Role of bone morphogenic protein-4 in gestational diabetes mellitus-related hypertension

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Abstract. Hyperglycaemia stimulates the synthesis and release of bone morphogenetic protein-4 (BMP-4) in vascular endothelial cells, which further induces peroxide production and inflammatory responses, leading to vascular endothelial dysfunction. However, the role of BMP-4 in gestational diabetes mellitus (GDM)-related vascular endothelial dysfunction remains unclear. In the present study, the hypothesis that the overexpression of BMP-4 would induce GDM-related hypertension by impairing vascular endothelial function was evaluated. An animal model of GDM was established in Sprague-Dawley (SD) rats. Based on blood pressure, rats were divided into control, GDM and GDM + hypertension (HT) groups. The expression levels of BMP-4, cyclooxygenase-2 (COX-2), nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 1 (NOX-1) and vascular cell adhesion molecule 1 (VCAM-1) in the endothelium of the abdominal aorta of rats in each group were determined via immunohistochemistry and western blotting. Pregnant SD rats were divided into four groups, separately infused with BMP-4, BMP-4 + noggin, noggin or vehicle by osmotic pumps, and blood pressure and vasorelaxation were examined. Immunohistochemistry indicated that the expression levels of the four proteins were lower in the control group than in the GDM and GDM + HT groups. The positive expression rate of VCAM-1 was significantly lower in the control group than in the GDM and GDM+HT groups,

and the differences were statistically significant (χ^2=17.325, P<0.05; χ^2=10.080, P<0.05). Western blotting revealed that the expression level of the COX-2 protein exhibited a sequential increase in the three groups. The expression level of COX-2 in the control and GDM groups was significantly lower than that in the GDM+HT group (3.358±1.286; P<0.05 and P<0.05, respectively). The expression level of VCAM-1 protein in the three groups also exhibited a significant sequential increase (F=31.732; P≤0.001). The expression level of VCAM-1 in the control and GDM groups was significantly lower than that in the GDM+HT group (2.698±0.223; P≤0.001 and P≤0.001, respectively). Infusion of BMP-4 increased systolic blood pressure (from 82 to 112 mmHg) and impaired vasorelaxation in pregnant SD rats after 2 weeks. Co-treatment with noggin completely blocked BMP-4-induced effects. Thus, the BMP-4/NOX-1/COX-2 signalling pathway may be involved in GDM-related hypertension, but VCAM-1 may be substantially associated with GDM-related hypertension. Furthermore, overexpression of BMP-4 could lead to hypertension by impairing endothelial function in pregnancy.

Introduction

Gestational diabetes mellitus (GDM) is defined as hyperglycaemia that is initially diagnosed during pregnancy (1). The prevalence of GDM in developed countries is 2-10% of all pregnancies and its incidence rate continues to rise annually (2). GDM is known to significantly increase the incidence of macrosomia, dystocia and operative delivery, and is closely associated with various adverse pregnancy outcomes and long-term health risks, such as miscarriage, foetal malformations, intrauterine death and development of type 2 diabetes in the mother and child (3-5).

Bone morphogenetic protein-4 (BMP-4) is a member of the transforming growth factor-β superfamily that plays important roles in embryonic development, angiogenesis and chondrogenesis (6-9). In various types of tissue, BMP-4 signalling has been associated with regulatory processes, such as cell development, differentiation, proliferation and apoptosis. Previous studies have found that hyperglycaemia stimulates the synthesis and release of BMP-4 in the vascular endothelium (10,11). BMP-4 acts as an autocrine and paracrine ligand to its receptor, a serine/threonine kinase receptor classified as types 1 and 2. Specifically, BMP-4 has been demonstrated to

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exhibit high affinity for type 1 receptors (8). After binding, activation of Nox1-dependent reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX-1) stimulates the synthesis of reactive oxygen species (ROS) (12,13). Increased intracellular ROS upregulates cyclooxygenase-2 (COX-2) expression via a p38 mitogen-activated protein kinase (MAPK)-dependent mechanism, as well as releasing prostaglandin 2α (PGF2α), thereby causing endothelium-dependent vasoconstriction and blocking endothelium-dependent vasodilation (14-17). Additionally, the increase in BMP-4 activates the expression of vascular cell adhesion molecule 1 (VCAM-1), which induces vascular endothelial inflammation and subsequent exacerbation of vascular endothelial dysfunction, thereby causing systemic vasoconstriction and multi-organ ischaemia, which results in elevated blood pressure and multiple organ damage (14,18).

