used in the trial.

General patient information

Sixty pregnant women who underwent pregnancy examination, and who delivered at Chancheng Central Hospital from September 2020 to September 2022, were selected and equally assigned to the control group (CG) and experimental group (EG). The pregnant women in EG were of an average age of 28.47 ± 5.02 years, mean body mass of 65.98 ± 5.14 kg, and mean BMI of 26.74 ± 2.10 kg/m^2. The pregnant women of CG were of an average age of 28.43 ± 5.35 years, mean body mass of 66.12 ± 5.24 kg, and mean BMI of 26.80 ± 2.14 kg/m^2. Baseline data of pregnant women in both groups were comparable.

Inclusion criteria

Pregnant women with a history of pregnancy-induced hypertension (PIH) [7], women who were in their second pregnancy, those with complete clinical data who were treated in the hospital throughout the whole period, those with natural pregnancy, and expectant mothers aged ≥ 18 years, were included in this study.

Exclusion criteria

Patients who met the following criteria were excluded: pregnant women who were unable to relate with others as a result of auditory impairment, speech problems, coma, or psychological issues; those with internal and surgical diseases e.g. hyperthyroidism, those with severe organic diseases or major organ dysfunction, patients with infection and diabetes, those who took hormone drugs during pregnancy, expectant women who conceived through assisted reproductive technology, and those who quit the study halfway.

Ethical considerations

This study received approval from the ethical authority of Foshan Fosun Chancheng Hospital (approval no. 20191126), and it was done in line with the guidelines of the Helsinki Declaration [8]. The pregnant women and their relatives received information about the aim, significance, content, and confidentiality of this research, and they signed informed consent forms.

Treatments

From the 12th week of gestation, the pregnant women in EG were administered 100 mg of aspirin (Hunan Zhongnan Pharmaceutical Company Limited) plus 1000 mg of calcium carbonate (Shanghai Tansuangai Factory Co. Ltd.; NMPA approval No. H31020140), while those in CG were given only 1000 mg of calcium carbonate daily. Both groups were treated up to the 35th week of gestation. During treatment, both groups received standardized perinatal health care while maintaining proper diets, appropriate rest, and moderate exercise. In addition, blood pressure and weight gain were closely monitored, and hematological and urinary indices, hepatic and renal functions, and blood clotting function were regularly measured every two weeks.

Treatment indices

Level of p38MAPK

Venous blood (2 mL) was taken from the elbow of each pregnant woman before and after treatment, and placed in a water bath box (37 °C; 3 h), followed by 5 min-centrifugation at 2000 rpm to separate serum. Then, the serum sample

was injected into sterilized glass test tubes for the measurement of p38MAPK levels with ELISA kits. The experimental protocol strictly followed the kit instructions.

Blood pressure

The blood pressure values of pregnant women were measured once a week from the 12th week of gestation. Before measurement, the pregnant women rested for 15 min to avoid the influence of exercise factors on blood pressure. Cuffs of suitable sizes were prepared for them, and they were in a sitting position, with legs flat on the floor. A mercury (Hg) sphygmomanometer was used to measure blood pressure. The blood pressure values of the pregnant women were recorded at the 12th, 27th, and 35th weeks of gestation.

Incidence of preeclampsia

Preeclampsia was diagnosed following the Guide for Diagnosis & Treatment of Hypertension and Preeclampsia in Pregnancy developed by the Group of Hypertensive Disorders in Pregnancy, Obstetrics and Gynecology Branch of the Chinese Medical Association [9]. The population of preeclampsia patients was recorded.

Maternal-infant outcomes

In addition to preeclampsia, the maternal-infant outcomes were premature delivery, cesarean section, postpartum hemorrhage, placental abruption, infants with low birth weights, perinatal death, fetal intracranial hemorrhage, newborns transferred to ICU, and neonatal Apgar score ≤ 7 points.

Statistical analysis

In this study, the data were processed using the SPSS 23.0 software, while graphs were prepared with GraphPad Prism 7 (GraphPad Software, San Diego, USA). Measurement data are expressed as mean ± SD. Laboratory results with skewed distribution were analyzed with the Friedman rank sum test. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Levels of p38MAPK

Figure 1 shows a lower p38MAPK level in EG than in CG at week 35 of pregnancy.

