

# Candesartan targeting of angiotensin II type 1 receptor demonstrates benefits for hypertension in pregnancy via the NF- $\kappa$ B signaling pathway

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**Abstract.** Hypertensive disorders may be a complication of pregnancy and are characterized by the high blood pressure. Evidence suggests that alterations in the renin-angiotensin-aldosterone system and the sympathetic nervous system are associated with gestational hypertension. Angiotensin II type 1 receptor (Ang-IIIR) is a potential target in the progression of gestational hypertension. Candesartan is selective Ang-IIIR antagonist that may act against vasoconstriction and reduces peripheral vascular resistance. The aim of the present study was to evaluate the efficacy of Candesartan and the underlying molecular mechanism of the nuclear factor- $\kappa$ B (NF- $\kappa$ B) signaling pathway in the progression of gestational hypertension in a mouse model. Expression and activity of Ang-IIIR was evaluated in a mouse model of gestational hypertension prior to and post-treatment of Candesartan both *in vitro* and *in vivo*. It was determined whether Candesartan treatment reduces higher blood pressure activated the renal renin-angiotensin system and a prognostic marker, soluble endoglin, and its associated gene in mice with gestational hypertension. Angiotensin-converting enzyme plasma levels and activity were also evaluated in the present study. Cytoplasmic and nuclear immunostaining of NF- $\kappa$ B and associated proteins transforming growth factor  $\beta$  (TGF- $\beta$ ) and endoglin was enhanced in vascular endothelial cells and mice with gestational hypertension. Soluble fms-like tyrosine kinase 1 (sFlt-1), insulin resistance homeostasis model assessment score and associated cardiovascular risk factors also were measured. Results demonstrated that

angiotensin and Ang-IIIR expression levels were upregulated in mice with gestational hypertension and were downregulated by Candesartan treatment. Renal renin-angiotensin and soluble endoglin were also improved in mice in the Candesartan-treated group. In addition, Candesartan treatment enhanced NF- $\kappa$ B activity, as well as TGF- $\beta$  and vascular endothelial growth factor expression which led to improved levels of sFlt-1, insulin resistance homeostasis and associated cardiovascular risk factors. Gestational hypertension was markedly improved by treatment of Candesartan compared with the control. In conclusion, the findings of the present study suggested that the NF- $\kappa$ B signaling pathway may be involved in with Candesartan-mediated Ang-IIIR for the treatment of gestational hypertension.

## Introduction

Gestational hypertension is a complication that specifically occurs in late pregnancy, and includes gestational hypertension, preeclampsia, eclampsia and chronic hypertension (1,2). A clinical investigation suggested that hypertensive disorder is a complication during pregnancy, is a common pregnancy-associated disease and is becoming increasingly present worldwide (3). However, the etiology of gestational hypertension is yet to be clearly investigated. Currently, insulin resistance, renin-angiotensin-aldosterone system (RAS) dysfunction, inflammation, obesity-induced interaction of neurohumoral and renal mechanisms are considered to be contributing factors of gestational hypertension (4,5). In addition, genetic factors that contribute to gestational hypertension have been well documented in a previous study (6). Furthermore, gestational hypertension seriously affects the life expectancy of the mother and fetus in the clinical setting (7,8). Gestational hypertension is one of the leading causes of maternal, fetal and neonatal morbidity and mortality (4,9). Reports have suggested that the incidence rate of gestational hypertension is approximately 7-12% in China, presenting a higher than the average incidence throughout the world (10).

Evidence has revealed that clinical assisted reproductive technology treatment, including artificial insemination and ovulation induction, may increase the incidence of gestational

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hypertension and preeclampsia (11). In addition, there are a large number of factors that may induce gestational hypertension, and have been found to serve a role in the etiology of these hypertensive disorders, including work stress, depression and anxiety (12,13). Reports also have indicated that angiotensin II type 1 receptor (Ang-II $\text{TR}$ ), vasodilation converting enzyme (VCE) and  $\alpha$ -1A adrenergic receptor ( $\alpha$ -ADR) serve crucial roles in the process of gestational hypertension and may be potential targets for the treatment of patients with gestational hypertension in clinical (3,12,13). Therefore, a large number of drugs targeting Ang-II $\text{TR}$  were introduced and their therapeutic effects for the treatment of gestational hypertension were evaluated.

Ang-II $\text{TR}$  has been demonstrated to be a potential target in the progression of gestational hypertension and higher expression levels of this receptor exist in vascular endothelial cells in patients with gestational hypertension, compared with healthy pregnant women (14,15). Antihypertensive agents targeting Ang-II $\text{TR}$  are the most common used drugs for remission patients with gestational hypertension (16,17). Candesartan ( $\text{C}_{24}\text{H}_{20}\text{N}_6\text{O}_3$ ), is an antihypertensive drug, and is a selective Ang-II $\text{TR}$  antagonist that acts by reducing peripheral vascular resistance and vasoconstriction (18,19). Candesartan is used to treat hypertension and prevents the constriction (narrowing) of blood vessels, including veins and arteries. In addition, fibrinolysis and insulin sensitivity was improved by treatment with Imidapril and Candesartan in patients with hypertension (20). The effect of Candesartan treatment on lipid metabolism in patients with hypertension has also been investigated in a retrospective longitudinal survey (21). However, the efficacy of Candesartan for gestational hypertension has not been previously studied.