Preeclampsia is the main cause of maternal and foetal morbidity and mortality (19) and affects 2-7% of pregnancies in non-diabetic women in developed countries (20,21). GDM further increases the risk of preeclampsia. The aetiology of preeclampsia remains elusive. However, GDM and preeclampsia share many risk factors (22). It is unclear whether a common aetiology underlies GDM and preeclampsia; however, various studies suggest that endothelial dysfunction occurs in GDM, as well as in preeclampsia (23-25).

Therefore, the present study hypothesized that BMP-4 is involved in GDM-related hypertension through impairment of endothelial function. The results indicated that the BMP-4/NOX-1/COX-2 signalling pathway may be involved in GDM-related hypertension, but that VCAM-1 may be substantially associated with GDM-related hypertension. Furthermore, overexpression of BMP-4 could lead to hypertension by impairing endothelial function in pregnancy.

Materials and methods

Establishing an animal model of GDM. The experimental protocol was approved by the Institutional Review Board of Shengjing Hospital of China Medical University (Shenyang, China). All the experiments were conducted according to the institutional guidelines for the humane treatment of laboratory animals. In the present study, a high-fat diet combined with intraperitoneal injection of streptozotocin (STZ; an antibiotic that produces pancreatic islet β-cell destruction) was employed to generate a rat model of GDM (26-28). A total of 70 (10 male and 60 female) Sprague-Dawley rats aged 9-10 weeks were purchased from Changsheng Biotechnology Co., Ltd. The mean weight of the rats was 301±52 g. All the animals were specific-pathogen free, housed at 21-22°C, under a relative humidity of 60-70% and a 12-h light/dark cycle. The female rats were randomly divided into two groups (30 rats per group), namely a high-fat diet group and a normal diet group. The high-fat diet group was fed a high-fat diet (Huafukang Medical Technology Co., Ltd.) with a daily fat content of 60%, whereas the normal diet group was fed a normal diet. All rats had free access to food and autoclaved water.

After one month, female and male rats were placed in cages at a 2:1 ratio and the vaginal plug or vaginal secretion smear of the female rats was examined the next morning.

The day on which sperm or vaginal plugs were observed in the rats was recorded as pregnancy day 0. Following the detection of pregnancy in the high-fat diet group, rats were starved for 12 h with provision of water *ad libitum* and then administered with an intraperitoneal injection of STZ (cat. no. S0130; MilliporeSigma) at a dosage of 30 mg/kg body weight (dissolved in 0.1 mmol/l sodium citrate, ready to use). The rats were then fed the high-fat diet. One week later, 100 μl blood from each rat was collected through the tail vein to evaluate fasting blood glucose and non-fasting blood glucose; measurements were conducted three times separately. Rats with ≥120 mg/dl fasting blood glucose and at the same time, ≥300 mg/dl non-fasting blood glucose were diagnosed with GDM (29-32); measurements were conducted three times separately. The normal diet group continued to receive the normal diet.

Monitoring blood pressure. After confirming pregnancy, the blood pressure of each rat was monitored every week using the tail-cuff method (using a Softron BP-98A and a Softron TMC-203). Three days before the formal measurement, the blood pressure was measured at a fixed time every day for adaptation to measurement conditions. The first three measurements were not recorded as a formal measurement and the measurement commenced only after the rats were stabilized. Blood pressure in each rat was measured three times and the mean value was recorded (14). Tail pressure was monitored every week until pregnancy day 20, as the average gestational period of rats is 21 days. Currently, no uniform standards exist for normal blood pressure and hypertension in pregnant rats. In the present study, hypertension was defined as systolic blood pressure elevated by >20 mmHg compared with the initial measurement (33-35). Based on the blood pressure values, rats with GDM were divided into a GDM group (GDM) and a GDM with hypertension group (GDM + HT). The blood pressure measurements were performed repeatedly to reduce error. The normal diet group served as the control group (CONT; Fig. 1).

Immunohistochemistry. Rats in all three groups were euthanized by exsanguination via abdominal aorta after anaesthetising by isoflurane inhalation (2.5-3.5% for the induction phase and 2.5-3.0% for the maintenance phase) on pregnancy day 20. Successful euthanasia was confirmed via respiratory and cardiac arrest. Following euthanasia, the abdominal aorta was collected immediately.

