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Original Article

Berberine alleviates preeclampsia possibly by regulating the expression of interleukin-2/interleukin-10 and Bcl-2/Bax

Ailan Wang[1], Qingcui Liu[1], Jin Zhang[1], Rui Zheng[2]

[1]Department of Obstetrics and Gynecology, The Children and Women's Healthcare Hospital, Laiwu City, Shandong Province, P. R. China; [2]Medical College, Qingdao University, Qingdao, Shandong Province, P. R. China

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Abstract: The present study is to investigate the effect of berberine on the expression of inflammatory factors interleukin (IL)-2 and IL-10, and the expression of apoptosis proteins Bcl-2 and Bax. A total of 70 SD rats were randomly divided into 7 equal groups, including normal non-pregnant group, normal pregnant group, preeclampsia group, preeclampsia + berberine (50, 100, and 200 mg/kg/day) groups, and preeclampsia + nifedipine (20 mg/kg/day) group. Blood pressure was measured before pregnancy, and on day 15 and 21 of pregnancy. Urines before pregnancy and on day 15 and 21 of pregnancy were collected for the determination of urine protein levels. Peripheral blood was collected from all rats on day 21 of pregnancy to measure the levels of blood urea nitrogen and creatinine. On day 21 of pregnancy, the weight of fetuses and placentas, and the number of normal fetuses were determined. Enzyme-linked immunosorbent assay was performed to determine the levels of IL-2 and IL-10 in plasma. Western blotting was used to measure the expression of Bcl-2 and Bax proteins in placenta of rats with preeclampsia. Treatment with berberine for seven days reduced blood pressure, urine proteins levels, and kidney function in rats with preeclampsia. Berberine improved the number of normal fetuses and the weight of fetuses and placentas from rats with preeclampsia. Berberine up-regulated IL-10 and down-regulated IL-2 in the peripheral blood of SD rats with preeclampsia. Berberine up-regulated Bcl-2 and down-regulated Bax in the placenta of SD rats with preeclampsia. Berberine increases the number and weight of normal fetuses in rats with preeclampsia, possibly by regulating the balance of IL-2 and IL-10, and inhibiting apoptosis.

Keywords: Berberine, preeclampsia, inflammatory factors, apoptosis

Introduction

Preeclampsia is a disease that is characterized by hypertension and albuminuria occurring after 20 weeks of pregnancy [1]. Preeclampsia is an important reason for the deaths of pregnant women and perinatal fetuses, with relatively high incidence [2]. However, the mechanism of preeclampsia is not clear yet. It is reported that preeclampsia may be related to the imbalance of inflammatory reaction and apoptosis [3]. Another study shows that cytokine interleukin (IL)-2 is up-regulated, but IL-10 is down-regulated during preeclampsia, breaking the inflammatory balance [4]. In addition, 15-50% cytotrophoblasts in fixation villi of preeclampsia patients undergo apoptosis, while nearly no apoptosis is detected in healthy pregnant women [5]. Bcl-2 and Bax are apoptotic proteins that participate in the pathological process of preeclampsia under the regulation by AP-2α [6].

Berberine is a quaternary ammonium salt from the protoberberine group of isoquinoline alkaloids, which is extracted from the rhizome of *Coptis chinensis*. Studies show that berberine lowers elevated blood total cholesterol, reduces platelet aggregation, alleviates arrhythmia, and resists inflammation. Berberine inhibits autoimmunity and humoral immunity, reduces the production of inflammatory factors, and enhances Bcl-2 expression, suggesting that berberine prevents the occurrence of preeclampsia by inhibiting inflammatory factors and apoptosis [7-9]. In the present study, we

investigate whether berberine alleviates preeclampsia, and its effect on the expression of IL-2 and IL-10, the expression of Bcl-2 and Bax proteins, and cell apoptosis.

