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Artesunate ameliorates high glucose-induced rat glomerular mesangial cell injury by suppressing the TLR4/NF-κB/NLRP3 inflammasome pathway

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ABSTRACT

Inflammatory response is important for the development and progression of diabetic nephropathy (DN). Artesunate (ART), an antimalarial drug, possesses anti-inflammatory effect and exhibits protective effect on chronic kidney diseases. However, the effect of ART on DN is unknown. The aim of the present study was to evaluate the effect and the molecular mechanism of ART on DN in an *in vitro* model. The rat mesangial cell line, HBZY-1, was induced by high glucose (HG; 30 mM D-glucose) in the presence or absence of ART (15 and 30 μg/ml) and incubated for 24 h. We found that HG induced the proliferation of HBZY-1 cells, while treatment with ART inhibited the cell proliferation. Treatment with ART inhibited HG-induced inflammatory cytokines production and expression of extracellular matrix (ECM). Besides, HG induced reactive oxygen species (ROS) and malondialdehyde (MDA) levels, and inhibited the superoxide dismutase (SOD) activity of HBZY-1 cells, and the effects were attenuated by ART treatment. ART decreased HG-induced the expression levels of toll-like receptor 4 (TLR4), myeloid differentiation primary response gene 88 (MyD88), nuclear factor κB (NF-κB) p-p65, and nod-like receptor protein 3 (NLRP3). Inhibition of the TLR4/NF-κB pathway suppressed NLRP3 inflammasome in HBZY-1 cells. In conclusion, ART exhibited protective effect on HG-induced HBZY-1 cells by inhibiting the inflammatory response, oxidative stress and ECM accumulation. The TLR4/NF-κB/NLRP3 inflammasome pathway was involved in the protective effect of ART. The results suggested that ART might be a potential therapy agent for the DN treatment.

1. Introduction

Diabetic nephropathy (DN) is one of the major complications of diabetes mellitus (DM) and a main cause of end-stage kidney diseases [1]. DN is pathologically characterized by accumulation of the glomerulur extracellular matrix (ECM), thickened glomerular basement membranes, increased mesangial hypertrophy, and glomerulosclerosis [2]. Fully understanding of the mechanism of DN development is beneficial for the treatment of DN.

Increasing researches have demonstrated that oxidative stress functions in the onset and development of DN [3]. Excessive oxidative stress activates multiple intracellular signaling pathways and stimulates transcription factors, thus resulting in the increased deposition of ECM and reduced matrix degradation, and finally leading to glomerulosclerosis and renal fibrosis [1]. In addition to oxidative stress, chronic low-grade inflammation and the activation of innate immune system also play crucial roles in DN pathogenesis [3]. It has been reported that increased levels of inflammatory cytokines often occur in DN, and usually contribute to the development of DN [1]. Therefore, it is necessary to explore a timely and appropriate antioxidative and anti-inflammatory agent for the DN treatment.

Artesunate (ART, Fig. 1A) is an important derivative of artemisinin extracted from the Chinese herb *Artemisia annua* [4]. ART is a safe and effective antimalarial drug which is recorded in the World Health Organization's List of Essential Medicines [5]. In recent years, researchers have found that ART possesses various activities, such as anti-tumor, antioxidative, antimalarial, and anti-inflammatory effects [6–10]. Li et al. reported that ART induced the conversion of α cells to functional β-like cells *in vitro* and increased pancreatic islet size in mice [11]. However, the effect of ART in DN remains unclear.

The aim of the present study was to evaluate the effect and the molecular mechanism of ART on DN in an *in vitro* model. The results indicated that ART alleviated high glucose-induced inflammatory responses, oxidative stress and ECM accumulation in rat glomerular mesangial cells by suppressing the toll-like receptor 4 (TLR4)/nuclear factor κB (NF-κB)/nod-like receptor protein 3 (NLRP3) inflammasome

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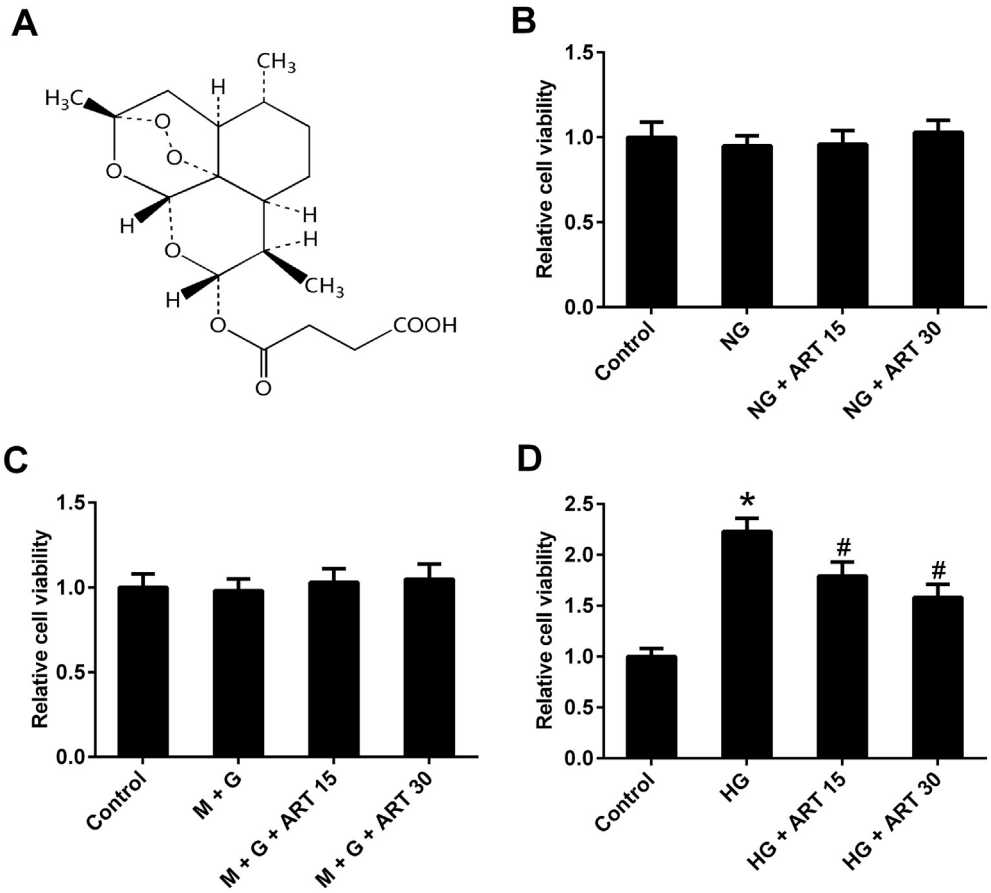