During sample collection, care was taken to avoid damage to the vascular endothelium. The tissue samples from the three groups of rats were fixed in 10% formaldehyde and ethanol at 25°C for 24 h. Following dehydration and paraffin embedding, 4-μm serial sections were obtained. After heating of paraffin-embedded sections in a 60°C incubator for 0.5-1 h, sections were deparaffinized with xylene at 25°C, rehydrated in a concentration descending ethanol series and immersed in endogenous peroxidase blocking buffer for 10 min. Next, antigen retrieval was performed using high-pressure antigen retrieval. Diluted primary antibodies, BMP-4 (cat. no. ab39973; 1:100), COX-2 (cat. no. ab15191; 1:2,000), VCAM-1 (cat. no. ab134047; 1:500) and NOX-1 (cat. no. ab131088; 1:1,000; all obtained from Abcam) were added and slides were incubated overnight
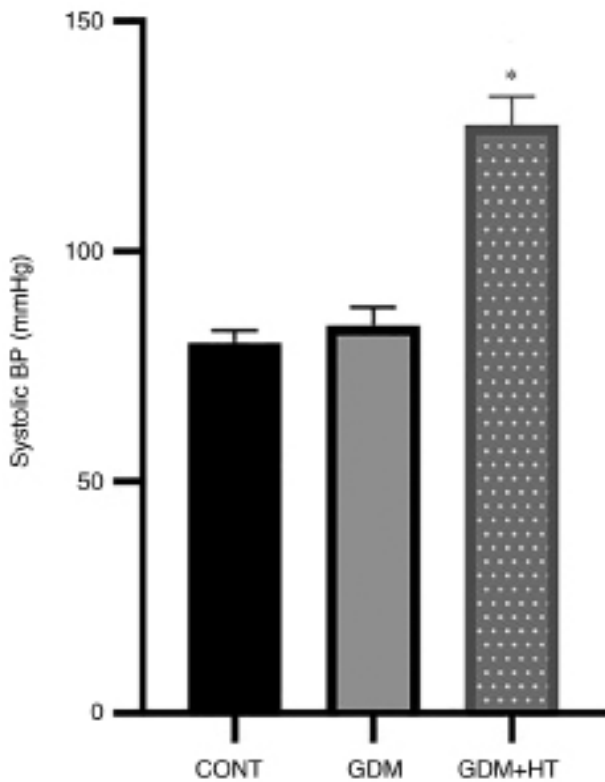
Figure 1. Comparison of systolic BP in CONT group (n=11), GDM group (n=11) and GDM+HT group (n=4). *P<0.05 vs. GDM and CONT groups. BP, blood pressure; CONT, control; GDM, gestational diabetes mellitus; HT, hypertension.

at 4°C. PBS treatment served as a negative control. Slides were re-heated at 37°C for 20 min and rinsed three times with PBS solution. The reaction enhancer was added and slides were incubated at 25°C for 20 min and rinsed three times with PBS solution. The horseradish enzyme labelled goat anti-rabbit IgG (cat. no. ZB-2301; 1:5,000; OriGene Technologies, Inc.) was added and slides were incubated at 25°C for 20 min. Slides were then rinsed three times with PBS solution. Subsequently, 3,3'-diaminobenzidine was used for colour development. Slides were rinsed with running water and haematoxylin was used for counterstaining of nuclei, while hydrochloric acid and ethanol were used for differentiation. Finally, slides were rinsed with running water, dehydrated and dried using an ethanol gradient, cleared with xylene and mounted with neutral gum.

Scoring criteria. The BMP-4, NOX-1, COX-2 and VCAM-1 proteins are located primarily in the cytoplasm of vascular endothelial cells and positive expression is indicated by brown or tan colouration in immunohistochemistry. Five high-magnification (x400) fields of view were randomly selected for each slide, and the staining intensity and number of positive cells were observed. A score was assigned based on the staining intensity as follows: 0, No staining; 1, light yellow; 2, brown; 3, tan. Another score was assigned based on the percentage of positive cells as follows: 0, All cells negative; 1, 1-10% positive cells; 2, 11-50% positive cells; 3, 51-75% positive cells; and 4, >76% positive cells. A positive immune reaction was defined as a product of the staining intensity score and the percentage of positive cells >3.

