

Ang-(1-7) protects HUVECs from high glucose-induced injury and inflammation via inhibition of the JAK2/STAT3 pathway

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Abstract. Angiotensin (Ang)-1-7, which is catalyzed by angiotensin-converting enzyme 2 (ACE2) from angiotensin-II (Ang-II), exerts multiple biological and pharmacological effects, including cardioprotective effects and endothelial protection. The Janus kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) pathway has been demonstrated to be involved in diabetes-associated cardiovascular complications. The present study hypothesized that Ang-(1-7) protects against high glucose (HG)-induced endothelial cell injury and inflammation by inhibiting the JAK2/STAT3 pathway in human umbilical vein endothelial cells (HUVECs). HUVECs were treated with 40 mmol/l glucose (HG) for 24 h to establish a model of HG-induced endothelial cell injury and inflammation. Protein expression levels of p-JAK2, t-JAK2,

p-STAT3, t-STAT3, NOX-4, eNOS and cleaved caspase-3 were tested by western blotting. CCK-8 assay was performed to assess cell viability of HUVECs. Apoptotic cell death was analyzed by Hoechst 33258 staining. Mitochondrial membrane potential (MMP) was obtained using JC-1. Superoxide dismutase (SOD) activity was tested by SOD assay kit. Interleukin (IL)-1 β , IL-10, IL-12 and TNF- α levels in culture media were tested by ELISA. The findings demonstrated that exposure of HUVECs to HG for 24 h induced injury and inflammation. This injury and inflammation were significantly ameliorated by pre-treatment of cells with either Ang-(1-7) or AG490, an inhibitor of the JAK2/STAT3 pathway, prior to exposure of the cells to HG. Exposure of the cells to HG also increased the phosphorylation of JAK2/STAT3 (p-JAK2 and p-STAT3). Increased activation of the JAK2/STAT3 pathway was attenuated by pre-treatment with Ang-(1-7). To the best of our knowledge, the findings from the present study provided the first evidence that Ang-(1-7) protects against HG-induced injury and inflammation by inhibiting activation of the JAK2/STAT3 pathway in HUVECs.

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Introduction

The incidence of diabetes mellitus (DM) is estimated to rise to 7.7% by 2030 globally (1) and is predicted to affect 591,900,000 individuals by 2035 (2). DM is a major and increasing health problem worldwide due to its increasing incidence, which can lead to a variety of complications, including diabetic retinopathy, diabetic nephropathy, diabetic neuropathy, diabetic foot and cardiovascular complications. Of note, cardiovascular complications are the major cause of diabetes-associated mortality (3). The vascular endothelium is central to the pathogenesis of diabetic complications, with vascular endothelial cells mainly involved in maintaining endothelial dysfunction;

cardiovascular homeostasis is considered an important factor in the pathogenesis of diabetes-associated vascular complications (4,5). Studies have revealed that hyperglycemia induces numerous pathological changes in vascular endothelial cell injury, including oxidative stress (6), inflammation (7), increased endothelial cell apoptosis (6,8) and mitochondrial membrane permeabilization (9). However, the associated molecular mechanism by which hyperglycemia results in vascular endothelial cell injury in DM remains to be elucidated.

As one of four protein-tyrosine kinases, Janus kinase (JAK)1, JAK2, JAK3 and Tyk2, JAK2 is an essential factor in cellular proliferation, differentiation, survival and senescence. JAK2 also regulates other signaling molecules, including the RAS, signal transducer and activator of transcription (STAT)5, STAT3 and phosphoinositide 3-kinase/Protein kinase B (PI3K/AKT) pathways (10). STAT3 is a member of the STAT family (STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b and STAT6), and its phosphorylation is induced by the activation of JAK2; phosphorylated (p-)STAT3 forms a dimer and translocates into the nucleus from the cytoplasm, where it binds to related sequences and alters the expression of various target genes. Therefore, the JAK2/STAT3 pathway is gradually being recognized as a membrane-to-nucleus pathway for a variety of stimulating responses *in vitro* and *in vivo* (11-13). Accumulating evidence has shown that the JAK2/STAT3 pathway is a cell survival signal, which contributes to cell proliferation, differentiation, growth and apoptosis (14-16). The JAK2/STAT3 pathway is also important in the progress of cardiovascular diseases. Manea *et al* (17) demonstrated that the JAK2/STAT3 pathway is a crucial regulator of the response of EAhy926 endothelial cells to diabetes-associated cardiovascular dysfunction. Hyperglycemia increases angiotensin (Ang)-II-induced vascular smooth muscle cell proliferation by increasing JAK2/STAT3 pathway transduction (18). These findings indicate that the JAK2/STAT3 pathway is important in cardiovascular diseases and endothelial dysfunction. However, the effects of the JAK2/STAT3 pathway on high glucose (HG)-induced endothelial dysfunction *in vivo*, and the associated mechanisms, remain to be elucidated.

There has been increasing focus on the protective effects of Ang-(1-7) against hyperglycemia-induced cardiovascular complication. The secretory level of plasma Ang-(1-7) was demonstrated to be low in patients with diabetic-induced cardiac dysfunction (19). The expression level of angiotensin-converting enzyme 2 (ACE2), which is responsible for the tissue degradation of Ang-II into Ang-(1-7), was also observed to be decreased in patients with diabetes (20). An increase in the expression levels of ACE2 markedly improved control of blood glucose levels and alleviated glomerular injury in streptozotocin (STZ)-induced diabetic mice (21), and Ang-(1-7) was reported to exert protective effects against diabetes-induced cardiovascular events (22,23). In addition, the Ang-(1-7) and ACE2/Ang-(1-7)/Mas receptor axis was revealed to have additional beneficial effects in preventing diabetes-induced cardiac dysfunction (24). These findings indicate that Ang-(1-7) is closely associated with hyperglycemia-induced cardiovascular dysfunction. However, the underlying mechanisms between Ang-(1-7) and hyperglycemia-induced cardiovascular dysfunction remain to be fully elucidated.

A previous study reported that Ang-(1-7) produced an inhibitory effect on the activation of JAK2/STAT3 (25). In diabetic nephropathy, Ang-(1-7) exerts renoprotective effects on diabetic nephropathy via inhibiting the STAT3 pathway, apparent as a reduction in inflammation, fibrosis, oxidative stress and lipotoxicity (26). The present study tested the hypothesis that exogenous Ang-(1-7) protects human umbilical vein endothelial cells (HUVECs) against HG-induced injury and inflammation by inhibiting the JAK2/STAT3 pathway.

Materials and methods

Materials. Ang-(1-7) was purchased from Sigma; Merck KGaA (Darmstadt, Germany) and stored at -20°C. The following 2',7'-dichlorofluorescein diacetate (DCFH-DH), Hoechst 33258, 5',6,6'-tetrachloro-1,1',3,3'-tetraethyl-imidacarbocyanine iodide (JC-1) and AG490 (an inhibitor of the STAT3/JAK2 pathway) were obtained from Sigma-Aldrich; Merck KGaA. The Cell Counting Kit-8 (CCK-8) was supplied by Dojindo Molecular Technologies, Inc. (Kumamoto, Japan). Fetal bovine serum (FBS) and Dulbecco's modified Eagle's medium (DMEM) were purchased from Gibco; Thermo Fisher Scientific, Inc. (Waltham, MA, USA). Anti-p-STAT3 antibody (cat. no. SAB4300033), anti-total (t-)STAT3 antibody (cat. no. SAB4300708), anti-p-JAK2 antibody (cat. no. SAB4300124), anti-t-JAK2 antibody (cat. no. SAB4501599), anti-caspase-3 antibody (cat. no. C5737) anti-NADPH oxidase 4 (Nox4) antibody (cat. no. SAB4503153) and anti-endothelial nitric oxide synthase (eNOS) antibody (cat. no. N2643) were supplied by Cell Signaling Technology, Inc. (Boston, MA, USA), horseradish peroxidase (HRP)-conjugated secondary antibody (cat. no. KC-5A08) and a BCA protein assay kit were obtained from KangChen Biotech, Inc. (Shanghai, China). Enhanced chemiluminescence (ECL) solution was purchased from Nanjing KeyGen Biotech Co., Ltd. (Nanjing, China). The reagents for reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis was obtained from Invitrogen; Thermo Fisher Scientific, Inc.).

Cell culture and treatments. The HUVECs were supplied by Sun Yat-sen University Experimental Animal Center (Guangzhou, China). The HUVECs were cultured in DMEM supplemented with 10% FBS under an atmosphere of 5% CO₂ and 37°C with 95% air.

To establish a model of HG-induced HUVEC injury, the cells were cultured in DMEM (5.5 mM glucose) for 12 h prior to the administration of 40 mM glucose (final concentration) for 24 h. The glucose concentration of the control group was 5.5 mM. To investigate the protective effect of exogenous Ang-(1-7) against HG (40 mM glucose)-induced injury, the cells were seeded at a density of 1×10⁴/ml and treated with HG in the presence or absence of Ang-(1-7) for 24 h at 37°C. In order to examine whether the STAT3/JAK2 pathway contributed to the protective effects of Ang-(1-7), and to further determine the mechanisms underlying the protective effects of Ang-(1-7), the HUVECs at a density of 1×10⁴/ml were co-treated with HG, 2 μmol/l Ang-(1-7) and 20 μmol/l AG490 (an inhibitor of the STAT3/JAK2 pathway), and incubated at 37°C.