In the present study, the therapeutic effects of Candesartan on gestational hypertension in a mouse model was investigated, in addition to the underlying molecular mechanisms by which it contributes to attenuating gestational hypertension induced by homocysteine. The aim of the present study was to characterize alterations in Ang-II $\text{TR}$ , soluble fms-like tyrosine kinase 1 (sFlt-1), insulin resistance homeostasis model assessment score and associated cardiovascular risk factors in mice with gestational hypertension after treatment with Candesartan. NF- $\kappa$ B activation and nuclear factor NF- $\kappa$ B p65 subunit (p65), inhibitor of NF- $\kappa$ B kinase subunit  $\beta$  (IKK- $\beta$ ) and NF- $\kappa$ B inhibitor  $\alpha$  (IkB $\alpha$ ) expression levels were also investigated in vascular endothelial cells in mice treated by Candesartan.

## Materials and methods

**Ethics statement.** The present study was carried out in strict accordance with the recommendations in the Guide for Shandong Provincial Hospital affiliated to Shandong University. All surgery and euthanasia were performed to minimize suffering. This study was approved by the ethics committee of Shangdong University and Taian City Central Hospital (Jinan, China).

**Cell culture and reagents.** Vascular endothelial cells were isolated from experimental mice and cultured in Minimum Essential media (Merck KGaA, Darmstadt, Germany)

supplemented with 10% fetal bovine serum (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Vascular endothelial cells were cultured at 37°C in a humidified atmosphere of 5%  $\text{CO}_2$ . Cells were treated with Candesartan and/or NF- $\kappa$ B activity inhibitor JSH23 (Sigma-Aldrich; Merck KGaA) for 12 h at 37°C.

**Western blot analysis.** Vascular endothelial cells were homogenized in radioimmunoprecipitation assay lysis buffer (Sigma-Aldrich; Merck KGaA) containing protease-inhibitor and centrifuged at 6,000  $\times$  g at 4°C for 10 min. The resulting supernatant was used for analysis of proteins. Protein concentration was determined with a bicinchoninic acid protein assay kit. Protein (10  $\mu\text{g}/\text{lane}$ ) was separated by 12.5% SDS-PAGE and subsequently transferred onto polyvinylidene fluoride membranes as previously described (22). For western blotting, membranes were incubated with the following primary antibodies for 12 h at 4°C: Superoxide dismutase (SOD; 1:1,000; cat. no. A91960Hu01; Shanghai Wuqi Biotechnology Co., Ltd., Shanghai, China) sFlt-1 (1:1,000; cat. no. ab230516), tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ; 1:1,000; cat. no. ab6671), interleukin-2 (1:1,000; cat. no. ab80780), reactive oxygen species (ROS; 1:1,000; cat. no. ab191522), transcription factor p65 (1:1,000; cat. no. ab16502), inhibitor of nuclear factor  $\kappa$ -B kinase subunit  $\beta$  (Ikk- $\beta$ ; 1:1,000; cat. no. ab17870), NF- $\kappa$ B inhibitor  $\alpha$  (IkB $\alpha$ ; 1:1,000; cat. no. ab16502) and  $\beta$ -actin (1:1,000; cat. no. ab8227; all Abcam, Cambridge, MA, USA) were added following blocking in 5% skimmed milk for 60 min at 37°C. After washing with PBS three times, horseradish peroxidase-conjugated goat anti-rabbit IgG monoclonal secondary antibodies (1:2,000; cat. no. PV-6001; OriGene Technologies, Inc., Beijing, China) were added for 2 h at 37°C. The results were visualized with chemiluminescence substrate (Roche Diagnostics, Basel, Switzerland). Band density was analyzed by Quantity One software (version 4.62; Bio-Rad Laboratories, Inc., Hercules, CA, USA).

**Animal study.** Female C57BL/6 mice (aged 8 weeks; 25-30 g) were purchased from Jackson Laboratory (Bar Harbor, ME, USA) and housed in a 12 h light-dark cycle at 23 $\pm$ 1°C with a relative humidity of 50 $\pm$ 5%. All mice had free to access food and water. The mouse model of gestational hypertension was established according to previous studies (23,24). Mice with gestational hypertension were divided into two groups and received treatment with Candesartan (0.2 mg/kg) or with PBS as a control once daily for a total of 7 days.

**Reverse transcription-quantitative polymerase chain reaction (RT-qPCR).** Total RNA was extracted from vascular endothelial cells by using an RNAeasy Mini kit (Qiagen GmbH, Hilden, Germany). RNA was reversed to cDNA using a one-step reverse transcription kit (Thermo Fisher Scientific, Inc.). Vascular endothelial growth factor (VEGF), transforming growth factor  $\beta$  (TGF- $\beta$ ), angiopoietin-1 (Ang-1), placental growth factor (PLGF), angiotensin, Ang-II $\text{TR}$ , cytokine-induced neutrophil chemoattractant 1 (CINC-1), lipopolysaccharide induced CXC chemokine (LIX), monokine,  $\alpha$ -ADR expression levels in vascular endothelial cells were determined by RT-qPCR with  $\beta$ -actin as an endogenous

Table I. Primer sequences used for reverse transcription-quantitative polymerase chain reaction.

Gene name	Reverse primer	Forward primer
Angiotensin	5'-GCAAGCGCAAGACCACTAAC-3'	5'-GCCGCTGTAATCCATCATGC -3'
Ang-IITR	5'-AAGAGAGCTTCCGTAAGGCG-3'	5'-GCATCCTCTTCAGTTACGTCC -3'
VEGF	5'-TTGCTGCTCTACCTCCAC-3'	5'-AATGCTTTCTCCGCTCTG-3'
TGF- $\beta$	5'-CCCCTGGAAAGGGCTCAACAC-3'	5'-TCCAACCCAGGTCCTTCCTAAAGTC-3'
Ang-1	5'-GAAGGAAACCGAGCCTATTAC-3'	5'-CCACAAGCATCAAACCACCA-3'
PLGF	5'-GTTTCAGCCCATCTGTGTCT -3'	5'-CTTCATCTTCTCCCGCAGAG -3'
CINC-1	5'-GAAGATAGATTGCACCGATG-3'	5'-CATAGCCTCTCACACATTTC-3'
LIX	5'-GCATCTCATCTGTTACAGC-3'	5'-GCAAGCGCAAGACCACTAAC-3'
Monokine	5'-AGGCCTCCTGGGCTTCAT-3'	5'-GGAGTAGAAGTCCCGCAGGAT-3'
$\alpha$ -ADR	5'-AAACCTGTCCAACCTACCTC-3'	5'-TAATCCTCGTCTCCTTCC -3'
$\beta$ -actin	5'-CGGAGTCAACGGATTTGGTC-3'	5'-AGCCTTCTCCATGGTCGTGA-3'