Western blotting. Rat aortas were harvested after the indicated treatment. Protein was extracted using 300 μl lysis buffer (cat. no. WLA019a; Wanleibio Co., Ltd.) supplemented with protease and phosphatase inhibitors. BCA method was used to determine the concentration of the protein and 50 μg protein was loaded per lane. Western blotting was performed according to standard methods: Protein separation by 10% gel electrophoresis, transferring the protein from the gel onto polyvinylidene fluoride membranes (Bio-Rad Laboratories, Inc.), the membranes were blocked using 3% bovine serum albumin (cat. no. B2064; Sigma-Aldrich; Merck KGaA) at 25°C for 1 h, and then were probed with BMP-4, COX-2, VCAM-1 and NOX-1 antibodies (dilution of all the antibodies was 1:1,000), β-actin antibody (cat. no. sc-1616; Santa Cruz Biotechnology, Inc.; 1:1,000) was used for the reference protein, the membranes were incubated overnight at 4°C, then the goat anti-rabbit IgG (cat. no. sc-2004; Santa Cruz Biotechnology, Inc.; 1:1,000) was added in the membranes and then the membranes were incubated at 25°C for 1 h, then an enhanced chemiluminescence method using chemiluminescent substrate (cat. no. 34577; Thermo Fisher Scientific, Inc.) was performed. The molecular weight of each protein shown on the immunoblot was estimated based on the Rainbow 245 Marker for Western Blotting Protein Standard (Beijing Solarbio Science & Technology Co., Ltd.) on a 10% SDS-PAGE gel. Quantification was performed based on the density of bands obtained using ImageJ v1.8.0 Software (National Institutes of Health). β-actin served as the loading control.

Assessment of blood pressure in rats infused with BMP-4. Following fertilization, female rats that were fed with a normal diet were randomly divided into four groups (n=4 rats per group) designated as A, B, C and D. Blood pressure was measured using the tail-cuff method on the day pregnancy was discovered and recorded as the initial blood pressure. In order to evaluate whether BMP-4 affected the blood pressure of pregnant rats, osmotic minipumps (Alzet®Osmotic Pumps; Durect Corp.) were implanted using 1% sodium pentobarbital as the anaesthetic (3 ml/kg via intraperitoneal injection) on the third day of pregnancy. The solution was composed of an infusion vehicle, recombinant human BMP-4 (cat. no. 314-BP-050; R&D Systems, Inc.) or recombinant human noggin (cat. no. 6057-NG-100; R&D Systems, Inc.) supplemented with 0.1% bovine serum albumin (cat. no. A8020, Solarbio) in 4 mmol/l HCl. Four groups were evaluated as follows: Group A was infused with 0.45 mg/kg BMP-4 + vehicle, group B was infused with 0.45 mg/kg noggin + vehicle, group C was infused with 0.45 mg/kg BMP-4 + vehicle and 0.45 mg/kg noggin + vehicle, two osmotic minipumps were used and implanted at different sites for infusion to avoid mixing, and group D was infused with vehicle alone and served as a control (14). These four groups of rats were administered with infusions continuously for 2 weeks. Blood pressure was monitored and recorded again on pregnancy day 18 and the changes in blood pressure among the four groups were compared.

Vascular reactivity testing. Rats in all four groups were euthanised by exsanguination via abdominal aorta after

anaesthetising by 2.5-3.0% isoflurane inhalation on pregnancy day 20. Following euthanasia, the thoracic aorta was collected immediately for vascular reactivity testing. During sample collection, care was taken to avoid damage to the vascular endothelium. After collection, the aorta was immediately placed in a petri dish containing Krebs-Henseleit (KH) solution (cat. no. PB180348; Procell Life Science & Technology Co., Ltd.) at 37°C. A mixture of 95% oxygen + 5% carbon dioxide gas was continuously injected into the KH solution. The fatty tissue on the surface of the blood vessels was removed and blood vessels were cut into rings (length, 4 mm). Two L-shaped stainless-steel hooks were passed through the lumen, with one end fixed and the other end connected to the tension transducer of a physiological recorder (PL3508 PowerLab 8/35; ADInstruments, Ltd.). Blood vessels were immersed in the KH solution and the physiological recorder was adjusted to apply a baseline tension of 1 g to the blood vessels to simulate physiological tension. The tension was equilibrated for 60 min, during which the KH solution was replaced every 15 min. After stabilisation of the tension, 10^{-5} mol/l phenylephrine was added to the container, and 10^{-9}-10^{-6} mol/l acetylcholine was added after vasoconstriction was equilibrated. The time interval between additions of different concentrations of acetylcholine was 2 min. The rings were continuously aerated while phenylephrine and acetylcholine were added. The relaxation of the thoracic aorta vascular ring of each group of rats was recorded.