RNA interference. HUVECs were transfected at 70% confluency using Lipofectamine transfection reagent (Life Technologies; Thermo Fisher Scientific, Inc.), with siRNA against NLRP3 (Ribo Biotechnology, Shanghai, China) or a physiologically irrelevant negative control siRNA. The siRNA sequences used in the present study were as follows: Sense, 5'-GCUUCAGCCACAUGACUUUTT-3'; and antisense, 5'-AAAGUCAUGUGG CUGAAGCTT-3'. Each siRNA was dissolved in nuclease-free water to achieve a final concentration of 20 μ M. A total of 5 μ l siRNA (20 μ M) and 5 μ l Lipofectamine were added to a 500 μ l buffer system. The mixtures were kept at room temperature for 30 min to form complexes, and equal amounts were added into wells of a 6-well plate. The cultures were incubated at 37°C in a 5% CO₂ incubator. The medium was replaced after 12 h with DMEM without the presence of siRNA or transfection reagent. Cells were collected at 12 h for analyses.

Western blot analysis. Following the indicated treatments, the HUVECs were harvested using a cell scraper and lysed with cell lysis solution (Beyotime Institute of Biotechnology, Haimen, China), at 4°C for 30 min. The total proteins were quantified using the BCA protein assay kit. Loading buffer was added to the cytosolic extracts and boiled for 5 min. Equal quantities of supernatant from each sample (20 μ g) were fractionated by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis, following which the total proteins were transferred onto polyvinylidene difluoride membranes. The membranes were blocked with 5% fat-free milk for 60 min in fresh blocking buffer containing 0.1% Tween20 in Tris-buffered saline (TBS-T) at room temperature, and incubated with either anti-p-STAT3 (1:1,000 dilution), anti-t-STAT3 (1:1,000 dilution), anti-Nox4 (1:1,000 dilution), anti-p-JAK2 (1:1,000 dilution), anti-t-JAK2 (1:1,000 dilution), anti-caspase-3 (1:1,000 dilution) and anti-eNOS (1:1,000 dilution) in freshly prepared TBS-T with 3% fat-free milk, overnight with gentle agitation at 4°C. The membranes were then washed for 15 min with TBS-T and incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibody (Kangchen Biotech, Inc.), at a 1:3,000 dilution, in TBS-T with 3% fat-free milk for 90 min at room temperature. The membranes were then washed three times with TBS-T for 15 min and the immunoreactive signals were visualized using an ECL detection. In order to quantify protein expression, the X-ray films were scanned and analyzed with ImageJ 1.47i software (National Institutes of Health, Bethesda, MA, USA). The experiment was repeated three times.

Measurement of cell viability. The HUVECs were seeded in 96-well plates at a concentration of 1x10⁴/ml, and incubated at 37°C, following which a CCK-8 assay was used to assess the cell viability of HUVECs. Following the indicated treatments, 10 μ l CCK-8 solution at a 1/10 dilution was added to each well and the plate was incubated for 1.5 h in the incubator. The absorbance at 450 nm was determined using a microplate reader (Molecular Devices LLC, Sunnyvale, CA, USA). The mean optical density (OD) of three wells in the indicated groups was used to calculate the percentage of cell viability according to the following formula: Cell viability (%) = (OD treatment group/OD control group) x 100%. The experiment was performed five times.

Hoechst 33258 nuclear staining for the analysis of apoptosis. Apoptotic cell death was analyzed using Hoechst 33258 staining followed by photofluorography. Firstly, the HUVECs were plated in 35 mm dishes at a density of 1x10⁶ cells/well. Following the indicated treatments, the cells were fixed with 4% paraformaldehyde in 0.1 mol/l phosphate-buffered saline (PBS; pH 7.4) for 10 min at 4°C. The slides were washed 5 times with PBS. Following staining by incubation with 5 mg/ml Hoechst 33258 for 30 min, the cells were washed 5 times with PBS. Finally, the cells were visualized under a fluorescence microscope (Bx50-FLA; Olympus Corporation, Tokyo, Japan). Viable HUVECs exhibited a uniform blue fluorescence throughout the nucleus and normal nuclear size, whereas apoptotic HUVECs demonstrated condensed, distorted or fractured nuclei. The experiment was repeated 5 times.

Examination of intracellular reactive oxygen species (ROS) generation. Intracellular ROS generation was determined by the oxidative conversion of cell-permeable oxidation of DCF-DH to fluorescent DCF. The HUVECs were cultured on a slide with DMEM. Following the above treatments, the slides were washed twice with PBS. Subsequently, 10 μ mol/l DCFH-DA solution in serum-free medium was added to the slides, and the cells were incubated at 37°C for a further 30 min. The slides were washed 5 times with PBS, and DCF fluorescence was measured over the entire field of vision using a fluorescence microscope connected to an imaging system (BX50-FLA; Olympus Corporation). The mean fluorescence intensity (MFI) from five randomly selected fields was measured using ImageJ 1.47i software and the MFI was used as an index of the level of ROS. The experiment was repeated 5 times.

Measurement of the mitochondrial membrane potential (MMP). The MMP was determined using the fluorescent dye, JC-1, a cell-permeable cationic dye, which preferentially enters mitochondria based on the highly negative MMP. Depolarization of MMP results in a loss of MMP from the mitochondria and a decrease in green fluorescence. The HUVECs were cultured on a slide with DMEM at a density of 1x10⁶ cells/well. Following the indicated treatments, the slides were washed 3 times with PBS; and the cells were incubated with 1 mg/l JC-1 at 37°C for 30 min in the incubator, washed briefly 3 times with PBS, and air-dried. The fluorescence was measured over the entire field of vision using a fluorescent microscope connected to an imaging system (BX50-FLA). The MFI of JC-1 from three randomly selected fields was analyzed using ImageJ 1.47i software, and the MFI was measured as an index of the levels of MMP. The experiment was repeated 3 times.

Measurement of superoxide dismutase (SOD) activity. SOD activity was analyzed using an SOD assay kit. Following the indicated treatments, the HUVECs were washed using PBS and lysed in ice-cold 0.1 M Tris/HCl (pH 7.4) containing 0.5% Triton, 5 mmol/l β -mercaptoethanol and 0.1 mg/ml phenylmethylsulfonyl fluoride. The lysates were clarified by centrifugation at 14,000 x g at 4°C for 5 min and cell debris was discarded. SOD activity was detected using a commercial SOD Assay kit according to the manufacturer's protocol

(Sigma-Aldrich; Merck KGaA). The absorbance values at 450 nm were measured using a microplate reader. The experiment was repeated 3 times.

Enzyme-linked immunosorbent assay (ELISA) for the detection of interleukin (IL)-1 β , IL-10, IL-12 and tumor necrosis factor (TNF)- α in culture supernatant. The HUVECs were seeded at 1×10^4 cells/well and cultured in 96-well plates. Following the indicated treatments, the levels of IL-1 β , IL-10, IL-12 and TNF- α in culture media were analyzed using ELISA according to the manufacturer's protocol. The experiments were repeated 5 times.

RT-qPCR analysis. Total cellular RNA was extracted from cell cultures using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The extracted RNAs were DNase-treated with RQ1 RNase-Free DNase (Promega Corporation, Madison, WI, USA). First strand cDNA was prepared using Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RT; cat. no. 1701; Promega Corporation). The RT-qPCR analysis was performed using the Rotor-GeneTM SYBR Green PCR kit (Qiagen GmbH, Dusseldorf, Germany) and the QuantiNova SYBR Green PCR kit (Qiagen GmbH) on a Rotor-Gene 6000 Rotary Analyzer (Qiagen GmbH), and determined using Rotor-Gene 6000 software version 2.3.3 (Qiagen GmbH). For each assay, a total of 8 ng cDNA was added to a final reaction volume of 25 μ l containing 1X Rotor-Gene SYBR Green PCR master mix or QuantiNova SYBR Green PCR master mix and 1 μ M of each forward and reverse primer. Quantitative gene amplifications were performed using the following thermocycling conditions: Initial denaturation for 5 min at 95°C, 40 cycles of denaturation at 95°C for 5 sec, and annealing and extension at 60°C for 20 sec. The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was used as endogenous control (or reference gene). A non-template reaction was used as a negative control. Three replicates were performed for all analysis.

The selection of the threshold intensity was set at a fixed intensity on the log-linear phase of the amplification curve for all the samples tested. Validation experiments, which included the generation of standard curves using a series of diluted cDNA samples, were performed to ensure primer efficiency, and target and reference gene amplification compatibility. Melt curve analysis and conventional agarose gel analysis were used alongside to verify the presence of a single amplicon. An interassay calibration scheme was used to minimize loading variation and to detect possible contamination with the inclusion of duplicate reactions and 'no-template' control, respectively, in each qPCR assay. Relative expression levels of the gene of interest were calculated using $2^{-\Delta\Delta C_q}$ method (27). All samples were normalized to GAPDH as the endogenous control (28).

The primers used were as follows: STAT3, forward 5'-CTT TGAGACCGAGGTGTATCACC-3'; and reverse 5'-GGT CAGATGTTGTACCAAGG-3'; JAK2, forward 5'-CCG GAATTCGCTTTGAGTCGGTTTCTCCGGTTCC-3'; and reverse 5'-TGCTCTAGACCTCATGCAGTCGCTGAATAA GTCC-3'. GAPDH, forward, 5'-CCACCCATGGCAAATTCC ATGGCA3'; and reverse 5'-TCTAGACGGCAGGTCAGGTCC ACC3'.