Materials and methods

Animals

SD rats (Animal Experimental Center of Luzhou Medical College, Luzhou, China; n = 70) were randomly divided into 7 equal groups, including normal non-pregnant group, normal pregnant group, preeclampsia group, preeclampsia + berberine (50, 100, and 200 mg/kg/day; Lot No. 20130107, Purifa, Chengdu, China) groups, and preeclampsia + nifedipine (20 mg/kg/day) group. Preeclampsia group was made by subcutaneous injection of N-nitro-L-arginine methylester (L-NAME, 200 mg/kg/day; Cayman Chemical Company, Ann Arbor, MI, USA) from day 13 of pregnancy for consecutive 4 days. All groups of pregnant rats were treated on day 15 of pregnancy. Normal non-pregnant group and normal pregnant group were given normal saline. Preeclampsia group was also treated with normal saline after the preeclampsia model was successfully built. Preeclampsia + berberine groups were treated with gastric lavage of berberine (50, 100, and 200 mg/kg/day) once a day for consecutive seven days. Preeclampsia + nifedipine group was treated with gastric lavage of nifedipine (20 mg/kg/day) once a day for consecutive seven days.

Blood pressure was measured before pregnancy, and on day 15 and 21 of pregnancy (BP-6 non-invasive blood pressure measuring instrument, Taimeng Science and Technology Company, Chengdu, China). Urines before pregnancy and on day 15 and 21 of pregnancy were collected for the determination of urine protein levels. Peripheral blood (3 ml) was collected from all rats on day 21 of pregnancy to measure the levels of blood urea nitrogen and creatinine for the evaluation of the functions of liver and kidney. On day 21 of pregnancy, the fetuses and placentas were taken out by cesarean section for determining the weight, and the number of normal fetuses was counted. All animal experiments were conducted according to the ethical guidelines of The Children and Women's Healthcare Hospital of Laiwu City.

Enzyme-linked immunosorbent assay (ELISA)

The levels of IL-2 and IL-10 in plasma were measured using the ELISA kit (USCN Life Science Inc., Wuhan, China). The procedure was carried out according to the manufacturer's manual. Absorbance at 450 nm was measured using a microplate reader (model 550, Rio-Rad, Hercules, CA, USA) within 15 min after stopping the reactions.

Western blotting

Placenta (50 mg) was added into precooled radio-immunoprecipitation assay (RIPA) lysis buffer (200 μl; Beyotime Institute of Biotechnology, Shanghai, China). After lysis for 10 min, the mixture was centrifuged at 12000×g/min and 4°C for 10 min (SORVALL Stratos, Thermo Fisher Scientific, Waltham, MA, USA). The supernatant was used to determine protein concentration by bicinchoninic acid (BCA) protein concentration determination kit (RTP7102, Real-Times Biotechnology Co., Ltd., Beijing, China). Protein samples (50 μg) were then mixed with equal volume of 2× sodium dodecyl sulfate loading buffer before denaturation in boiling water bath for 5 min. Afterwards, 10 μl samples were subject to sodium dodecyl sulfate-polyacrylamide gel electrophoresis at 100 V (PAC300, Bio-Rad, Hercules, CA, USA). The resolved proteins were transferred to polyvinylidene difluoride membranes on ice (300 mA, 2 h) and blocked with 50 g/L skimmed milk at room temperature for 1 h. Then, the membranes were incubated with rabbit anti-human Bcl-2 antibody (1:5000), rabbit anti-human Bax antibody (1:5000) and rabbit anti-human β-actin antibody (1:5000) (Cell Signaling Technology, Danvers, MA, USA) at 4°C overnight. After extensive washing with phosphate-buffered saline with Tween 20 for 3 times of 15 min, the membranes were incubated with rabbit anti-human horseradish peroxidase-conjugated antibody (1:1000; Cell Signaling Technology, Danvers, MA, USA) for 1 h at room temperature before washing with phosphate-buffered saline with Tween 20 for 3 times of 15 min. Then, the membrane was developed with enhanced chemiluminescence detection kit (Sigma-Aldrich, St. Louis, MO, USA) for imaging. Image pro-plus 6.0 (Media Cybernetics, Inc., Rockville, MD, USA) software was used to