Statistical analysis. SPSS 23.0 software (IBM Corp.) was used for statistical analysis and data are expressed as means ± standard error. Student's t-test was used for comparisons between two groups and one-way analysis of variance was used for comparisons between multiple groups. Tukey's post hoc test was performed for pairwise comparisons between groups. The χ^2 test or Fisher's exact probability test was performed for comparisons of countable data. P<0.05 was considered to indicate a statistically significant difference.

Results

Vascular endothelium in GDM rats shows higher positive expression rates of VCAM-1. As shown in Fig. 2, the positive expression rates of BMP-4 in the GDM, GDM + HT and control groups were 100% (11/11), 100% (4/4) and 72.7% (8/11), respectively. The positive expression rates of NOX-1 in the GDM, GDM + HT and control groups were 100% (11/11), 100% (4/4) and 81.8% (9/11), respectively. The positive expression rates of COX-2 in the GDM, GDM + HT and control groups were 100% (11/11), 100% (4/4) and 72.7% (8/11), respectively. The positive expression rates of VCAM-1 in the GDM, GDM + HT and control groups were 100% (11/11), 100% (4/4) and 10% (1/10), respectively. The expression levels of all four proteins in the control group were lower than those in the GDM and GDM + HT groups. The positive expression rate of VCAM-1 in the control group was significantly lower than that in the GDM and GDM + HT groups; the differences were statistically significant (χ^2=17.325, P<0.05 and χ^2=10.080, P<0.05, respectively).
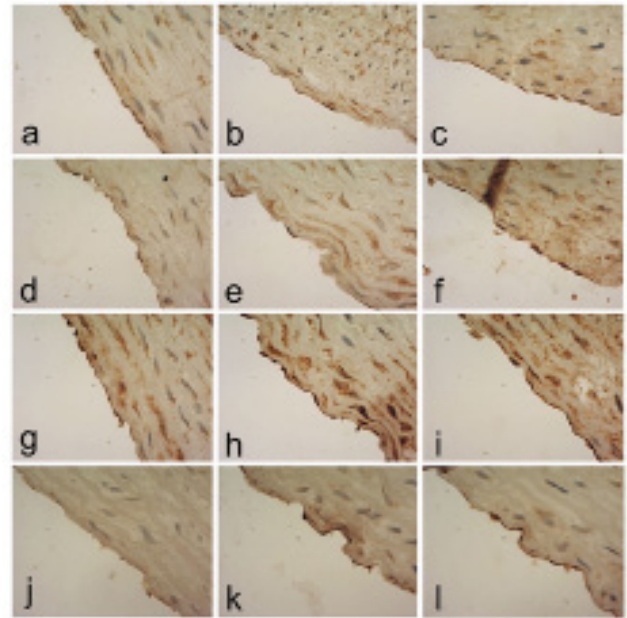

Figure 2. Immunohistochemistry of BMP-4, COX-2, NOX-1 and VCAM-1 expression in the vascular endothelium of rats in CONT group (n=11), GDM group (n=11) and GDM+HT group (n=4). Panels a, b and c demonstrate the expression of BMP-4 in the CONT, GDM and GDM + HT groups, respectively. Panels d, e and f demonstrate the expression of COX-2 in the CONT, GDM, and GDM + HT groups, respectively. Panels g, h and i demonstrate the expression of NOX-1 in the CONT, GDM and GDM + HT groups, respectively. Panels j, k and l demonstrate the expression of VCAM-1 in the CONT, GDM and GDM + HT groups, respectively. Magnification, x400. BMP-4, bone morphogenetic protein-4; COX-2, cyclooxygenase-2; NOX-1, nicotinamide adenine dinucleotide phosphate oxidase 1; VCAM-1, vascular cell adhesion molecule 1; CONT, control; GDM, gestational diabetes mellitus; HT, hypertension.