control (25) (Invitrogen; Thermo Fisher Scientific, Inc.). All forward and reverse primers were synthesized by Invitrogen; Thermo Fisher Scientific, Inc. (Table I). The thermocycling conditions were as follows: Initial denaturation at 94°C for 2 min, followed by 35 cycles of 95°C for 30 sec, 54°C for 30 sec and 72°C for 10 min. The reaction volume (50  $\mu$ l) contained 50 ng genomic cDNA, 200  $\mu$ M dNTPs, 200  $\mu$ M primers, 2.5 U Taq DNA polymerase (Thermo Fisher Scientific, Inc.) and 2.5 U SYBR-Green (Thermo Fisher Scientific, Inc.). Relative mRNA expression levels were calculated by the  $2^{-\Delta\Delta C_q}$  method (26). The results are expressed as the n-fold way compared to control.

**Immunohistochemical staining.** Immunohistochemical staining was performed using the avidin-biotin-peroxidase technique. Paraffin-embedded tumor tissue sections (5  $\mu$ m) were fixed with 10% paraformaldehyde for 2 h at 37°C were prepared and subsequently deparaffinized in xylene and washed in PBS with Tween-20 (1%) three times at room temperature, followed by epitope retrieval using Tris-EDTA buffer solution (pH 9.0; Sigma-Aldrich; Merck KGaA; cat. no. SRE0063) for 60 min at 65°C. The paraffin sections were subjected to hydrogen peroxide (3%) for 10-15 min, and were subsequently blocked with 5% bovine serum albumin (Sigma-Aldrich; Merck KGaA) for 10-15 min at 37°C. Finally, the sections were incubated with rabbit anti-mouse p65 (1:1,000; cat. no. ab16502), Ikk- $\beta$  (1:1,000; cat. no. ab17870), Ikb $\alpha$  (1:1,000; cat. no. ab16502; all Abcam) at 4°C for 12 h. All sections were washed three times and incubated with Alexa Fluor<sup>®</sup> 488-conjugated goat anti-rabbit IgG secondary antibody (1:1,000; cat. no. ab150077; Abcam) for 1 h at 37°C and were observed in six random fields of view under a fluorescence microscope at x40 magnification.

**Enzyme-linked immunosorbent assay (ELISA).** Plasma concentration levels of angiotensin, VCE and  $\alpha$ -ADR from mice with gestational hypertension were detected by ELISA after receiving treatment with Candesartan, using commercialized ELISA kits for ACE (cat. no. DL-ACE2-Mu),  $\alpha$ -ADR (cat. no. KL-EL001391SH; both Shanghai Kanglang Biotechnology Co., Ltd., Shanghai, China), according to the

manufacturer's protocol. The absorbance was measured at a wavelength of 450 nm on an ELISA plate reader.

**Analysis of Th1/Th2.** Plasma concentration levels Th1/Th2 were measured using Th1/Th2 kit (LHC0015M, Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol.

**Activity of NF- $\kappa$ B.** Activity of NF- $\kappa$ B in vascular endothelial cells from healthy mice and mice with gestational hypertension was analyzed after receiving treatment with Candesartan. The activity of NF- $\kappa$ B was determined with the NF- $\kappa$ B p65 Transcription Factor Assay Kit (cat. no. 10007889; Shenzhen Xinbo Sheng Biological Technology Co., Ltd., Shenzhen, China) according to the manufacturer's instructions.

**Statistical analysis.** All data are presented as mean  $\pm$  standard error of three replicates within the same experiment and performed using GraphPad Prism software 5.0 (GraphPad Software, Inc., La Jolla, CA, USA). Statistical differences between experimental groups were analyzed by Student's t-test or a one-way analysis of variance followed by Tukey's post-hoc test.  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**Effects of Candesartan treatment on expression of Ang-IITR in vascular endothelial cells.** To assess the impact of Candesartan on angiotensin release and Ang-IITR expression in a gestational hypertension model system, isolated endothelial cells were incubated with Candesartan. Angiotensin expression levels were upregulated in control vascular endothelial cells, whereas Candesartan significantly inhibited angiotensin release compared with control mice; there was no significant compared with healthy mice (Fig. 1A). *In vitro* assays indicated that homocysteine also induced angiotensin release compared with the control group, whereas Candesartan treatment inhibited angiotensin release compared with the control group (Fig. 1B). In addition, Ang-IITR expression levels (Fig. 1C) and angiotensin plasma concentration levels (Fig. 1D) were upregulated in control group compared with the healthy