Statistical analysis. All data are presented as the mean \pm standard error of the mean. Differences between groups were analyzed using one-way analysis of variance with SPSS 13.0 (SPSS, Inc., Chicago, IL, USA) software, and followed by an LSD post hoc comparison test. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Ang-(1-7) and AG490 attenuate HG-induced decreased cell viability in HUVECs. The HUVECs were treated with different concentrations of glucose (10, 20, 30, 40, 50 and 60 mmol/l glucose) for 24 h, and it was found that glucose induced cell cytotoxicity (Fig. 1A). A glucose concentration of 40 mmol/l was considered a suitable concentration for use in the following experiments. To examine the cytoprotective effect of Ang-(1-7) against HG-induced cytotoxicity in HUVECs, a dose-response study with varying doses of Ang-(1-7) (0.5, 1, 2, 4, 6, 8 and 10 μ M) was performed to calculate the cardioprotection dose of Ang-(1-7). As shown in Fig. 1B, exposure of the HUVECs to 40 mM glucose (HG) for 24 h induced cytotoxicity, as indicated by the decrease in cell viability. However, the cytotoxic effect of HG on HUVECs was markedly inhibited by pre-treatment of the cells with Ang-(1-7) for 30 min. The maximum inhibitory effect was observed with 2 μ M Ang-(1-7). Alone, 10 μ M Ang-(1-7) did not significantly alter the viability of the HUVECs. For this reason, the HUVECs were pre-treated with 2 μ M Ang-(1-7) for 30 min prior to exposure to HG in all subsequent experiments.

As shown in Fig. 1C, exposure of the HUVECs to HG for 24 h induced cytotoxicity, which led to a decrease in cell viability. However, this decreased cell viability was markedly repressed by pre-treatment with 2 μ M Ang-(1-7) or 20 μ M AG490. Alone, neither 2 μ M Ang-(1-7) or 20 μ M AG490 affected the viability of HUVECs.

JAK2 siRNA inhibits expression of the JAK2/STAT3 pathway and attenuates the HG-induced decrease in cell viability of HUVECs. To observe the effects of JAK2 siRNA on the expression of the JAK2/STAT3 pathway, the HUVECs were treated with JAK2 siRNA. As shown in Fig. 2, JAK2 siRNA significantly inhibited the expression levels of p-JAK2 (Fig. 2A and B) and p-STAT3 (Fig. 2C and D). In addition, JAK2 siRNA attenuated the HG-induced decrease in cell viability in the HUVECs, as shown in Fig. 2E.

HG activates the JAK2/STAT3 pathway in HUVECs. The present study also examined the effects of HG on the STAT3/JAK2 pathway, including the phosphorylation of STAT3 and JAK2. As shown in Fig. 3A-D, the effect of glucose on the HUVECs were examined. The cells were exposed to the indicated concentrations (20, 30, 40, 50 and 60 mM) of glucose for 24 h, and exposure to glucose significantly upregulated the expression levels of (Fig. 3A and C) pSTAT3, peaking at 60 mM glucose. However, the expression of t-STAT3 was not affected by the indicated concentrations of glucose. Based on these results, the effect of time on the expression of p-STAT3 was examined. As shown in Fig. 3B and D, following exposure of HUVECs to 40 mM glucose for the indicated times (3, 6, 12, 18, 24, 36 and 48 h), the expression levels of p-STAT3 were

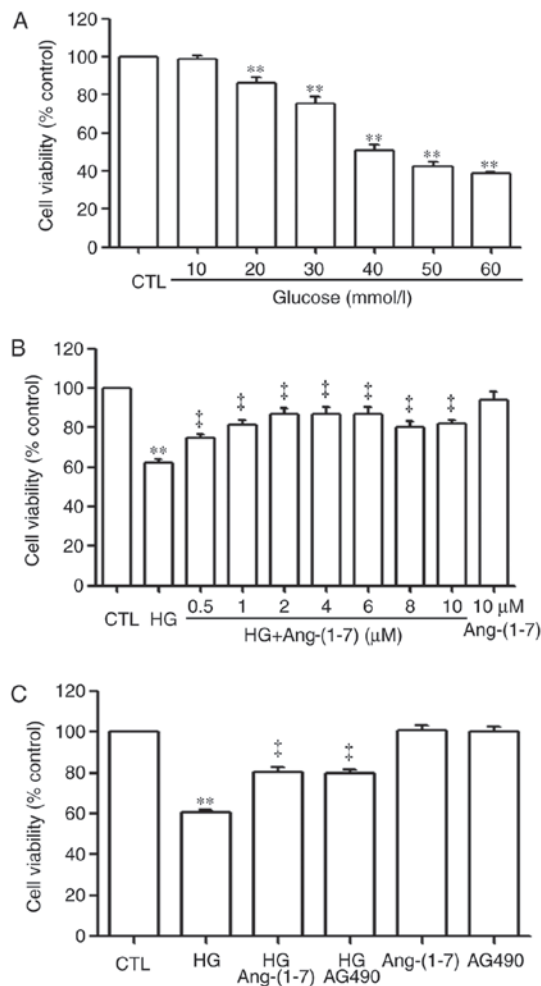


Figure 1. Ang-(1-7) and AG490 alleviate HG-induced cardiomyocyte cytotoxicity in HUVECs. Cell viability was detected using the Cell Counting Kit-8 assay. (A) HUVECs were treated with different concentrations of glucose (10, 20, 30, 40, 50 and 60 mmol/l glucose) for 24 h. (B) HUVECs were treated with HG for 24 h in the absence or presence of pre-treatment with the indicated concentrations (0.5, 1, 2, 4, 6, 8 and 10 μ M) of Ang-(1-7) for 30 min prior to exposure of cells to HG for 24 h. (C) Cells were pre-treated with or without 2 μ M Ang-(1-7) or 20 μ M AG490 (inhibitor of Janus kinase 2/signal transducer and activator of transcription 3 pathway) for 30 min prior to exposure of cells to 40 mM glucose for 24 h. **P<0.01, vs. CTL group; *P<0.01, vs. HG group. HG, high glucose (40 mM glucose); Ang-(1-7), angiotensin-(1-7); HUVECs, human umbilical vein endothelial cells.

significantly upregulated, reaching a peak at 36 h, whereas the expression of t-STAT3 remained unchanged. Similarly, exposure of the cells to 40 mM glucose increased the expression levels of p-JAK2, as shown in the dose-response experiment (Fig. 3E) and time-response experiment (Fig. 3F), respectively. The mRNA expression levels of STAT3 (Fig. 3G) and JAK2 (Fig. 3H) were also markedly increased.

Ang-(1-7) downregulates HG-induced activation of the JAK2/STAT3 pathway in HUVECs. To observe effects of Ang-(1-7) on the activation of the JAK2/STAT3 pathway induced by HG, the HUVECs were pre-treated with 2 μ M Ang-(1-7) for 30 min, prior to exposure to HG for 24 h. As shown in Fig. 4, exposure of the cells to 40 mM glucose significantly increased the expression levels of p-STAT3 (Fig. 4A and B) and p-JAK2 (Fig. 4A and C). However, the increased

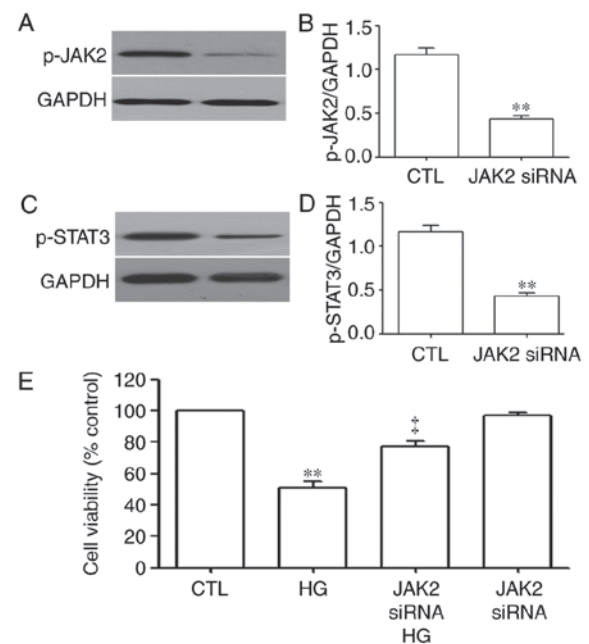


Figure 2. JAK2 siRNA inhibits the expression of JAK2/STAT3 and attenuates HG-induced decreased cell viability in HUVECs. HUVECs were exposed to JAK2 siRNA for 24 h. Western blot analysis was performed to detect the expression levels of (A) JAK2 with (B) densitometric analysis, and for the expression of (C) STAT3 with (D) densitometric analysis. (E) Cells were co-conditioned with HG and JAK2 siRNA for 24 h. Cell viability was detected using a Cell Counting Kit-8 assay. **P<0.01 vs. CTL group. JAK2, Janus kinase 2; STAT3, signal transducer and activator of transcription 3; HUVECs, human umbilical vein endothelial cells; HG, high glucose; siRNA, small interfering RNA; CTL, control; p, phosphorylated.

phosphorylation of the JAK2/STAT3 pathway was reduced by pre-treatment with 2 μ M Ang-(1-7).