Table 1. Effect of berberine on the blood pressure of SD rats with preeclampsia

Groups	Progestational blood pressure (mmHg)	Blood pressure on day 15 (mmHg)	Blood pressure on day 21 (mmHg)
Normal non-pregnant	114.50 ± 7.37	117.60 ± 5.67**	119.60 ± 8.23**
Normal pregnant	118.10 ± 5.07	121.60 ± 6.17**	127.30 ± 3.82**
Preeclampsia	117.30 ± 5.42	161.40 ± 7.05	162.80 ± 6.05
Preeclampsia + berberine (50 mg/kg/day)	116.60 ± 7.54	156.70 ± 6.11	141.10 ± 7.07*
Preeclampsia + berberine (100 mg/kg/day)	117.90 ± 6.07	158.40 ± 7.93	137.20 ± 5.08*
Preeclampsia + berberine (200 mg/kg/day)	116.90 ± 3.04	157.33 ± 5.61	129.20 ± 7.15**
Preeclampsia + nifedipine (20 mg/kg/day)	118.10 ± 7.52	160.23 ± 6.91	121.20 ± 4.18**

Note: *, $P < 0.05$; **, $P < 0.01$ compared with preeclampsia group.

Table 2. Effect of berberine on the level of urine protein in SD rats with preeclampsia

Groups	Progestational urine proteins (mg/day)	Urine protein on day 15 (mg/day)	Urine protein on day 21 (mg/day)
Normal non-pregnant	5.14 ± 0.41	4.79 ± 0.61**	4.81 ± 0.67**
Normal pregnant	5.27 ± 0.34	5.77 ± 0.45**	5.64 ± 0.71**
Preeclampsia	5.30 ± 0.33	11.61 ± 1.42	13.9 ± 0.83
Preeclampsia + berberine (50 mg/kg/day)	5.14 ± 0.27	11.32 ± 0.78	8.02 ± 0.91*
Preeclampsia + berberine (100 mg/kg/day)	5.21 ± 0.33	11.44 ± 1.46	7.56 ± 0.74**
Preeclampsia + berberine (200 mg/kg/day)	5.22 ± 0.18	11.75 ± 0.97	6.88 ± 0.78**
Preeclampsia + nifedipine (20 mg/kg/day)	5.34 ± 0.49	11.07 ± 0.34	6.75 ± 0.19**

Note: *, $P < 0.05$; **, $P < 0.01$ compared with preeclampsia group.

Table 3. Effect of berberine on kidney function of SD rats with preeclampsia

Groups	Blood urea nitrogen (mmol/L)	Creatinine (μmol/L)
Normal non-pregnant	5.37 ± 0.61**	34.75 ± 6.44**
Normal pregnant	5.32 ± 0.74**	35.12 ± 4.73**
Preeclampsia	11.32 ± 1.31	49.56 ± 3.42
Preeclampsia + berberine (50 mg/kg/day)	6.78 ± 1.07*	37.54 ± 4.67*
Preeclampsia + berberine (100 mg/kg/day)	6.15 ± 0.97**	35.42 ± 5.01**
Preeclampsia + berberine (200 mg/kg/day)	5.91 ± 0.48**	34.76 ± 4.87**
Preeclampsia + nifedipine (20 mg/kg/day)	5.67 ± 0.52**	34.16 ± 3.41**

Note: *, $P < 0.05$; **, $P < 0.01$ compared with preeclampsia group.

acquire and analyze imaging signals. The relative contents of Bcl-2 and Bax proteins were calculated using β-actin as reference.