Fig. 1. ART inhibited HG-induced proliferation of HBZY-1 cells. (A) Chemical structure of ART. (B–D) HBZY-1 cells were treated with normal glucose (NG, 5.5 mM D-glucose), mannitol plus glucose (M + G, 24.5 mM mannitol and 5.5 mM D-glucose), or high glucose (HG, 30 mM D-glucose) in the presence or absence of ART (15 and 30 μg/ml). Cell proliferation was assessed using MTT assay. $^{*}p < 0.05$ versus control group, $^{\#}p < 0.05$ versus HG group.

pathway.

2. Materials and methods

2.1. Cell culture

Rat mesangial cell line, HBZY-1, was purchased from Cell Bank of Chinese Academy of Sciences (Shanghai, China). HBZY-1 cells were cultured in DMEM supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin, and 100 mg/ml streptomycin in a humidified incubator containing 5% CO_2 at 37 °C. The medium was replaced every 2 days.

2.2. Cell proliferation detection assay

HBZY-1 cells were seeded in 96-well plate with the density of 5×10^4 cell/well. Cells were treated with normal glucose (5.5 mM D-glucose), or 24.5 mM mannitol and 5.5 mM D-glucose, or high glucose (30 mM D-glucose) in the presence or absence of ART (15 or 30 μg/ml). After 24 h, the supernatant was removed and 20 μl of 5 mg/ml MTT solution was added and incubated for 4 h. Subsequently, 150 μl DMSO was added to dissolve the formed formazan. The absorbance value was detected at 490 nm using a microplate spectrophotometer (Tecan, Switzerland).

2.3. Quantitative real time-PCR (qRT-PCR)

Total mRNA was extracted from HBZY-1 cells using the Trizol reagent (Life Technologies). The reverse transcription was performed using a reverse transcription kit (Takara Biotechnology Co., Ltd., Dalian, China) according to the manufacturer's instructions. Then 50 ng cDNA was used for the amplification using an ABI7900HT machine (Applied Biosystems, Carlsbad, CA, USA). The experimental conditions and primer sequences were as follows: denaturation 95 °C for 30s, annealing 55 °C for 20s, extension 72 °C for 1 min, 40 cycles. Relative expression of genes was calculated using the $2^{-\Delta\Delta CT}$ method with β-actin as the internal control. Interleukin (IL)-6, 5′-GTAA GTAC GTCG GTCC CG-3' (forward); 5′-AGTA GGTC AGTA TTGG GCCG C-3' (reverse). IL-1β, 5′-AGGC TGCT CTGG GATT C-3' (forward); 5′-GCCA CAAC AACT GACG C-3' (reverse). Tumor necrosis factor α (TNF-α), 5′-GAAC CTGT CGGA GTGA C-3' (forward); 5′-CTGA TGAC AGTA CGAG G-3' (reverse). Laminin, 5′-GAAT CTGT GGAG TGGT C-3' (forward); 5′-TAGA GATC GTAA GGAT CTTA-3' (reverse). Collagen IV, 5′-TTAC GGCC TAGC CTTG AG-3' (forward); 5′-CGCA GTAA CGGA GAAC AG -3' (reverse). Fibronectin, 5′-GGTA ACCT CGTA GAGG CG-3' (forward); 5′-GTTA GATA CCTG GGAT A-3' (reverse). β-actin, 5′-GTCG ACAA CGGC TCCG GC-3' (forward); 5′-GGTG TGGT GCCA GATT TTCT-3' (reverse).

2.4. Enzyme-linked immunosorbent assay (ELISA)

After different treatments, the production levels of IL-6, IL-1β, and TNF-α in HBZY-1 cell supernatant were measured by commercial ELISA kits (Boster Biotechnology Co., Wuhan, China) according to the manufacturer's instructions. For detection of concentration of laminin, collagen IV, and fibronectin in cell supernatant, specific ELISA kits (Boster Biotechnology Co.) were used according to the manufacturer's instructions. The absorbance value at 450 nm was determined using a microplate spectrophotometer (Tecan, Switzerland).

2.5. Detection of oxidative stress

The reactive oxygen species (ROS) level was evaluated using a Reactive Oxygen Species Assay Kit of DCFH-DA (Jiancheng Bioengineering Institute, Nanjing, China) according to the