HG upregulates the expression level of caspase-3 and down-regulates the expression level of eNOS in HUVECs. The present study also examined the effects of HG on the expression levels of caspase-3 and eNOS in HUVECs. As shown in Fig. 5A and E, The HUVECs were exposed to (A) the indicated concentrations (20, 30, 40, 50 and 60 mM) of glucose for 24 h, or (B) with 40 mM glucose for the indicated durations (3, 6, 12, 18, 24, 36 and 48 h). (C) Exposure to different glucose concentrations markedly increased the expression levels of caspase-3, peaking at 60 mM glucose. Based on these results, the effect of time on the expression was examined. As shown in Fig. 5D, when the HUVECs were exposed to 40 mM glucose for the indicated durations (3, 6, 12, 18, 24, 36 and 48 h), the expression levels of caspase-3 were significantly upregulated, reaching a peak at 12 and 18 h. By contrast, exposure of the cells to HG decreased the expression levels of eNOS, as shown in the dose-response experiment (Fig. 5E) and time-response experiment (Fig. 5F), respectively.

Ang-(1-7) and AG490 downregulate the increased expression of caspase-3 and upregulate the decreased expression of eNOS induced by HG in HUVECs. To observe the effects of Ang-(1-7) and AG490 on the increased expression level of caspase-3 and upregulating the decreased expression level of eNOS induced by HG, the HUVECs were pre-treated with 2 μ M Ang-(1-7), as shown in Fig. 6A-C, or 20 μ M AG490 for 30 min (Fig. 6D-F), prior to exposure to HG for

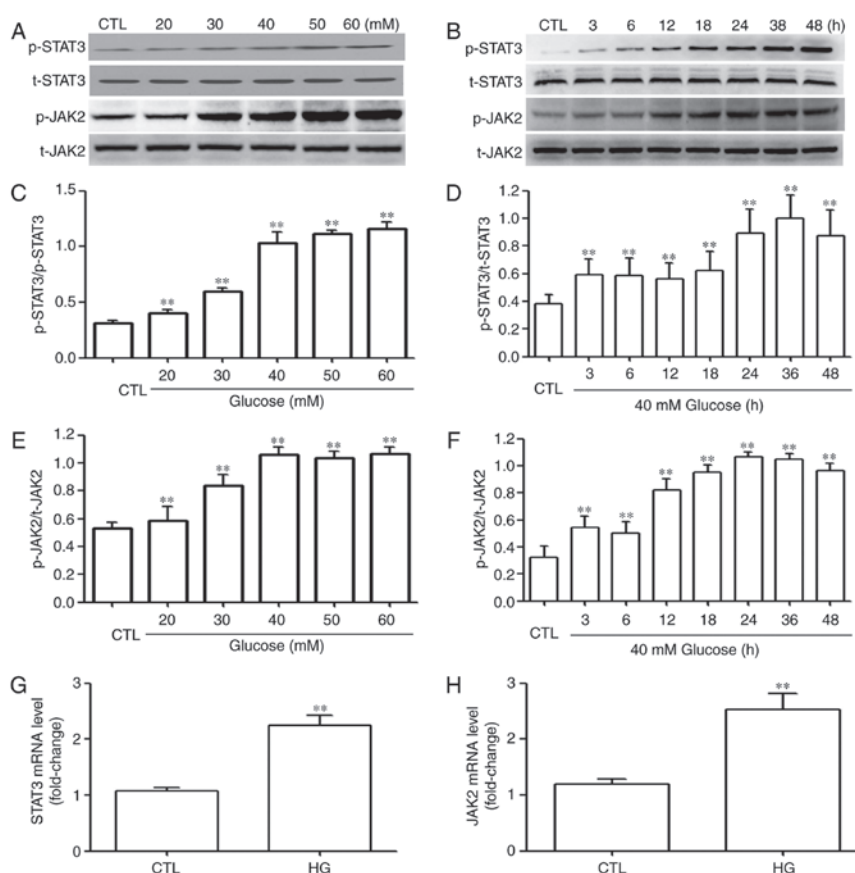


Figure 3. HG induces activation of the STAT3/JAK2 pathway in HUVECs. (A) HUVECs were exposed to the indicated concentrations of glucose (20, 30, 40, 50 and 60 mmol/l, respectively) for 24 h or (B) were exposed to HG for the indicated durations (3, 6, 12, 18, 24, 36 and 48 h, respectively), followed by western blot analysis for the expression of STAT3 and JAK2. Densitometric analysis was performed to determine levels of (C and D) STAT3 and (E and F) JAK2 under different concentrations of glucose and different durations of HG, respectively. mRNA levels of (G) STAT3 and (H) JAK2 were assessed. ** $P < 0.01$ vs. CTL group. JAK2, Janus kinase 2; STAT3, signal transducer and activator of transcription 3; HUVECs, human umbilical vein endothelial cells; HG, high glucose; siRNA, small interfering RNA; CTL, control; p, phosphorylated; t, total.

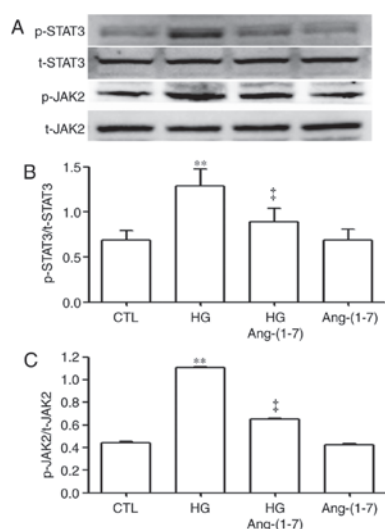


Figure 4. Ang-(1-7) ameliorates the phosphorylation of the STAT3/JAK2 pathway induced by HG in HUVECs. HUVECs were treated with HG in the absence or presence of co-treatment with 2 μ M Ang-(1-7) for 24 h. (A) Expression levels of STAT3 and JAK2 were detected using western blot assays. (B) Densitometric analysis of changes in the expression levels of STAT3 and (C) JAK2 in the indicated groups. ** $P < 0.01$, compared with the CTL group; † $P < 0.01$ vs. HG group. JAK2, Janus kinase 2; STAT3, signal transducer and activator of transcription 3; HUVECs, human umbilical vein endothelial cells; Ang-(1-7), angiotensin-(1-7); p, phosphorylated; t, total; HG, high glucose (40 mmol/l glucose); CTL, control.

24 h. As shown in Fig. 6, exposure of the cells to 40 mM glucose significantly increased the expression levels of caspase-3 (Fig. 6A, C, D and F). However, the increased expression level of caspase-3 was reduced by pre-treatment with 2 μ M Ang-(1-7) (Fig. 6A and B) or 20 μ M AG490 (Fig. 6D and F) for 30 min prior to exposure to HG for 24 h. Secondly, the exposure of cells to 40 mM glucose significantly decreased the expression levels of eNOS (Fig. 6A, B, D and E). However, the decreased expression levels of eNOS were upregulated following pre-treatment with 2 μ M Ang-(1-7) (Fig. 6A and B) or 20 μ M AG490 (Fig. 6D and E) for 30 min prior to exposure to HG for 24 h.

Ang-(1-7) and AG490 suppress HG-induced apoptosis in HUVECs. As shown in Fig. 7Aa and b, exposure of HUVECs to 40 mM glucose for 24 h induced typical apoptosis, which was manifested as the nuclear condensation and fragmentation condensation of chromatin, and the shrinkage of nuclei and apoptotic bodies. However, pre-treatment of the cells with 2 μ M Ang-(1-7) for 30 min prior to exposure to HG for 24 h mitigated the HG-induced increase in the number of cells undergoing apoptosis (Fig. 7Ac). In addition, pre-conditioning of the cells with 20 μ M AG490 for 30 min prior to exposure to HG for 24 h also ameliorated the HG-induced apoptosis of cardiac cells (Fig. 7Ad). Alone, 2 μ M Ang-(1-7) (Fig. 7Ae) or