Statistical analysis

All results were analyzed using SPSS 16.0 (IBM, Armonk, NY, USA). Measurement data were expressed as means ± standard deviation. Comparison of mean data of multiple groups was performed using single factor analysis of variance, while intergroup comparison was performed using SNK method. $P < 0.05$ was considered statistically significant.

Results

Treatment with berberine for seven days reduces blood pressure, urine proteins levels, and kidney function in rats with preeclampsia

To investigate the effect of berberine on the physiology of rats, blood pressure, the levels of urine proteins and the levels of blood urea nitrogen and creatinine were measured. The data showed that progestational blood pressure and blood pressure on day 15 of rats treated with berberine (50, 100, and 200 mg/kg/day) were not significantly different from those of rats in preeclampsia group ($P > 0.05$). However, blood pressure on day 21 of rats treated with berberine was significantly lower than that of rats in preeclampsia group ($P < 0.05$). Compared with rats in preeclampsia group, the levels of urine proteins, blood urea nitrogen and creatinine on day 21 were signifi-

Table 4. Effect of berberine on the development of fetuses from SD rats with preeclampsia

Groups	No. of normal fetuses	Fetus weight (g)	Placenta weight (g)
Normal non-pregnant	0	0	0
Normal pregnant	9.74 ± 0.37**	3.11 ± 0.24**	0.58 ± 0.06*
Preeclampsia	7.41 ± 0.32	2.32 ± 0.18	0.41 ± 0.02
Preeclampsia + berberine (50 mg/kg/day)	9.15 ± 0.07	2.91 ± 0.07*	0.49 ± 0.03
Preeclampsia + berberine (100 mg/kg/day)	9.42 ± 0.35	3.04 ± 0.15**	0.51 ± 0.04
Preeclampsia + berberine (200 mg/kg/day)	9.77 ± 0.33*	3.07 ± 0.21**	0.54 ± 0.07**
Preeclampsia + nifedipine (20 mg/kg/day)	9.64 ± 0.44*	3.13 ± 0.11**	0.55 ± 0.05**

Note: *, P < 0.05; **, P < 0.01 compared with preeclampsia group.

Table 5. Effect of berberine on serum concentrations of interleukin-2 and interleukin-10 in SD rats with preeclampsia

Groups	Interleukin-2 (pg/ml)	Interleukin-10 (ng/ml)
Normal non-pregnant	217.38 ± 12.45	13.67 ± 3.21
Normal pregnant	132.48 ± 11.37**	15.94 ± 1.24**
Preeclampsia	387.64 ± 27.48Δ	7.32 ± 1.13Δ
Preeclampsia + berberine (50 mg/kg/day)	311.02 ± 11.56	9.91 ± 0.07*
Preeclampsia + berberine (100 mg/kg/day)	297.18 ± 19.34	12.04 ± 0.15**
Preeclampsia + berberine (200 mg/kg/day)	276.41 ± 22.54*	13.07 ± 0.21**
Preeclampsia + nifedipine (20 mg/kg/day)	251.65 ± 31.36*	13.21 ± 0.11**

Note: *, P < 0.05; **, P < 0.01 compared with preeclampsia group; Δ, P < 0.05 compared with normal pregnant group.

cantly reduced (P < 0.05) (**Tables 1**-**3**). The results suggest that treatment with berberine for seven days reduces blood pressure, urine proteins levels, and kidney function in rats with preeclampsia.

Berberine improves the number of normal fetuses and the weight of fetuses and placentas from rats with preeclampsia

To test the effect of berberine on the development of rat fetuses, the number of normal fetuses and the weight of fetuses and placentas were determined. The data showed that the number of normal fetuses from rats with preeclampsia was significantly lower than that from normal pregnant rats (P < 0.05). In addition, the weight of fetuses and placentas from rats with preeclampsia was significantly reduced compared with that from normal pregnant rats (P < 0.05). Of note, rats with preeclampsia treated with berberine had significantly increased number of normal fetuses and enhanced fetus and placenta weight compared with rats with preeclampsia (P < 0.05) (**Table 4**). These results indicate that berberine improves the number of normal fetuses and the weight of fetuses and placentas from rats with preeclampsia.