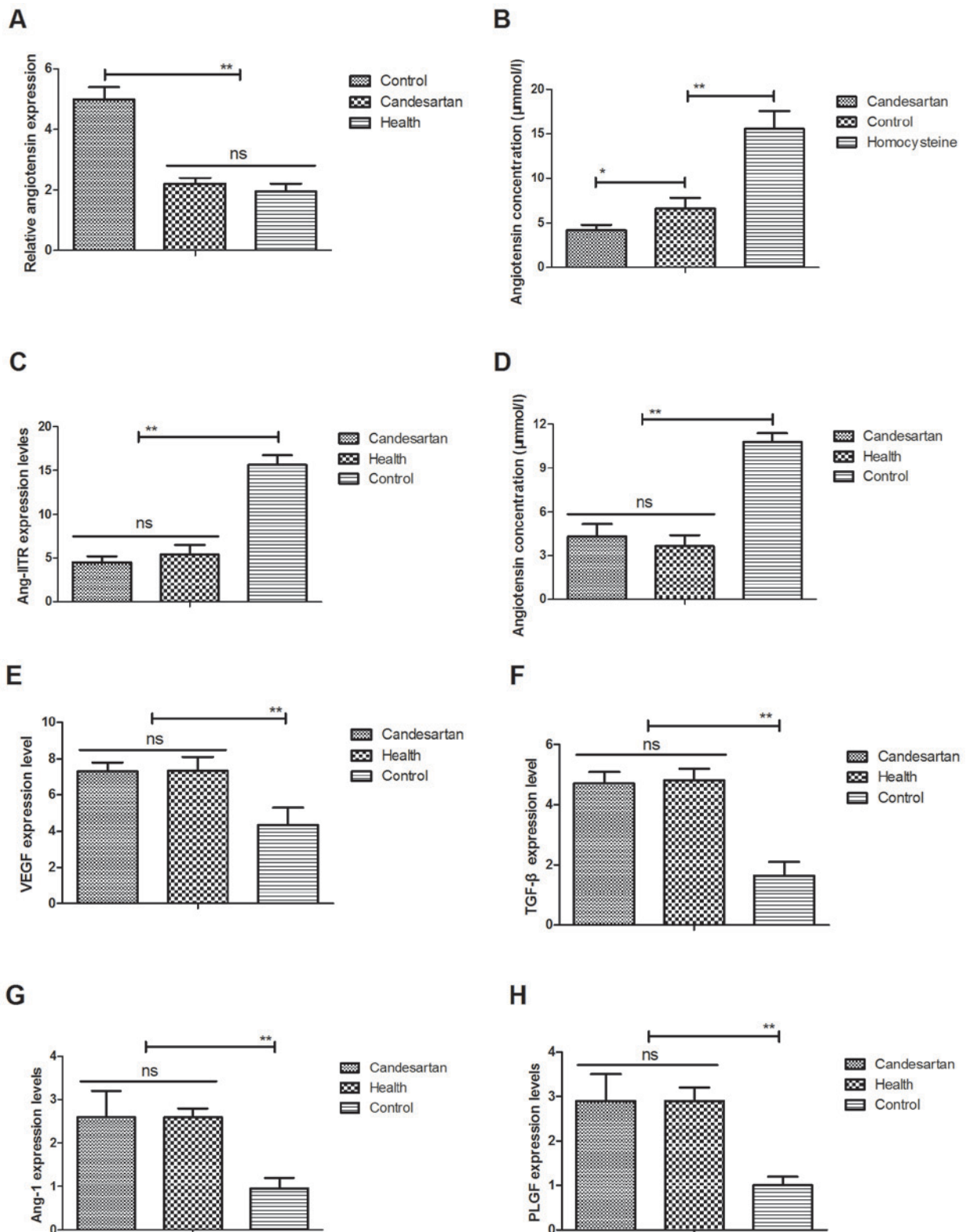


Figure 1. Effects of Candesartan on Ang-IIIR and expression levels of hypertension-related proteins in vascular endothelial cells. (A) Angiotensin expression levels in control, healthy or Candesartan-treated vascular endothelial cells. (B) Angiotensin release in vascular endothelial cells after treatment with homocysteine or Candesartan. (C) Ang-IIIR expression levels and (D) angiotensin plasma concentration levels in control or Candesartan-treated mice with gestational hypertension. Analysis of (E) VEGF, (F) TGF- $\beta$ , (G) Ang-1 and (H) PLGF expression levels in control, healthy or Candesartan-treated vascular endothelial cells. The data are presented as the mean  $\pm$  standard error. \* $P$ <0.05 and \*\* $P$ <0.01 vs. control. ns, no significant difference; VEGF, vascular endothelial growth factor; TGF- $\beta$ , transforming growth factor- $\beta$ ; Ang-1, angiotensin II type 1 receptor.

group and were downregulated after Candesartan treatment *in vivo* compared with the control group. Furthermore, we analyzed expression levels of hypertension-related proteins.

As demonstrated in Fig. 1E-H, VEGF, TGF- $\beta$ , Ang-1 and PLGF expression levels were upregulated by Candesartan compared with the control group. Taken together, these results



suggested that Candesartan treatment may be beneficial for the downregulation of angiotensin release and Ang-IITR expression in vascular endothelial cells and mice with gestational hypertension induced by homocysteine.

*Candesartan treatment markedly improves sFlt-1, insulin resistance, and cardiovascular risk factors.* Gestational hypertension is characterized by widespread endothelial cell dysfunction, the progressive elevation of insulin resistance and higher expression of cardiovascular risk factors (27). Western blot analyses of sFlt-1 revealed that Candesartan treatment significantly decreased sFlt-1 expression levels compared with control vascular endothelial cells (Fig. 2A). Insulin resistance of mice was significantly improved in mice with gestational hypertension after treatment with Candesartan compared with the control group (Fig. 2B). In addition, renal prorenin receptor expression levels were also downregulated by Candesartan compared with the control group (Fig. 2C). Furthermore, cardiovascular risk factors including inflammation and oxidative stress were studied in the vascular endothelial cells after treatment with Candesartan. As demonstrated in Fig. 2D-F, tumor necrosis factor- $\alpha$  and interleukin-2 expression and the balance of Th1/Th2 was downregulated by Candesartan compared with the control group, in the serum of mice with gestational hypertension. Western blot analysis of oxidative stress demonstrated that expression levels of SOD (Fig. 1G) and ROS (Fig. 1H) were downregulated by Candesartan compared with control vascular endothelial cells. Taken together, these results suggested that Candesartan treatment markedly improved sFlt-1, insulin resistance and cardiovascular risk factors.