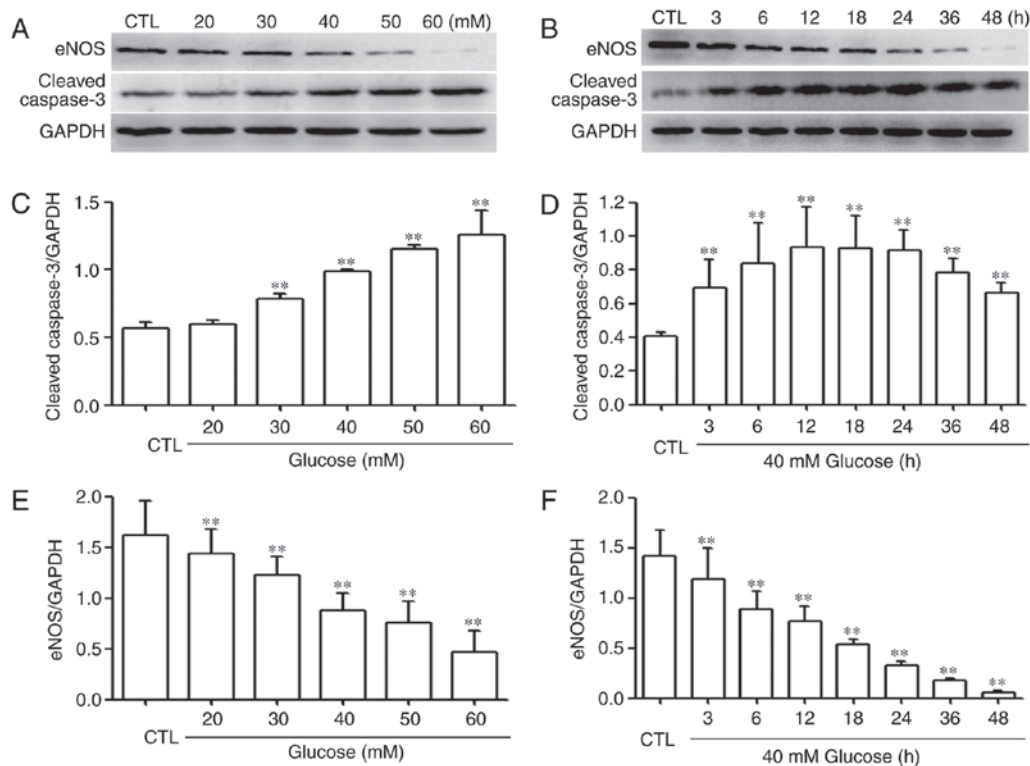


Figure 5. HG upregulates the expression levels of caspase-3 and downregulates the expression levels of eNOS in HUVECs. HUVECs were exposed to (A) the indicated concentrations of glucose (20, 30, 40, 50 and 60 mmol/l, respectively) for 24 h, or (B) HG for the indicated durations (3, 6, 12, 18, 24, 36 and 48 h, respectively). Expression levels of caspase-3 and eNOS were measured using western blot assays. Densitometric analysis of the expression of caspase-3 under (C) different glucose concentrations and (D) with HG for different durations. Densitometric analysis of the expression of eNOS under (E) different glucose concentrations and (F) with HG for different durations, respectively. ** $P < 0.01$ vs. CTL group. HUVECs, human umbilical vein endothelial cells; eNOS, endothelial nitric oxide synthase; Ang-(1-7), angiotensin-(1-7); GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HG, high glucose (40 mmol/l glucose); CTL, control.

20 μ M AG490 (Fig. 7Af) did not significantly alter the number of apoptotic cells. Quantification of results is shown in Fig. 7B.

Ang-(1-7) and AG490 reduce the oxidative stress induced by HG in HUVECs. The results demonstrated that oxidative stress contributed to HG-induced HUVEC injury. As shown in Figs. 8 and 9, exposure of the HUVECs to 40 mM glucose for 24 h resulted in oxidative stress, as evidenced by an increase in the generation of ROS (Fig. 8Ab and B), a decrease in SOD activity (Fig. 9A) and increases in the expression level of Nox4 in the dose-response experiment (20, 30, 40, 50 and 60 mM glucose; Fig. 9B and C) and time-response experiment (3, 6, 12, 18, 24, 36 and 48 h; Fig. 9D and E). However, pre-treatment of the cells with 2 μ M Ang-(1-7) for 30 min prior to exposure to HG for 24 h mitigated the HG-induced increase in ROS generation (Fig. 8Ac and B), increased the HG-induced decrease in SOD activity (Fig. 9A) and decreased the expression level of Nox4 (Fig. 9F and G). To determine whether the JAK2/STAT3 pathway was involved in HG-induced oxidative stress, the HUVECs were pre-treated cells with 20 μ M AG490 for 30 min prior to exposure to HG for 24 h. The resulting data showed that pre-treatment of the cells with 20 μ M AG490 for 30 min prior to exposure to HG for 24 h decreased the generation of ROS (Fig. 8Ad and B), upregulated SOD activity (Fig. 9A) and reduced the expression levels of Nox4 (Fig. 9F and G) in the HUVECs. Treatment with Ang-(1-7) or AG490 alone did not alter the basal level of ROS, activity of SOD or expression level of Nox4 in the HUVECs.

Ang-(1-7) and AG490 inhibit the HG-induced dissipation of MMP in HUVECs. It was shown that the exposure of HUVECs to 40 mM glucose for 24 h elicited mitochondrial damage, as manifested by the dissipation of MMP (Fig. 10Aa and b). The dissipation of MMP was reduced by pre-treatment of the cells with 2 μ M Ang-(1-7) for 30 min prior to exposure to HG for 24 h (Fig. 10Ac), which demonstrated that Ang-(1-7) protected the HUVECs against HG-induced mitochondrial damage. Similarly, pre-treatment of HUVECs with 20 μ M AG490 for 30 min prior to exposure to HG for 24 h attenuated the HG-induced dissipation of MMP (Fig. 10Ad). The quantitative results are shown in Fig. 10B.

Ang-(1-7) and AG490 suppress the HG-induced increased production of pro-inflammatory cytokines in HUVECs. As shown in Fig. 11, the levels of IL-1 β (Fig. 11A), IL-10 (Fig. 11B), IL-12 (Fig. 11C) and TNF- α (Fig. 11D) were markedly increased in the HG-induced HUVECs, compared with those in the control group ($P < 0.01$). However, these increased levels of IL-1 β , IL-10, IL-12 and TNF- α were significantly suppressed by pre-treatment of the HUVECs with 2 μ M Ang-(1-7) for 30 min prior to exposure to HG for 24 h. This suggested an inhibitory effect of Ang-(1-7) on the production of pro-inflammatory cytokines, including IL-1 β , IL-10, IL-12 and TNF- α , induced by HG. Similarly, pre-treatment of the HUVECs with 20 μ M AG490 for 30 min prior to exposure to HG for 24 h decreased the enhanced production of IL-1 β , IL-10, IL-12 and TNF- α .

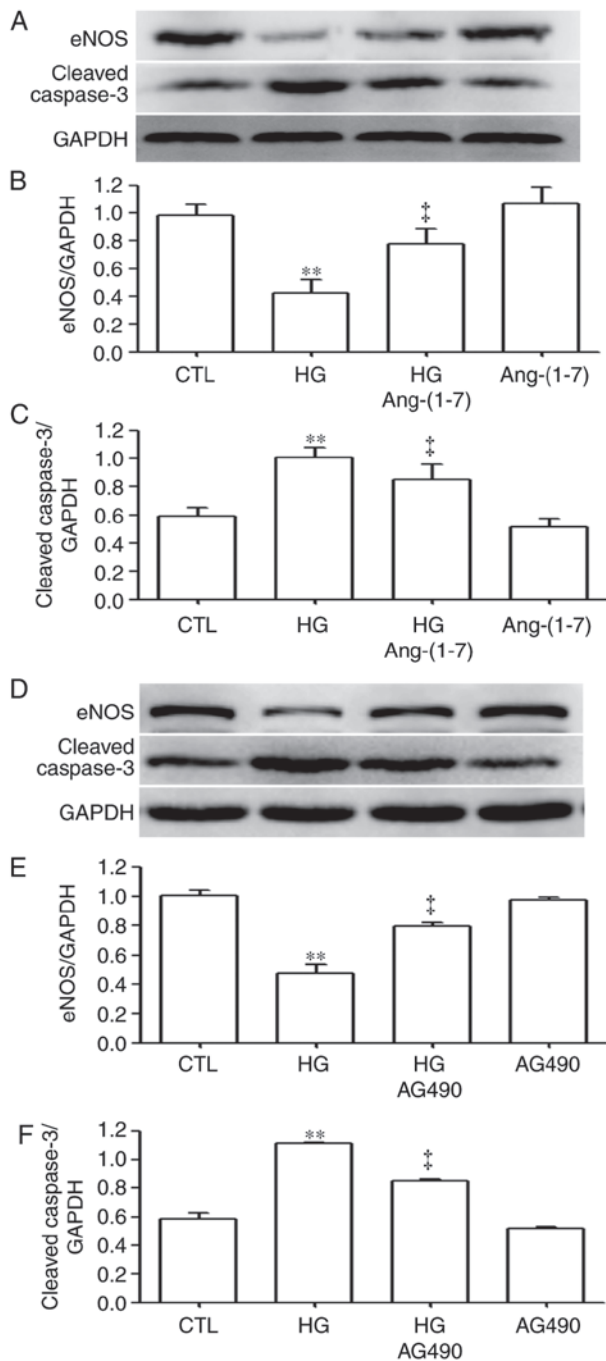


Figure 6. Ang-(1-7) and AG490 downregulate the increased expression level of caspase-3 and upregulate the decreased expression level of eNOS induced by HG in human umbilical vein endothelial cells. Cells were co-conditioned with HG and 2 $\mu\text{mol/l}$ Ang-(1-7) or 20 $\mu\text{mol/l}$ AG490 (inhibitor of the Janus kinase 2/signal transducer and activator of transcription 3 pathway) for 24 h. (A) Cells were treated with 2 $\mu\text{mol/l}$ Ang-(1-7) followed by densitometric analysis of the expression of (B) eNOS and (C) caspase-3. (D) Cells were treated with 20 $\mu\text{mol/l}$ AG490, followed by densitometric analysis for the expression of (E) eNOS and (F) caspase-3. ** $P < 0.01$ vs. CTL group; * $P < 0.01$, compared with the HG-treated group. eNOS, endothelial nitric oxide synthase; Ang-(1-7), angiotensin-(1-7); GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HG, high glucose (40 mmol/l glucose); CTL, control.