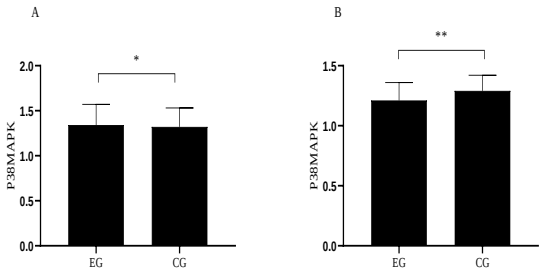

Figure 1: Comparison of p38MAPK levels. (A): p38MAPK levels at the 12th week of gestation, (B): p38MAPK levels at week 35th of pregnancy. *$P > 0.05$, p38MAPK level in EG vs p38MAPK level in CG at week 12 of pregnancy; **$p < 0.001$, p38MAPK level in EG vs p38MAPK level in CG at the 35th week of gestation

Blood pressure values

There were lower blood pressure values in EG than in CG at the 27th and 35th weeks of gestation ($p < 0.05$), as demonstrated in Figure 2.

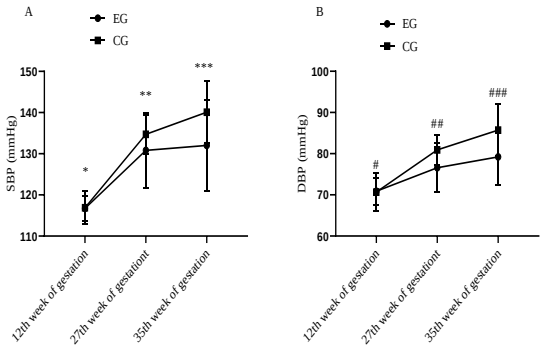

Figure 2: Comparison of blood pressure (mmHg). (A) Systolic blood pressure (SBP); (B) Diastolic blood pressure (DBP). *$P > 0.05$, SBP in EG vs SBP in CG at week 12 of pregnancy; **$p < 0.05$, SBP in EG vs SBP in CG at week 27 of pregnancy; ***$p < 0.001$, SBP in EG vs SBP in CG at week 35 of pregnancy; #$p > 0.05$, DBP in EG vs DBP in CG at week 12 of pregnancy; ##$p < 0.001$, DBP in EG vs DBP in CG at week 27 of pregnancy; ###$p < 0.001$, DBP in EG vs DBP in CG at the 35th week of gestation

Incidence of preeclampsia

Preeclampsia occurred in 5 women (16.7 %) in EG, and in 13 women (43.3 %) in CG, with a lower incidence of preeclampsia in EG than in CG ($\chi^2 = 5.079$, $p < 0.05$).

Maternal-infant outcomes

The numbers of newborns through premature delivery and cesarean section, and Apgar score

Table 1: Comparison of maternal-infant outcomes (n = 30; (%))

Outcome	EG	CG	χ^2	*P*-value
Premature delivery	0(0.0)	4(13.3)	4.286	0.038
Cesarean section	2(6.7)	8(26.7)	4.320	0.038
Postpartum hemorrhage	0(0.0)	1(3.3)	1.017	0.313
Placental abruption	0(0.0)	1(3.3)	1.017	0.313
Low birth weight infants	1(3.3)	3(10.0)	1.071	0.301
Perinatal death	0(0.0)	0(0.0)	-	-
Fetal intracranial hemorrhage	0(0.0)	1(3.3)	1.017	0.313
Newborns transferred to ICU	1(3.3)	2(6.7)	0.351	0.554
Neonatal Apgar score ≤ 7	0(0.0)	5(16.7)	5.455	0.020

≤ 7 in EG were lower than those in CG ($p < 0.05$; Table 1).