Berberine up-regulates IL-10 and down-regulates IL-2 in the peripheral blood of SD rats with preeclampsia

To determine the levels of IL-2 and IL-10 in the plasma of rats with preeclampsia, ELISA was performed. The data showed that the concentration of anti-inflammatory factor IL-10 in the plasma of normal pregnant group was significantly higher than that of normal non-pregnant group (P < 0.05), while the plasma concentration of proinflammatory factor IL-2 in normal pregnant group was significantly lower than that of normal non-pregnant group (P < 0.05). In addition, IL-10 concentration in preeclampsia group was significantly lower than that in normal pregnant group (P < 0.05), while IL-2 concentration in preeclampsia group was significantly higher than that in normal pregnant group (P < 0.05). Compared with preeclampsia group, treatment with berberine significantly enhanced the concentration of IL-10 (P < 0.05), and significantly reduced the concentration of IL-2 (P < 0.05) (**Table 5**). The results suggest that berberine up-regulates IL-10 and down-regulates IL-2 in the peripheral blood of SD rats with preeclampsia.

Berberine up-regulates Bcl-2 and down-regulates Bax in the placenta of SD rats with preeclampsia

To measure the expression of Bcl-2 and Bax in the placenta of rats with preeclampsia, Western

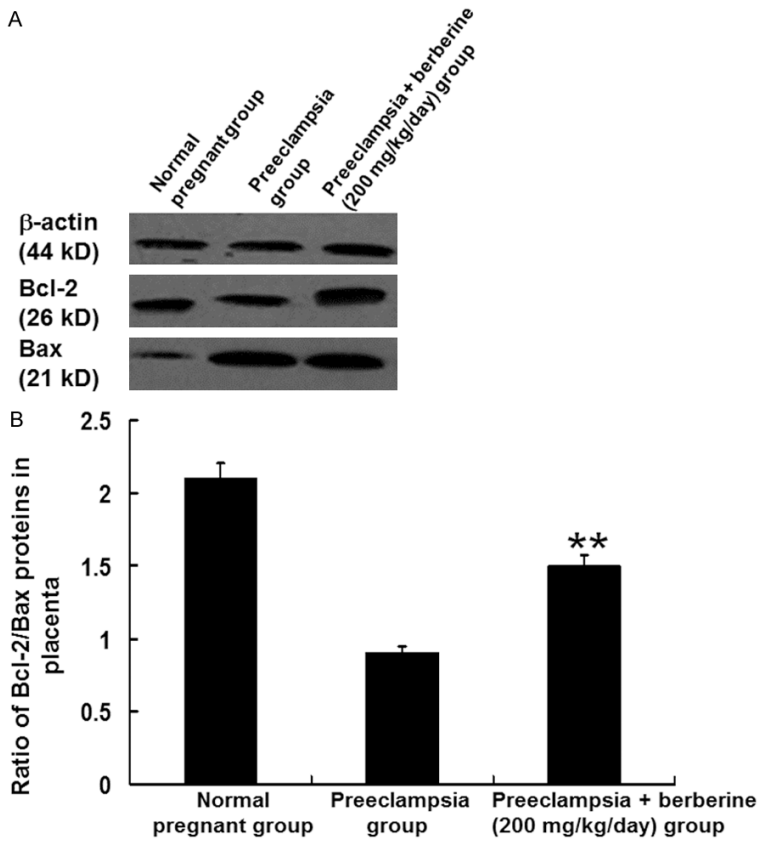

Figure 1. Expression of Bcl-2 and Bax in the placenta of SD rats. A. Levels of Bcl-2 and Bax proteins determined by Western blotting. B. Ratio of Bcl-2/Bax proteins in placenta. In normal pregnant group, rats were given normal saline; In preeclampsia group, rats were treated with normal saline after the preeclampsia model was successfully built; In preeclampsia + berberine (200 mg/kg/day) group, rats were treated with gastric lavage of berberine (200 mg/kg/day) once a day for consecutive seven days. **, $P < 0.01$ compared with Group 1.