Discussion

In the present study, the data revealed several novel findings indicating that the JAK2/STAT3 signaling pathway is relevant

to the potential mechanisms responsible for HG-induced HUVEC injury and inflammation, and the effect of Ang-(1-7) on protecting vascular endothelium. First, in the HUVECs, the JAK2/STAT3 signaling pathway was involved in the HG-induced HUVEC injury and inflammation. Secondly, Ang-(1-7) exerted endothelial protection against HG-induced injury and inflammation. Finally, the protective effects on the vascular endothelium by Ang-(1-7) were associated with inhibition of the JAK2/STAT3 pathway.

The data obtained demonstrated that the exposure of HUVECs to HG for 24 h led to injury and inflammation, as characterized by an increase in apoptotic cells, expression levels of caspase-3 (a death effector domain), oxidative stress (demonstrated by increased ROS production), decreased activation of SOD, increased expression of Nox4 (an important component of the NADPH oxidase family), increased expression level of eNOS, decreased cell viability and dissipation of MMP, and the upregulation of secretion of inflammatory cytokines (IL-1 β , IL-6, IL-12 and TNF- α). These results are consistent with those of previous studies (5,6-9,29-31) and demonstrated that the damage in HG-induced HUVEC injury and inflammation was extensive. However, the associated mechanism remains to be fully elucidated.

The JAK2/STAT3 pathway is known to mediate survival signals, which contribute to cell proliferation, differentiation, growth and apoptosis (14-16). Similarly, the effects of the JAK2/STAT3 pathway on the cardiovascular system are also important and have been widely examined. Accumulating evidence has demonstrated that the JAK2/STAT3 pathway is involved in the progress of various stimulation-induced cardiovascular complication (32-36), including apoptosis (32,33), reticulum stress (32), ROS (35,36), contractile dysfunction (36) and inflammation (37). Currently, the potential roles of the JAK2/STAT3 pathway in hyperglycemia-induced cardiovascular complication remain to be fully elucidated. Fiaschi *et al* identified a novel role for STAT3 as a crucial signaling molecule of collagen I production in cardiac fibroblasts induced by a diabetic environment (38). In addition, in streptozotocin (STZ)-induced diabetic rats, wortmannin (an inhibitor of PI3K) and AG490 (an inhibitor of JAK2), synergistically mitigate myocardial ischemia reperfusion injuries (39). This indicates that PI3K/Akt and JAK2/STAT3 are synergistically involved in myocardial injury in diabetes.

As the effects of the JAK2/STAT3 pathway in HG-induced HUVEC injury and inflammation remain to be fully elucidated, the present study aimed to elucidate the mechanism. Firstly, the role of HG on activation of the JAK2/STAT3 pathway in HUVECs was investigated. The results showed that exposure of the HUVECs to HG upregulated the expression levels of p-JAK2 and p-STAT3, indicating that HG activated the JAK2/STAT3 pathway in HUVECs. Secondly, the associated roles of JAK2/STAT3 pathway activation were examined in HG-stimulated injury. The data indicated that co-treatment of the HUVECs with HG and AG490, an inhibitor of the JAK2 pathway, significantly alleviated HG-induced injuries, including apoptosis, cytotoxicity, mitochondrial damage and oxidative stress, as evidenced by a decrease in the number of apoptotic cells, decreased expression levels of caspase-3 and Nox4, and an increase in the activation of SOD, cell viability, expression of eNOS and dissipation of MMP. These

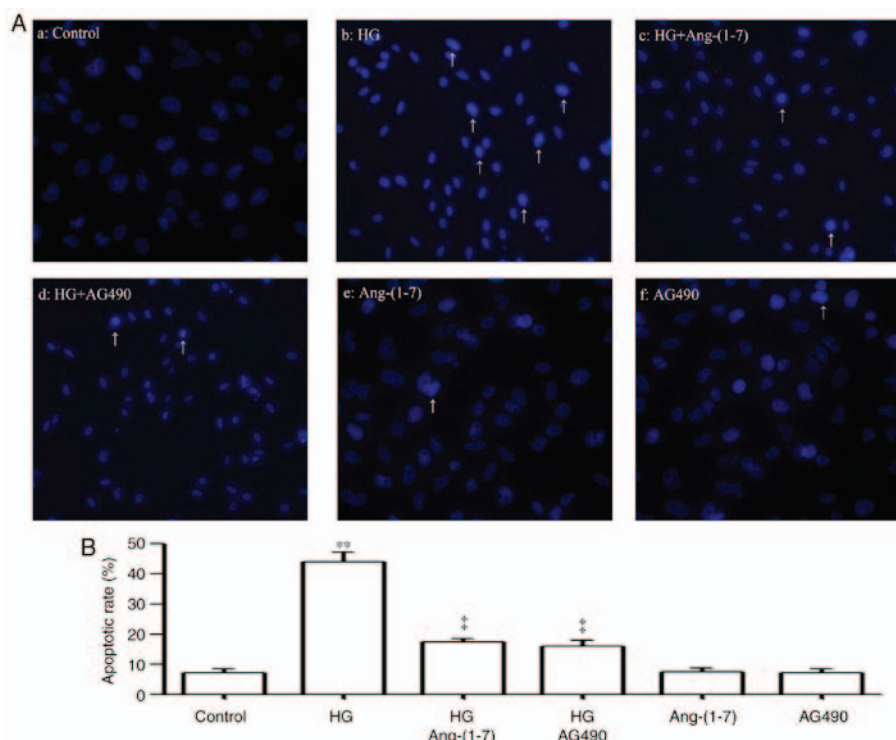


Figure 7. Ang-(1-7) and AG490 suppress HG-induced apoptosis in HUVECs. (A) Hoechst 33258 nuclear staining followed by fluorescence imaging was performed to examine cell apoptosis. (a) Control group; (b) HUVECs exposed to HG for 24 h; (c) HUVECs co-treated with HG and 2 $\mu\text{mol/l}$ Ang-(1-7) for 24 h; (d) HUVECs co-treated with HG and 20 $\mu\text{mol/l}$ AG490 (inhibitor of the Janus kinase 2/signal transducer and activator of transcription 3 pathway) for 24 h. (e) HUVECs treated with 2 $\mu\text{mol/l}$ Ang-(1-7) for 24 h; (f) HUVECs treated with 2 $\mu\text{mol/l}$ Ang-(1-7) for 24 h. (B) Apoptotic rates were analyzed using ImageJ 1.47i software. Images are captured at x200 magnification. ** $P < 0.01$ vs. CTL group; $^{\#}P < 0.01$, compared with the HG-treated group. HUVECs, human umbilical vein endothelial cells; Ang-(1-7), angiotensin-(1-7); HG, high glucose (40 mmol/l glucose); CTL, control.

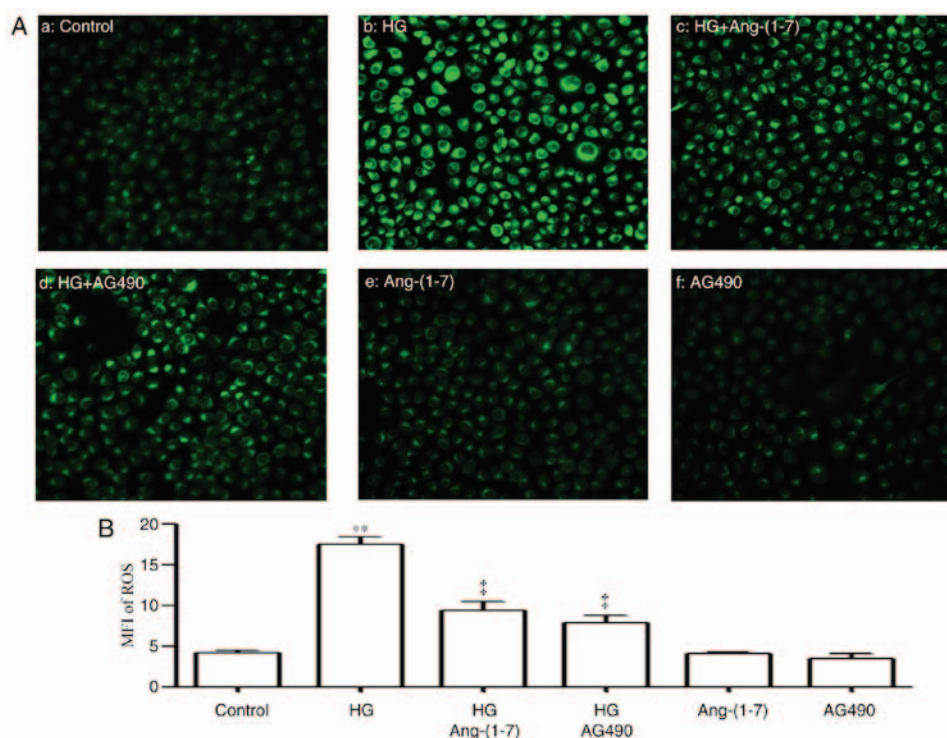


Figure 8. Ang-(1-7) and AG490 alleviate HG-induced increased ROS generation in HUVECs. (A) DCFH-DA staining followed by photofluorography was used to measure intracellular ROS levels. (a) Control group; (b) HUVECs exposed to HG for 24 h; (c) HUVECs co-treated with HG and 2 $\mu\text{mol/l}$ Ang-(1-7) for 24 h; (D) HUVECs co-treated with HG and 20 $\mu\text{mol/l}$ AG490 (inhibitor of the Janus kinase 2/signal transducer and activator of transcription 3 pathway) for 24 h. (E) HUVECs treated with 2 $\mu\text{mol/l}$ Ang-(1-7) for 24 h; (F) HUVECs treated with 2 $\mu\text{mol/l}$ Ang-(1-7) for 24 h. (B) Quantitative analysis of the MFI of DCFH-DA using ImageJ 1.47i software. Images are captured at x100 magnification. ** $P < 0.01$, compared with the CTL group; $^{\#}P < 0.01$ vs. HG-treated group. HUVECs, human umbilical vein endothelial cells; Ang-(1-7), angiotensin-(1-7); HG, high glucose, 40 mmol/l glucose; DCFH-DA, 2',7'-dichlorofluorescein diacetate; MFI, mean fluorescence intensity; ROS, reactive oxygen species; CTL, control.