The expression levels of BMP-4, COX-2 and VCAM-1 proteins in rats with GDM related hypertension are higher compared with those without hypertension. The expression level of the BMP-4 protein in the control, GDM and GDM + HT groups exhibited a sequentially increasing tendency; however, the difference observed among the groups was not statistically significant (F=2.651, P=0.13). Similarly, the difference in the expression levels of the NOX-1 protein among the three groups was not statistically significant (F=2.122, P=0.18). By contrast, the expression levels of COX-2 protein in the three groups exhibited a sequential increase and the difference was observed to be statistically significant (F=8.560, P<0.05). The difference in the expression levels of the COX-2 protein between the control (1.000±0.255) and the GDM (1.434±0.344) groups was not identified to be statistically significant (P=0.78), but the expression levels in the control and GDM groups were significantly lower than those in the GDM+ HT group (3.358±1.286, P<0.05 and P<0.05, respectively). The expression levels of the VCAM-1 protein in the three groups also exhibited a sequential increase, with a statistically significant difference (F=31.732, P≤0.001). The difference in the expression levels of the VCAM-1 protein between the control (1.000±0.297) and GDM (1.525±0.347) groups was not statistically significant (P=0.11), but the expression levels in the control and GDM groups were significantly lower than those in the GDM + HT group (2.698±0.223, P≤0.001 and P≤0.001, respectively; Fig. 3).
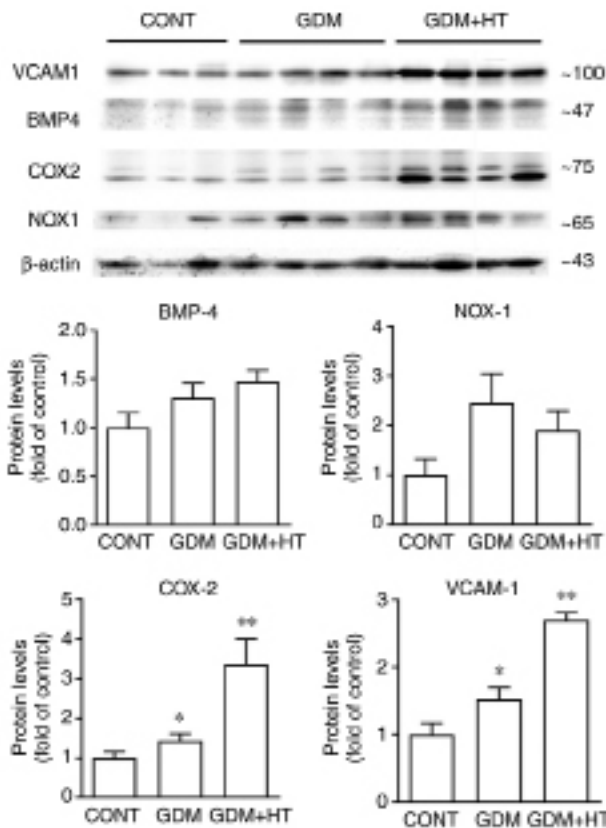
Figure 3. Western blot analysis of BMP-4, COX-2, NOX-1 and VCAM-1 expression levels in the vascular endothelium of rats in CONT group (n=3), GDM group (n=4) and GDM+HT group (n=4). *P<0.05 vs. GDM + HT group; **P<0.05 vs. CONT group. The white line on the blot indicates gels that were cropped due to loading of the incorrect sample. BMP-4, bone morphogenetic protein-4; COX-2, cyclooxygenase-2; NOX-1, nicotinamide adenine dinucleotide phosphate oxidase 1; VCAM-1, vascular cell adhesion molecule 1; GDM, gestational diabetes mellitus; HT, hypertension; CONT, control.

BMP-4 induces elevated blood pressure in pregnant SD rats. Fig. 4 demonstrates that infusion of BMP-4 significantly increased the blood pressure of rats by 30 mmHg above the control values (from 82.3±5.0 to 112.3±6.3 mmHg; P<0.05).

The specificity of the hypertensive effect of BMP-4 was evaluated by infusing noggin, a BMP-4 antagonist, either alone or in combination with BMP-4. As shown in Fig. 4, noggin co-delivery prevented the BMP-4-induced hypertension in pregnant SD rats.

BMP-4 abrogates the endothelium-dependent vasodilation of the vascular endothelium in pregnant rats. To evaluate the effect of BMP-4 on vascular endothelial function in pregnant rats, the diastolic activity of the thoracic aorta of rats in each group were assessed. The results demonstrated that the maximum relaxation in the control group after adding acetylcholine was 88.90±1% of the maximum contractile tension, whereas the maximum relaxation in the BMP-4-infused group after adding acetylcholine was 68.96±0.8% of the previous maximum contractile tension, which was significantly smaller than that in the control group (P<0.05). Accordingly, it was observed that the blood vessels in the noggin-infused group were nearly the same as those in the control group (Fig. 5 and Table I).
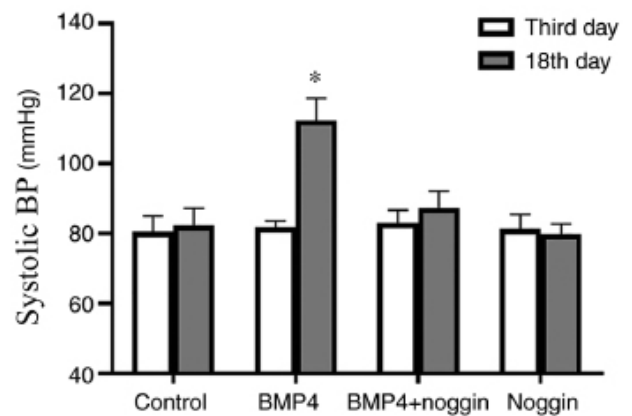