*Candesartan regulates soluble endoglin via the NF- $\kappa$ B signaling pathway.* To analyze the mechanism of Candesartan-mediated benefits for gestational hypertension, the NF- $\kappa$ B signaling pathway was investigated. Soluble endoglin expression was analyzed in vascular endothelial cells. As demonstrated in Fig. 3A, endoglin expression was downregulated by Candesartan compared with the control. Cytokine mRNA expressions levels of CINC-1 (Fig. 3B), LIX (Fig. 3C) and monokine (Fig. 3D) were decreased after treatment with Candesartan compared with the control. In addition, it was observed that p65, IKK- $\beta$  and I $\kappa$ B $\alpha$  expression levels were upregulated by Candesartan in vascular endothelial cells, compared with the control (Fig. 3E). NF- $\kappa$ B activity was upregulated by Candesartan in vascular endothelial cells compared with the control (Fig. 3F). Inhibition of NF- $\kappa$ B activity by JSH23 reversed Candesartan-mediated reduction of sFlt-1 and endoglin expression levels (Fig. 3G, H). Taken together, the data suggested that Candesartan may mediate gestational hypertension via the endoglin-mediated NF- $\kappa$ B signaling pathway.

*Analysis of the efficacy of Candesartan treatment on mice with gestational hypertension induced by homocysteine.* To determine the therapeutic effects of Candesartan for gestational hypertension, a mouse model of gestational hypertension was established using homocysteine. As demonstrated in Fig. 4A, hypertension was decreased by Candesartan treatment compared with the control. Plasma concentrations of VCE and  $\alpha$ -ADR were also downregulated by Candesartan treatment compared with the control, as determined by RT-qPCR (Fig. 4B and C).

Candesartan treatment also upregulated NF- $\kappa$ B activation and nuclear translocation *in vivo* (Fig. 4D and E). Immunostaining assays revealed that p65, IKK- $\beta$  and I $\kappa$ B $\alpha$  expression levels in vascular endothelial cells were increased after 14-day treatment with Candesartan (Fig. 4F). In addition, ELISA assays demonstrated that Candesartan treatment significantly inhibited aldosterone (Fig. 4G) and renin (Fig. 4H) release in serum in mice with gestational hypertension. Taken together, these results suggested that Candesartan treatment may be beneficial for the treatment of gestational hypertension induced by homocysteine.

## Discussion

Reports have indicated angiotensin release and Ang-IITR expression serve a crucial role in the initiation and development of gestational hypertension, which respectively target angiotensin and  $\alpha$ -ADR (28,29). Research also suggests that VCE and  $\alpha$ -ADR are potential targets for the treatment of gestational hypertension (30). The underlying mechanism of formation of gestational arterial hypertension for women during pregnancy have been investigated and results have indicated that Ang-IITR contributes to the progression of gestational hypertension (31). Therefore, Ang-IITR may be a potential target and studies have revealed the efficacy of an Ang-IITR antagonist for hypertension in the clinic (32-34). In the present study, the therapeutic effects of Candesartan (an Ang-IITR antagonist) was investigated in pregnant mice with hypertension, which was induced by homocysteine. Although various previous studies have investigated the efficacy of Candesartan for the treatment of hypertension, the therapeutic effects of Candesartan for gestational hypertension is infrequently reported (35,36).

Candesartan is in a class of drugs called angiotensin II receptor antagonists. Candesartan prevents the constriction (narrowing) of veins and arteries and has been used in the treatment of essential hypertension (37). Currently, the classical RAS has been studied extensively for decades and has yielded numerous effective therapies for gestational hypertension and gestational hypertension-induced complications (19,38). Research has indicated that Ang-IITR blockers are antihypertensive drugs that may be an efficient way to improve the renin-angiotensin system by binding to Ang-IITR, leading to improvement of RAS, gestational hypertension and gestational hypertension-associated target organ damage in hypertensive animal models (39-41). Therefore, the present study determined whether Candesartan may have therapeutic effects on gestational hypertension. Candesartan treatment markedly improved gestational hypertension in a mouse model of gestational hypertension induced by homocysteine. The findings of the present study have suggested that Candesartan improves gestational hypertension via the NF- $\kappa$ B signaling pathway.

Previous studies revealed that the NF- $\kappa$ B signaling pathway may be involved in the biochemical metabolism of vascular endothelial cells and progression of gestational hypertension (42,43). Henke *et al* (44) demonstrated that vascular endothelial cell-specific NF- $\kappa$ B inhibition not only attenuates hypertension, but may also improve hypertension-induced renal damage. Alonso *et al* (45) revealed that an angiotensin II- and NF- $\kappa$ B-dependent mechanism may increase connexin 43 in murine arteries by targeting of renin-dependent