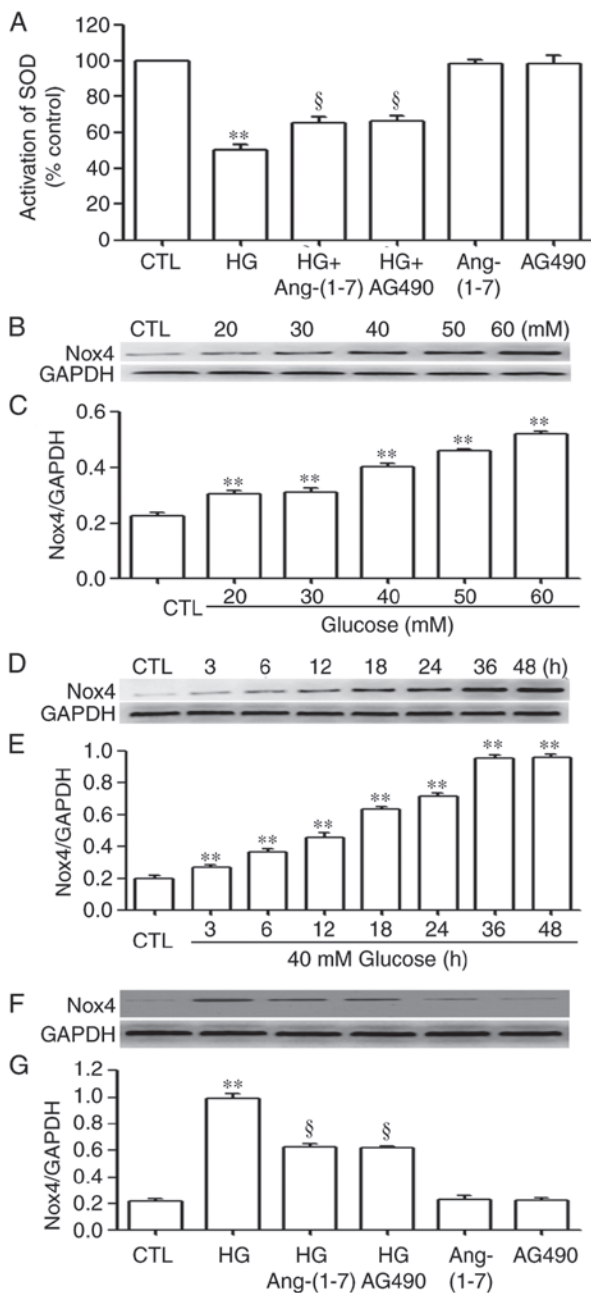


Figure 9. Ang-(1-7) and AG490 downregulate the increased expression level of Nox4 and increase SOD activity induced by HG in HUVECs. (A) SOD activity was examined using an SOD assay kit. (B) HUVECs were exposed to the indicated concentrations of glucose (20, 30, 40, 50 and 60 mmol/l, respectively) for 24 h and (C) levels of Nox4 were determined using densitometric analysis. (D) HUVECs were exposed to 40 mM glucose for the indicated times (3, 6, 12, 18, 24, 36 and 48 h, respectively) and (E) levels of Nox4 were determined using densitometric analysis. (F) HUVECs were co-conditioned with HG and 2 μ mol/l Ang-(1-7) or 20 μ mol/l AG490 (inhibitor of the Janus kinase 2/signal transducer and activator of transcription 3 pathway) for 24 h, and (G) expression levels of Nox4 were measured using western blot analysis, followed by (F) densitometric analysis. Data are presented as the mean \pm standard error of the mean (n=3). **P<0.01 vs. CTL group; §P<0.01, compared with the HG-treated group. HUVECs, human umbilical vein endothelial cells; Ang-(1-7), angiotensin-(1-7); HG, high glucose (40 mmol/l glucose); Nox4, NADPH oxidase 4; SOD, superoxide dismutase; CTL, control.

results suggested that JAK2/STAT3 activation was involved in HG-stimulated injury in HUVECs. In addition, as it has been demonstrated that hyperglycemia is involved in vascular

endothelium inflammation *in vitro* and *in vivo* (29,40-43), the present study further examined the role of JAK2/STAT3 activation on the HG-induced inflammatory response in HUVECs. Similar to the results of previous studies (29,40-43), it was found that exposure of the HUVECs to HG promoted inflammatory responses, as indicated by the upregulated production of IL-1 β , IL-6, IL-12 and TNF- α . However, the increased production of IL-1 β , IL-6, IL-12 and TNF- α was decreased by AG490. This suggested that the JAK2/STAT3 pathway was involved in the HG-induced production of pro-inflammatory factors (IL-1 β , IL-6, IL-12 and TNF- α). The above data provide definitive and novel evidence that activation of the JAK2/STAT3 pathway contributed to HG-induced injury and inflammation in HUVECs.

An important finding of the present study relates to the various endothelial protective effects of Ang-(1-7) on HG-induced injury and inflammation in HUVECs. Firstly, it was found that Ang-(1-7) markedly alleviated HG-induced cytotoxicity, as characterized by increased cell viability. These results are supported by previous findings that toxicity induced by various factors, including lipopolysaccharide, long-term hypoxia and STZ-induced diabetes is alleviated by Ang-(1-7) (44-46). Secondly, Ang-(1-7) can exert anti-apoptotic effects against HG-induced apoptosis in HUVECs. According to previous studies, the anti-apoptotic effect of Ang-(1-7) is widely recognized, and is associated with various systems and organs, including the endocrine system (46), reproductive system (47), circulatory system (48), respiratory system (49), urinary system (50) and motor system (51). These data demonstrate that Ang-(1-7) protects cells against the injury induced by various factors by exerting anti-apoptotic effects. In the present study, data indicated that Ang-(1-7) protected the HUVECs against HG-induced apoptosis, as indicated by decreases in cell apoptosis and the expression of caspase-3. Ang-(1-7) is also involved in the oxidative stress induced by HG in HUVECs. In the circulatory system, Ang-(1-7) exerts cardiovascular protective effects by attenuating oxidative stress in cardiac (53), cardiomyocyte autophagy (52), hypertension (53) and vascular remodeling (54). Additionally, AVE0991, an analog of Ang-(1-7), attenuates cardiac hypertrophy via inhibiting oxidative stress (55). Ang-(1-7) also mediates the endothelial protection of signaling by reducing the oxidative stress induced by diabetes (56). Consistent with these previous findings, the results of the present study demonstrated that Ang-(1-7) protected HUVECs against HG-induced apoptosis by reducing oxidative stress, as characterized by a decrease in the production of ROS and expression level of Nox4, an important component of the NADPH oxidase family, and an increase in the activation of SOD. The findings of the present study also revealed that Ang-(1-7) had mitochondrial protective effects against HG-induced mitochondrial injury (a loss of MMP), which was comparable with a previous study showing that Ang-(1-7) alleviated the loss of MMP during H₂O₂-induced in pancreatic β cells (57). The endothelial protective effect of Ang-(1-7) is also associated with its anti-inflammatory effect. In previous studies, Ang-(1-7) exerted anti-inflammatory effects in cardiomyocytes (58) and pulmonary microvascular endothelial cells (59) of the circulatory system. Similarly, the anti-inflammatory effect of Ang-(1-7) has been shown to contribute to endothelial protection in endothelial cells (60,61).