Figure 4. Chronic infusion of BMP-4 in pregnant rats increases systolic BP. Noggin prevented this increase. Results are presented as means ± standard deviation (n=4 per group). *P<0.05 vs. Control. BMP-4, bone morphogenetic protein-4; BP, blood pressure.

Discussion

BMP-4, as a member of the transforming growth factor-β superfamily, was first discovered to mediate bone growth and serves as an essential signalling molecule for human embryonic development (6,7). Noggin is an endogenous antagonist that counteracts with BMP-4 (7). BMP-4 interacts with type 1 BMP receptors to form a binding complex and trigger downstream signalling (8), which stimulates SMAD-dependent and -independent pathways, including the activation of NADPH oxidases that result in ROS overproduction, p38 MAPK activation and COX-2 upregulation (14-16). Previous *in vivo* and *in vitro* studies have demonstrated that BMP-4 activates the NOX1-dependent NADPH oxidase pathway in vascular endothelial cells and promotes the expression of inflammatory factors, which leads to vascular endothelial dysfunction and elevated blood pressure (14,16,36-45); however, to the best of our knowledge, there have been no studies on the role of BMP-4 during pregnancy. The present study attempted to identify the role of BMP-4 in GDM-related hypertension and endothelial dysfunction. In order to investigate the effect of BMP-4 on endothelial function, the change in vasorelaxation after adding BMP-4 and its antagonist requires measurement (14,16). However, in order to change endothelial function, the duration of treatment with BMP-4 or its antagonist must be more than two weeks and even up to four weeks. Unfortunately, the gestational period of rats is only 21 days and the duration for establishing the GDM animal model accounts for 7-10 days, meaning there is insufficient time to infuse BMP-4 and its antagonist to evaluate their effect on endothelial function in GDM rats. Accordingly, the present study was divided into two parts; one part evaluated the correlation between BMP-4 and GDM-related hypertension and the other investigated the role of BMP-4 on endothelial function during pregnancy.

During the first part of the study, a GDM rat model was established and the expression levels of BMP-4 pathway-related proteins and VCAM-1 proteins were evaluated in the endothelium of the abdominal aorta of rats. The results indicated that BMP-4, NOX-1, COX-2 and VCAM-1 proteins were expressed in the endothelium of the abdominal aorta of pregnant rats, which, to the best of our knowledge, has not previously been
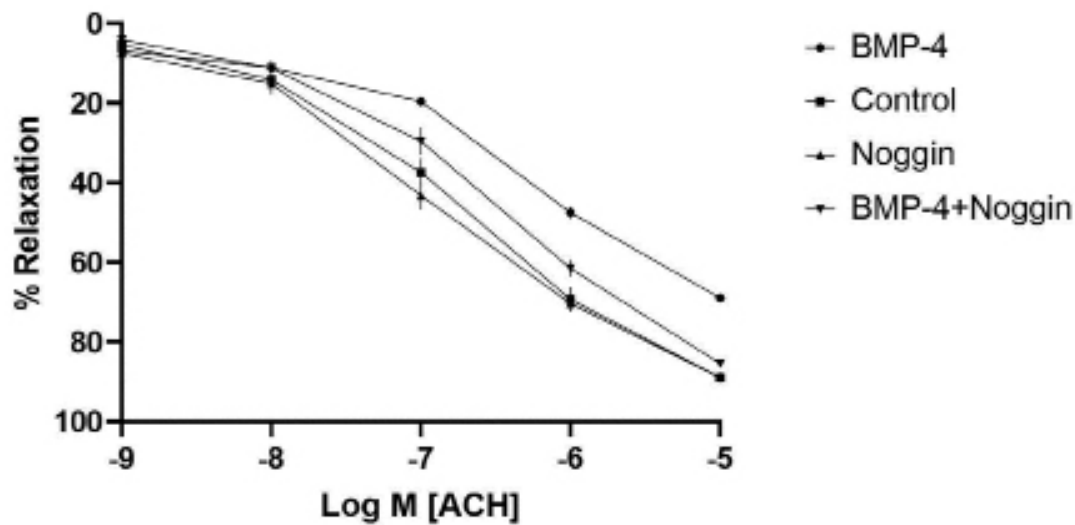
Figure 5. Chronic infusion impairs endothelium-dependent vasorelaxation in rats. Aortic rings were isolated from rats treated with BMP-4, noggin or vehicle for 2 weeks as presented in Fig. 4. After pre-contraction with phenylephrine, concentration-dependent relaxations of the aortic rings following stimulation by acetylcholine were examined in the organ chambers. The complete data set is presented in Table I.