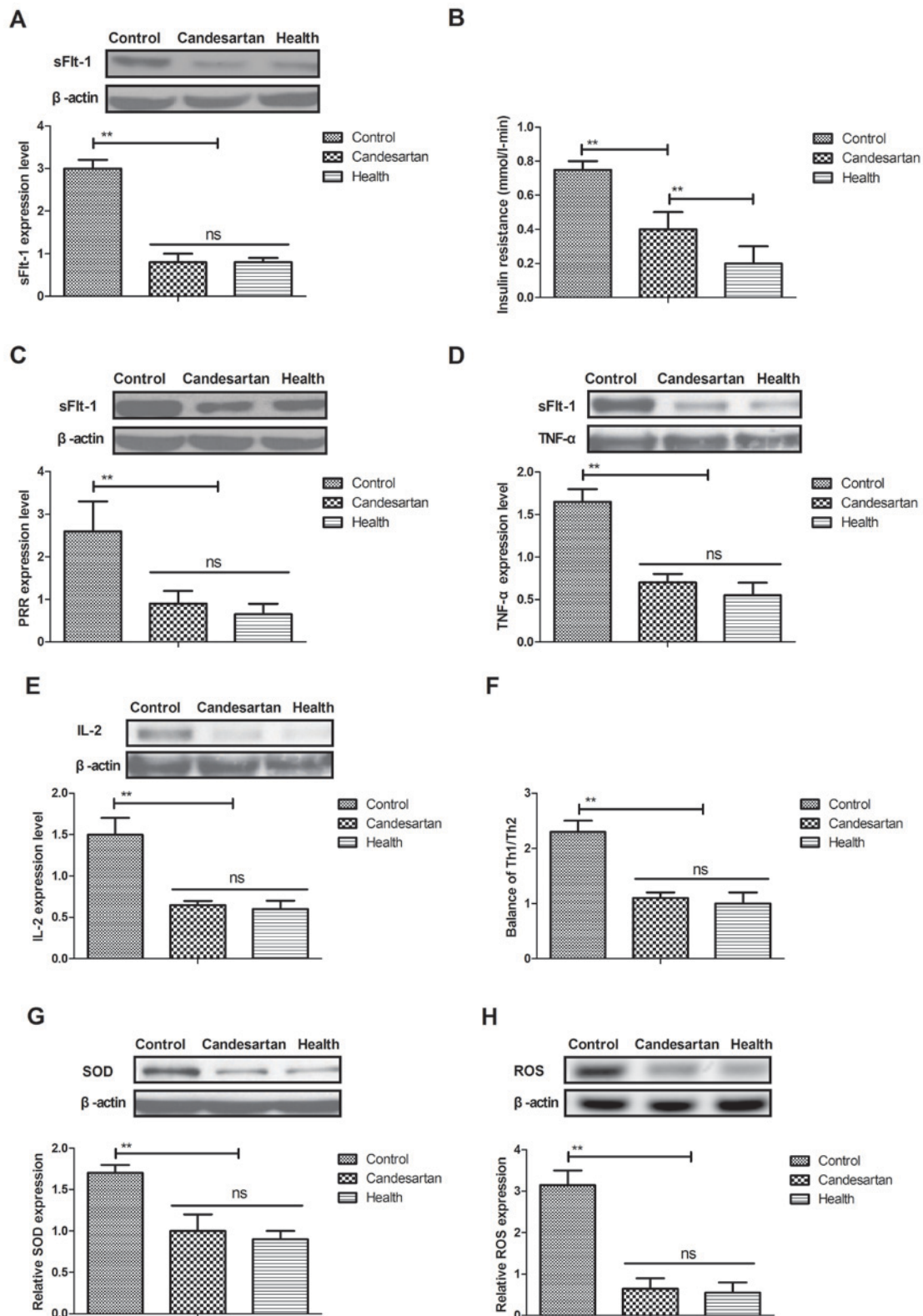


Figure 2. Analysis of sFlt-1, insulin resistance and cardiovascular risk factors in mice with gestational hypertension after treatment with Candesartan. (A) Expression levels of sFlt-1 in control, healthy or Candesartan-treated vascular endothelial cells. (B) Insulin resistance of control, healthy or Candesartan-treated mice with gestational hypertension. (C) Expression levels of PRR in control, healthy or Candesartan-treated vascular endothelial cells. Expression levels of (D) TNF- $\alpha$  and (E) IL-2, and (F) balance of Th1/Th2 in the serum of control, healthy or Candesartan-treated mice with gestational hypertension. Expression levels of (G) SOD and (H) ROS in control, healthy or Candesartan-treated vascular endothelial cells. The data are presented as the mean  $\pm$  standard error. \*\* $P$ <0.01 vs. control. ns, no significant difference. sFlt-1, soluble fms-like tyrosine kinase 1; PRR, prorenin receptor; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; IL-2, interleukin-2; Th1, T-helper cell class 1; Th2, T-helper cell class 2; SOD, superoxide dismutase; ROS, reactive oxygen species.

hypertension. In addition, the NF- $\kappa$ B signaling pathway may be involved in obesity-induced hypertension (46). The

NF- $\kappa$ B pathway is also involved in C-reactive protein-induced effects on pulmonary arterial endothelial cells in chronic