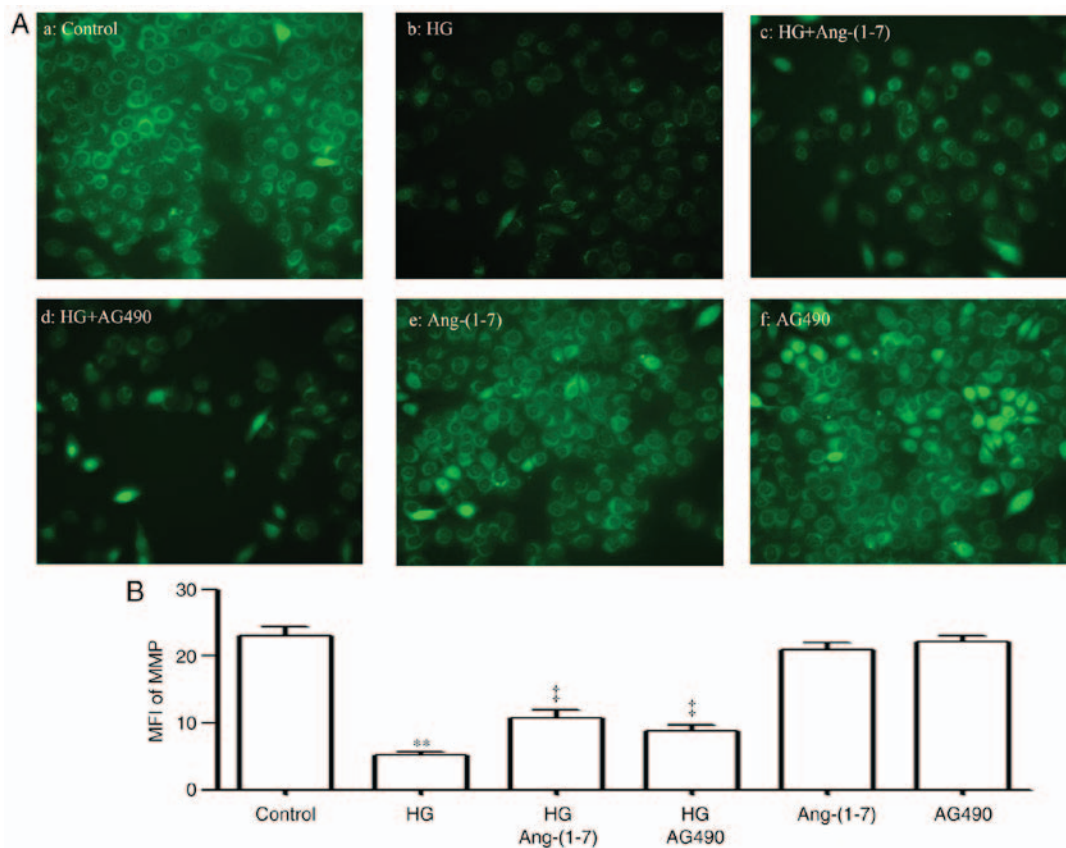


Figure 10. Ang-(1-7) and AG490 inhibit HG-induced dissipation of MMP in HUVECs. (A) Following the indicated treatments, MMP was assessed by Rh123 staining followed by photofluorography. (a) Control group; (b) HUVECs exposed to HG for 24 h; (c) HUVECs co-treated with HG and 2 μ mol/l Ang-(1-7) for 20 h; (d) HUVECs co-treated with HG and 20 μ mol/l AG490 (inhibitor of the Janus kinase 2/signal transducer and activator of transcription 3 pathway) for 24 h; (e) HUVECs treated with 2 μ mol/l Ang-(1-7) for 24 h; (f) HUVECs treated with 2 μ mol/l Ang-(1-7) for 24 h. (B) Quantitative analysis of the MFI of Rh123 using ImageJ 1.47i software. Data are shown as the mean \pm standard error of the mean (n=3). **P<0.01, compared with the CTL group; †P<0.01 vs. HG-treated group. HUVECs, human umbilical vein endothelial cells; MMP, mitochondrial membrane potential; Ang-(1-7), angiotensin-(1-7); HG, high glucose (40 mmol/l glucose); MFI, mean fluorescence intensity; CTL, control.

However, whether Ang-(1-7) inhibits HG-induced inflammation in HUVECs remains to be elucidated. In the present study, Ang-(1-7) significantly inhibited the HG-induced expression of inflammatory factors (IL-1 β , IL-6, IL-12 and TNF- α). This indicates that the endothelial protective effect of Ang-(1-7) was involved in its anti-inflammatory effect. It was also found that Ang-(1-7) ameliorated the expression level of eNOS induced by HG in the HUVECs. Exposure of the HUVECs to HG for 24 h upregulated the expression level of eNOS. These data were supported by previous studies (62,63). Co-treatment of the HUVECs with HG and Ang-(1-7) considerably elevated the expression level of eNOS. However, the association between Ang-(1-7) and eNOS remains to be elucidated and requires further investigation *in vitro* and *in vivo*.

Another important finding of the present study involves the effects of inhibition of the JAK2/STAT3 pathway on the endothelial protective effects of exogenous Ang-(1-7) against HG-induced multifarious endothelial cell injury and inflammation. Several studies have reported that Ang-(1-7) exerts endothelial protective effects (44,47,55,58,59). The present study examined whether Ang-(1-7) protected HUVECs against HG-induced injury and inflammation by suppressing the JAK2/STAT3 pathway. The results demonstrated that exogenous Ang-(1-7) widely antagonized JAK2/STAT3 pathway

activation and inflammatory factors (IL-1 β , IL-6, IL-12 and TNF- α). In addition, similar to the inhibitory effects of AG490, an inhibitor of the JAK2 pathway, as indicated above, co-treatment of the HUVECs with HG and Ang-(1-7) mitigated HG-induced endothelial cell injury and inflammation. These results showed that the inhibitory effect of the JAK2/STAT3 pathway may be a crucial mechanism responsible for the endothelial protective effects of exogenous Ang-(1-7) against HG-induced endothelial cell injury and inflammation. Similarly, previous evidence demonstrates that exogenous Ang-(1-7) reduced Ang II-induced (25) or monocrotaline-induced (64) cell injury through inhibition of the JAK2/STAT3 pathway. Previous findings have also indicated that Ang-(1-7) exerts cardioprotective protection against HG-induced injury (65). These studies support the results of the present study.

In conclusion, the present study provided novel evidence that the JAK2/STAT3 pathway contributed to HG-induced endothelial cell injury and inflammation, and that exogenous Ang-(1-7) exerted endothelial protection against HG-induced endothelial cell injury and inflammation via the inhibitory effect on the JAK2/STAT3 pathways. These findings may assist in the development of novel therapeutic methods for the prevention and treatment of hyperglycemia-associated endothelial cell injury and inflammation.

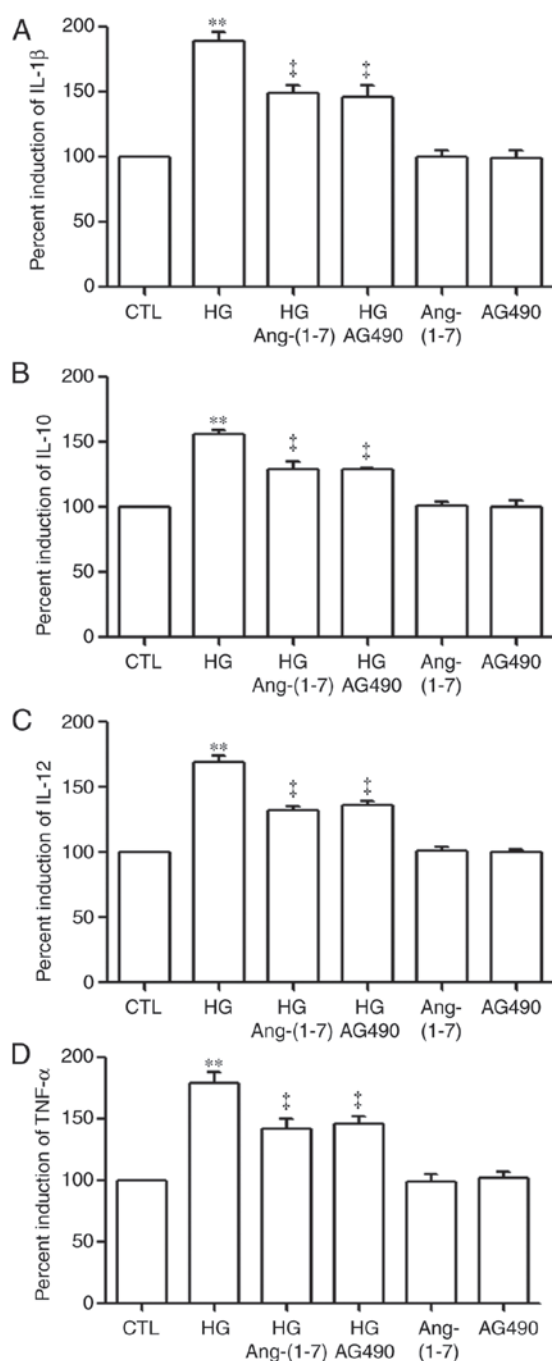


Figure 11. Ang-(1-7) and AG490 alleviate HG-induced production of IL-1 β , IL-10, IL-12 and TNF- α in human umbilical vein endothelial cells. The cells were treated with HG for 24 h with or without co-conditioning with 2 μ mol/l Ang-(1-7) or 20 μ mol/l AG490 (inhibitor of the Janus kinase 2/signal transducer and activator of transcription 3 pathway) for 24 h. An enzyme-linked immunosorbent assay was performed to determine the levels of (A) IL-1 β , (B) IL-10, (C) IL-12 and (D) TNF- α in cell supernatants. Data are shown as the mean \pm standard error of the mean (n=3). **P<0.01 vs. CTL group; *P<0.01, compared with the HG-treated group. Ang-(1-7), angiotensin-(1-7); HG, high glucose (40 mmol/l glucose). IL, interleukin; TNF- α , tumor necrosis factor- α ; CTL, control.

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Competing interests

The authors declare that they have no competing interests.

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