blotting was employed. The data showed that Bcl-2 and Bax protein expression in placenta tissues from preeclampsia group was lower and higher than those in normal pregnant group, respectively ($P < 0.05$). In addition, Bcl-2 expression in preeclampsia + berberine (200 mg/kg/day) group was significantly higher than that in preeclampsia group ($P < 0.05$), but was significantly lower than that in normal pregnant group ($P < 0.05$). Bax expression in preeclampsia + berberine (200 mg/kg/day) group was significantly lower than that in preeclampsia group ($P < 0.05$), but was significantly higher than that in normal pregnant group ($P < 0.05$) (**Figure 1**). These results indicate that berberine up-regulates Bcl-2 and down-regulates Bax in the peripheral blood of SD rats with preeclampsia.

Discussion

Preeclampsia is a disease that specifically occurs during pregnancy. It may be related to heredity, abnormal immunity, placental vascular formation disorder, placental oxidative stress reaction, and apoptosis [10]. Currently, the animal models of preeclampsia are usually induced by vascular growth factor inhibitors or oxidative stress [11]. In the present study, preeclampsia rat models were induced by subcutaneous injection with L-NAME (200 mg/kg/day). The rats induced by L-NAME (200 mg/kg/day) demonstrated characteristic blood pressure, urine protein level and growth of preeclampsia rats.

Since Wegmann et al. proposed that abnormal immune occurs during pregnancy [12], researches show that preeclampsia is a kind of excessive inflammatory response [13, 14]. IL-2 is a kind of Th1 cytokine that participates in inflammatory responses, antitumor activity and anti-graft rejection [15]. IL-10 is a kind of important Th2 cytokine, which is generated by Th2 cells and mononuclear macrophages. IL-10 can inhibit the production of proinflammatory cytokines, the secretion of IL-2 from Th1 cells, and immune responses of cells [16]. Studies show that patients with preeclampsia have enhanced level of IL-2 and reduced level of IL-10 compared with normal subjects, suggesting that the immune balance of Th1/Th2 moves towards Th1 [17, 18]. In the present study, the concentration of anti-inflammatory factor IL-10 in pregnant rats was significantly increased while that of IL-2 was decreased, suggesting that the immune balance of Th1/Th2 was restored.

Bcl-2 and Bax proteins are the members of the Bcl-2 family with opposite functions. As an anti-apoptosis protein, the increase of Bcl-2 protein

usually suggests enhanced anti-apoptosis function of cells. As a pro-apoptotic protein, the increase of Bax protein expression indicates promoted cell apoptosis. Therefore, changes in Bcl-2 and Bax indirectly reflect the apoptosis level of cells [19]. The present study showed that Bax protein expression in placenta of preeclampsia rats treated with berberine (200 mg/kg) was lower than that in untreated preeclampsia rats but higher than that in normal pregnant group. By contrast, Bcl-2 expression in placenta of preeclampsia rats treated with berberine (200 mg/kg) was higher than that in untreated preeclampsia rats but lower than that in normal pregnant group. In conclusion, berberine is capable of increasing the number and weight of normal fetuses in preeclampsia rats, probably by regulating the expression of IL-2/IL-10 and Bcl-2/Bax.

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Disclosure of conflict of interest

None.

Address correspondence to: Qingcui Liu, Department of Obstetrics and Gynecology, The Children and Women's Healthcare Hospital, No. 12 Fengchengxi Street, Laiwu 271100, Shandong Province, P. R. China. Tel: 86-13563476938; E-mail: lqc444@126.com

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