DISCUSSION

Extravillous trophoblasts invade uterine decidua, uterine shallow myometrium, and spiral arterioles after implantation of fertilized eggs, and physiologically reshape the spiral arterioles [10]. If an abnormal invasion of extravillous trophoblasts occurs, shallow placental implantation and insufficient uterine-placenta perfusion may appear. This will lead to long-term hypoxia and ischemia of the syncytiotrophoblasts, and the release of a large number of inflammatory factors, thereby triggering oxidative stress and vascular endothelial injury [11]. In addition, the secretion of prostaglandin is reduced, while the secretion of thromboxane A_2 is enhanced, leading to an imbalance in the ratio of prostaglandin to thromboxane A_2, abnormal function, and a hypercoagulable state of the blood. Therefore, there is aggravated disturbance of microcirculation, as well as damage to major organs such as the heart, brain, and lungs of pregnant women, resulting in microcirculation disturbance in the uterus and placenta [12]. Microcirculation disturbance in the uterus and placenta induces adverse pregnancy outcomes, an example of which is preeclampsia. The Chinese Guidelines for the Diagnosis and Treatment of Pregnancy-Induced Hypertension [13] and the American College of Obstetricians and Gynecologists [14] recommend the application of low-dose aspirin as an anticoagulant in high-risk pregnant women in order to prevent preeclampsia. Aspirin irreversibly inhibits the synthesis of cyclooxygenase, regulates the ratio of prostaglandin to thromboxane A_2, reduces hypoxia and ischemia in placental tissues, and prevents platelet aggregation, thereby effectively improving coagulation function in pregnant women, and preventing fibrin deposition [15]. Moreover, improved coagulation and the absence of fibrin deposition reduce the possibility of pregnancy complications and other adverse pregnancy outcomes. Therefore, the number of newborns with premature delivery, cesarean section, and the Apgar score ≤ 7 was lower in EG than in CG.

A previous study has suggested that the protective effect of aspirin against preeclampsia lies in maintaining the balance in the ratio of prostaglandin to thromboxane A_2 so as to ensure microcirculation in the placenta [16]. However, a recent study has further revealed that aspirin accelerated the release of cytokines from extravillous trophoblasts, reduced their apoptosis, and ultimately improved the infiltration capacity of extravillous trophoblasts [17]. The p38MAPK is widely present in the cytoplasm of extravillous trophoblasts. It is activated by various extracellular stress reactions, and it plays a key role in hypoxia-induced preeclampsia through enzymes and cascades related to extracellular signals. It has been reported that the p38MAPK mRNA in the placenta of patients with preeclampsia is significantly higher than that in normal pregnant women, while the expression of p38MAPK in women with serious preeclampsia is higher than that in those having moderate preeclampsia. These data suggest that increased transcription and translation of the p38MAPK gene impair vascular endothelial growth factors and accelerate apoptosis of extravillous trophoblasts, thereby affecting the infiltration capacity of the trophoblasts and inducing preeclampsia [18]. At present, there are limited studies on the effect of aspirin on the p38MAPK signaling pathway. This study has revealed that the p38MAPK level in EG at the 35^{th} week of gestation was lower than that of CG, indicating that aspirin treatment decreased the level of p38MAPK. The decreased p38MAPK level indicated an increased number of villous vessels, reduced fibrinoid necrosis, inhibited inflammatory aggregation and chemotaxis of neutrophils, and decreased capacity of oxygen free radicals and proteolytic enzymes to damage the vascular endothelium [19]. Thus, pregnancy-induced hypertension is alleviated by

enhancement of the infiltration ability of cells and mitigation of vascular endothelial injury. Therefore, blood pressure levels at the 27th and 35th weeks of gestation were lower in EG than in CG. Blood pressure is positively correlated with the incidence of preeclampsia, with higher blood pressure levels indicating a higher incidence [20].

Therefore, a decreased blood pressure level is conducive for reducing the occurrence of preeclampsia. At the same time, due to the reduced infiltration of cells and alleviation of vascular endothelial injury, the incidence of preeclampsia was lower in EG than in CG. This indicates that aspirin indeed reduced the incidence of preeclampsia through modulation of the p38MAPK signaling pathway.

Limitations of the study

It is worth noting that although this study investigated the influence of aspirin on the p38MAPK signal route by analyzing changes in p38MAPK levels in pregnant women, it did not investigate the specific molecular mechanism involved. Therefore, the molecular mechanism through which aspirin affected the p38MAPK signaling pathway needs further research to provide more scientific foundations for its practical application.

CONCLUSION

Aspirin exerts a protective effect against preeclampsia through the p38MAPK signaling pathway. Thus, aspirin treatment may be suitable for the reduction of the incidence of preeclampsia and improvement in maternal-infant outcomes. However, further clinical trials are recommended prior to application in clinical practice.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Min Su and Binying Zhou conceived and designed the study, and drafted the manuscript. Min Su, Binying Zhou, Manhua Zhen, and Jiasi Liu collected, analyzed, and interpreted the experimental data. Binying Zhou and Manhua Zhen revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

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