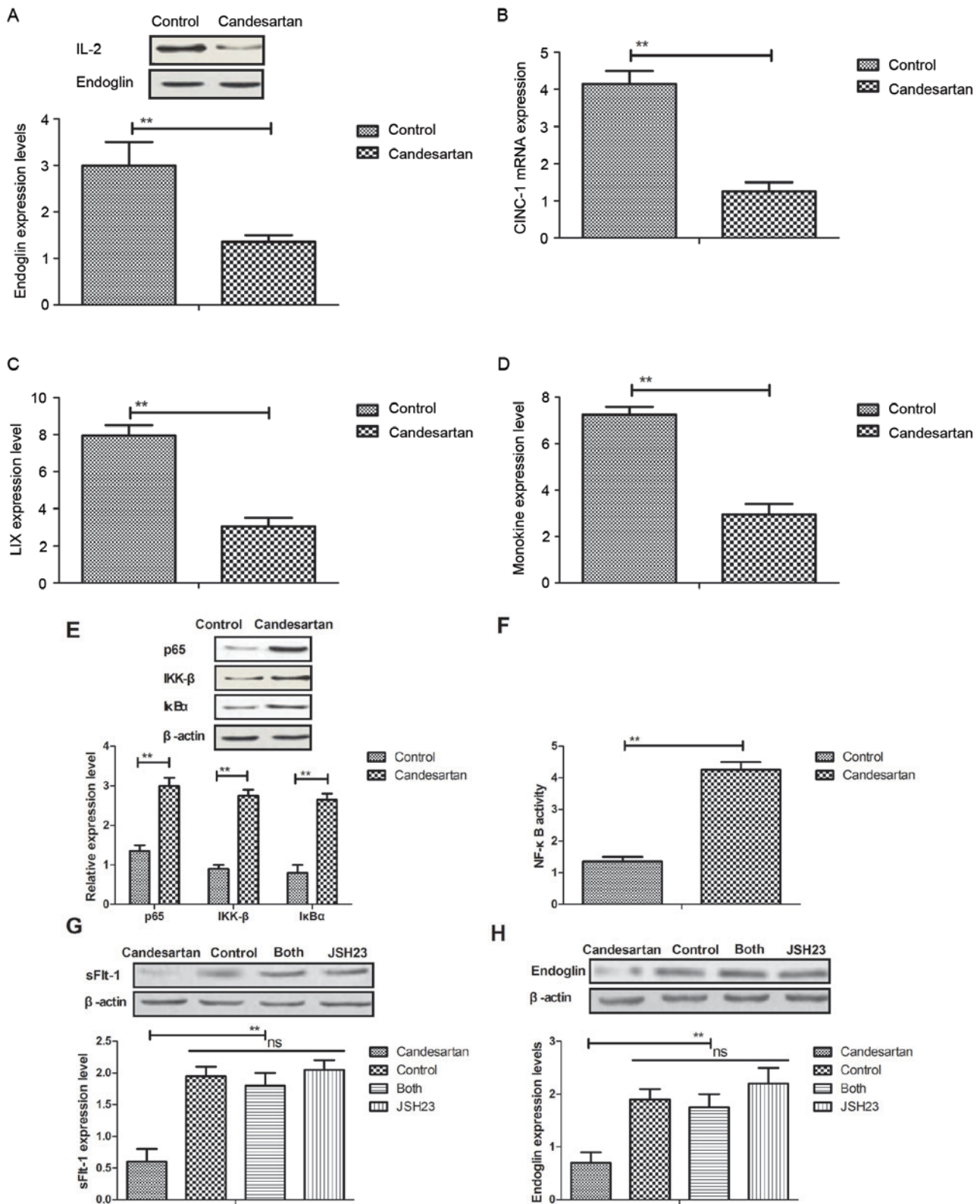


Figure 3. Candesartan regulates gestational hypertension via the NF- $\kappa$ B signaling pathway. (A) Expression levels of endoglin in control or Candesartan-treated vascular endothelial cells. mRNA expression levels of (B) CINC-1, (C) LIX and (D) monokine in control or Candesartan-treated vascular endothelial cells. (E) Expression levels of p65, IKK- $\beta$  and I $\kappa$ B $\alpha$  in control or Candesartan-treated vascular endothelial cells. (F) NF- $\kappa$ B activity in control or Candesartan-treated vascular endothelial cells. Inhibition of NF- $\kappa$ B activity by JSH23 reversed Candesartan-mediated reduction of (G) sFlt-1 and (H) endoglin expression levels. The data are presented as the mean  $\pm$  standard error. \*\*P<0.01 vs. control. NF- $\kappa$ B, nuclear factor- $\kappa$ B; CINC-1, cytokine-induced neutrophil chemoattractant 1; LIX, lipopolysaccharide induced CXCL chemokine; IKK- $\beta$ , inhibitor of nuclear factor- $\kappa$ B kinase subunit  $\beta$ ; I $\kappa$ B $\alpha$ , nuclear factor of  $\kappa$  light polypeptide gene enhancer in B-cells inhibitor  $\alpha$ ; sFlt-1, soluble fms-like tyrosine kinase.

thromboembolic pulmonary hypertension. Furthermore, the effect of antihypertensive drugs on the expression of NF- $\kappa$ B

in monocrotaline-induced pulmonary arterial hypertension of rats have been investigated by Li *et al* (47). These reports