shown. Moreover, the expression levels of these proteins in the endothelium of the abdominal aorta of rats with GDM were higher than those in pregnant rats without GDM. Additionally, the expression levels of BMP-4, COX-2 and VCAM-1 proteins were higher in rats with GDM-related hypertension when compared with those in rats with GDM with normal blood pressure, and the level of VCAM-1 expression was the highest among all three proteins. Therefore, it is hypothesized that BMP-4 pathway-related proteins are involved in GDM-related hypertension and VCAM-1 may be substantially associated with GDM-related hypertension.

In the second part of the present study, pregnant rats were subjected to slow infusions of BMP-4. The results revealed that the blood pressure of the BMP-4 group was significantly higher than that of the vehicle group, thereby indicating that BMP-4 could cause elevated blood pressure in pregnant rats. Noggin inhibited the BMP-4-induced increase in blood pressure. Further analysis of the effect of BMP-4 on vascular reactivity showed that BMP-4 abrogated the endothelium-dependent vasodilation in the vascular endothelium in pregnant rats, which led to vascular endothelial dysfunction and further hypertension. Whereas noggin blocked the destructive effect of BMP-4 on the endothelium-dependent vasodilation in the vascular endothelium. Accordingly, it is hypothesized that BMP-4 pathway proteins and VCAM-1 may participate in the hypertension associated with GDM, with VCAM-1 potentially playing a substantial role in hypertension. Therefore, the aetiology of GDM-related hypertension may be the endothelial dysfunction induced by overexpression of BMP-4 pathway proteins and VCAM-1.

However, there were certain limitations of the present study. Since the specific pathogenesis of GDM remains unclear, the GDM animal model that was established may not entirely simulate human GDM. Furthermore, data obtained from the aorta may not apply to other vascular beds, such as mesenteric arteries, which are resistance vessels and contribute to blood pressure markedly more than the aorta. Therefore, further studies on smaller arterial vessels are required in future. In

Table I. Effects of BMP-4 and noggin on vasorelaxation responses in pregnant Sprague-Dawley rats.

Group	Maximal relaxation (%)
Vehicle	88.90±1
BMP-4	68.96±1[a]
BMP-4 + noggin	85.41±1
Noggin	88.99±1

[a]P<0.0001 vs. vehicle, noggin and BMP-4 + noggin. BMP-4, bone morphogenetic protein-4.

addition, noggin, as a well-known inhibitor of BMP-4, may also have numerous other effects such as reducing the serum glucose levels (46), that could influence the experiments. Finally, due to the limited sample size, the results of the present study require confirmation by further studies using a larger sample size.

However, the present study demonstrated that BMP-4 may cause vascular endothelial dysfunction by disrupting the endothelium-dependent vasodilation in pregnant rats, thereby causing elevated blood pressure. Noggin, as an antagonist of BMP-4, may block the destructive effect of BMP-4 on endothelium-dependent vasodilation. Therefore, it is hypothesized that upregulation of the BMP-4 pathway proteins induced by hyperglycaemia in GDM may be involved in the mechanism of GDM-related vascular endothelial dysfunction and hypertension, and VCAM-1 may be substantially associated with GDM-related vascular endothelial dysfunction and hypertension. The present results may provide insight into the aetiology and treatment of GDM-related hypertension or preeclampsia. The action of BMP-4 raises the possibility that noggin and its related compounds may serve as therapeutic agents for GDM-related hypertension or preeclampsia. In the future, the authors aim

to conduct clinical studies, evaluating the expression levels of BMP-4 and its related proteins in humans to test our hypothesis, in order to identify a novel therapeutic strategy for GDM-related hypertension or preeclampsia.

Availability of data and materials

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

Authors' contributions

BC conceptualized and designed the study. He also performed the experiments and wrote the initial draft of the manuscript. JD supervised the design of the study and critically reviewed the manuscript drafts. BC and JD confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The experimental protocol was approved by the Ethics Committee of Shengjing Hospital of China Medical University.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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