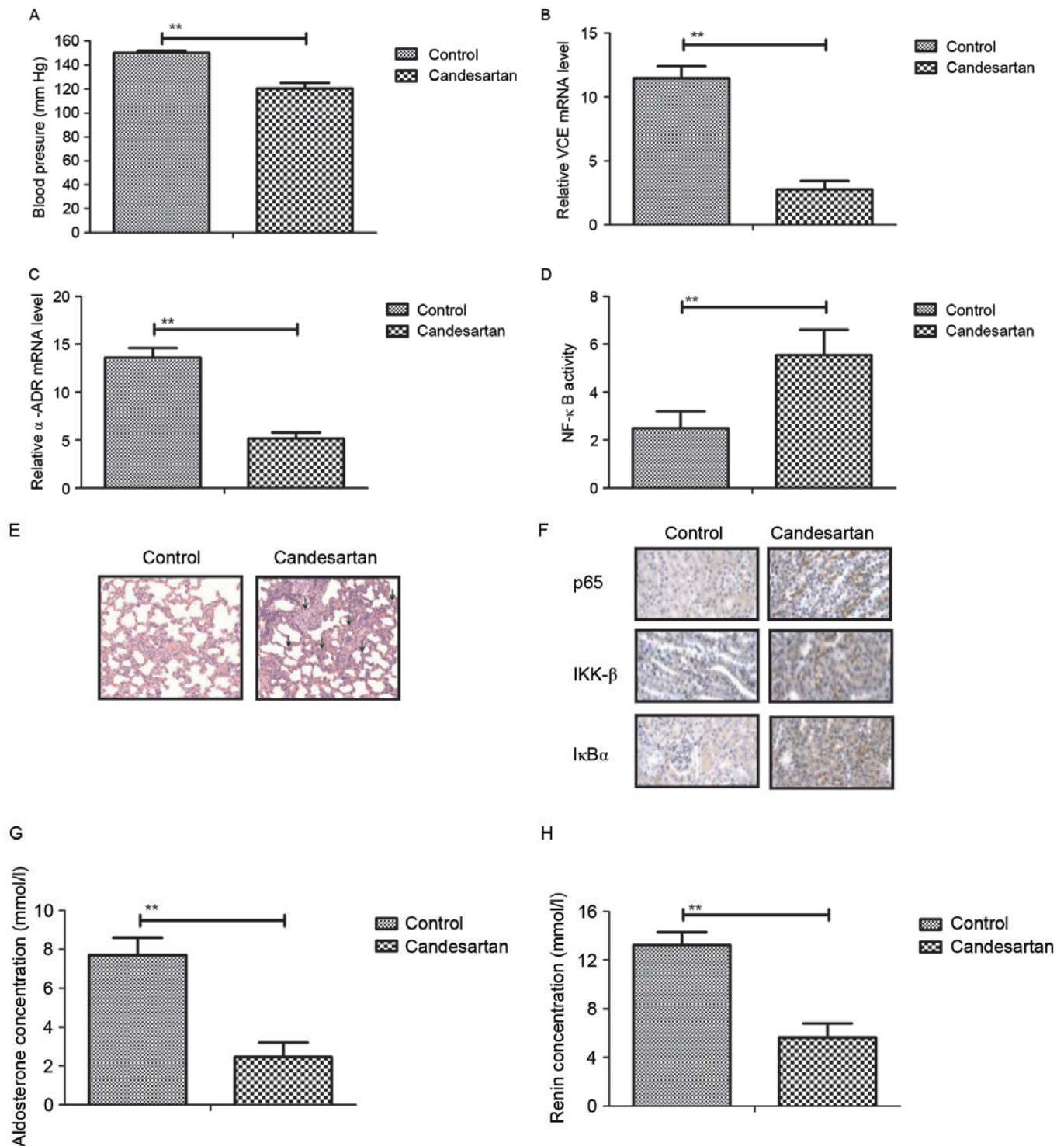


Figure 4. *In vivo* therapeutic effects of Candesartan on mice with gestational hypertension induced by homocysteine. (A) Blood pressure was reduced after Candesartan treatment, suggesting an improvement in gestational hypertension compared with the control. Plasma concentration levels of (B) VCE and (C)  $\alpha$ -ADR in control or Candesartan-treated mice with gestational hypertension. (D) NF- $\kappa$ B activity and (E) nuclear translocation of NF- $\kappa$ B were analyzed in control or Candesartan-treated vascular endothelial cells. (F) Immunostaining for p65, IKK- $\beta$  and I $\kappa$ B $\alpha$  expression levels in control or Candesartan-treated vascular endothelial cells. ELISA assays analyze concentration levels of (G) aldosterone and (H) renin release in serum in control or Candesartan-treated mice with gestational hypertension. The data are presented as the mean  $\pm$  standard error. \*\* $P < 0.01$  vs. control. VCE, vasodilation converting enzyme;  $\alpha$ -ADR,  $\alpha$ -adrenergic receptor; NF- $\kappa$ B, nuclear factor- $\kappa$ B; IKK- $\beta$ , inhibitor of nuclear factor- $\kappa$ B kinase subunit  $\beta$ ; I $\kappa$ B $\alpha$ , nuclear factor of  $\kappa$  light polypeptide gene enhancer in B-cells inhibitor  $\alpha$ .

suggested that the NF- $\kappa$ B signaling pathway may be associated with the initiation and progression of hypertension.

The results of the present study suggested that the NF- $\kappa$ B signaling pathway influences Candesartan-mediated soluble

endoglin and cytokine (CINC-1, LIX and monokine) expressions levels in vascular endothelial cells. Blocking of the NF- $\kappa$ B signaling pathway suppressed Candesartan-mediated improvement of sFlt-1 and endoglin expression levels. sFlt-1



and endoglin are two important predictive biomarkers of gestational hypertension (48,49). Alterations in sFlt-1 and endoglin expression levels may predict the progression of gestational hypertension (50,51). The results of the present study suggested that sFlt-1 and endoglin expression levels are altered after Candesartan treatment. Notably, gestational hypertension is markedly decreased after Candesartan treatment once daily for a total of 14 days.

To conclude, the present study investigated the clinical efficacy of Candesartan in mice with gestational hypertension induced by homocysteine. Although previous studies have reported the direct effects of Candesartan on hypertension, the present study investigated the effects of Candesartan in gestational hypertension and gestational hypertension-associated factors (52,53). This study suggested that Candesartan has a novel role in gestational hypertension management and an increasing number of preclinical reports show promising results (54-56). Of note, our results suggested that the NF- $\kappa$ B signaling pathway is involved in Candesartan-mediated initiation and progression of gestational hypertension. Overall, our findings suggested that mice with gestational hypertension treated by Candesartan exhibited beneficial effects for inflammation, oxidative stress and hypertension. However, future work should be performed in a larger cohort to support the data of the present study.

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#### Availability of data and materials

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

#### Author's contributions

XZ performed the experiments. XW designed the experiments and analyzed the data.

#### Ethics approval and consent to participate

The present study was carried out in strict accordance with the recommendations in the Guide for Shandong Provincial Hospital affiliated to Shandong University. All surgery and euthanasia were performed to minimize suffering. This study was approved by the ethics committee of Shandong University and Taian City Central Hospital (Jinan, China).

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare that they have no competing interests.

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