Original Paper

High Mobility Group Box1 Inhibitor Glycyrrhizic Acid Attenuates Kidney Injury in Streptozotocin-Induced Diabetic Rats

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Key Words

High mobility group box 1 • Diabetic kidney disease • Inflammation • Glycyrrhizic acid

Abstract

Background/Aims: High mobility group box 1 (HMGB1) is an important mediator of the inflammatory response. It has been implicated in the pathogenesis of autoimmune diseases, atherosclerosis, and obesity. However, the effects of HMGB1 on diabetic nephropathy remain unclear. Here, we investigated the potential roles and mechanisms of an HMGB1 inhibitor, glycyrrhizic acid (GA), in renal injury with the streptozotocin (STZ)-induced rat model. **Methods:** The diabetic rat was generated by intraperitoneal injection of STZ and then treated with the HMGB1 inhibitor GA or saline for 8 weeks. Rats were randomly divided into three groups: the normal control and saline group (Control), the diabetic rats with saline group (Diabetic) and the diabetic rats plus GA group (Diabetic+GA). Peripheral blood was obtained for measurements of blood glucose, TNF- α , IL-6 and IL-1 β . The mRNA levels of proinflammatory cytokines (TNF- α , IL-6 and IL-1 β), chemokines (MCP-1), intercellular adhesion molecules (ICAM-1) and TGF- β 1 in the kidneys were evaluated by quantitative real-time PCR. The protein levels of phosphorylated(p) and total(t) p38 MAPK, JNK, ERK, and NF- κ B were measured by western blot. **Results:** We found that diabetic rats showed obvious renal lesions, an elevated urinary albumin/creatinine ratio (UACR) and increased expression levels of TGF- β 1 and Col-IV in the kidneys, accompanied by significantly enhanced expression levels of HMGB1, receptor for advanced glycation end products (RAGE) and toll-like receptor 4 (TLR-4) in the kidney tissue. Furthermore, the GA treatment significantly reduced the UAC levels, ameliorated renal injury, and decreased the TNF- α , IL-6, IL-1 β , MCP-1, ICAM-1, TGF- β 1 and Col-IV levels. Importantly, the expression levels of HMGB1, RAGE and TLR4 in the kidney tissues of the diabetic rats were also inhibited by the GA treatment. Furthermore, the GA treatment significantly reduced the phosphorylation levels of ERK and p38 MAPK and suppressed NF- κ B translocation from the cytoplasm to the nucleus. **Conclusion:** Our findings indicate that the HMGB1 inhibitor GA may

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improve renal injury and inflammatory responses in diabetic rats by regulating RAGE/TLR4-related ERK and p38 MAPK/NF- κ B activation.

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Introduction

Diabetic nephropathy (DN), a microvascular complication of diabetes, is the most common cause of end-stage renal disease (ESRD) in developed countries, and the prevalence of DN is dramatically increasing in developing countries [1]. DN is characterized by functional and structural changes, such as proteinuria, decline in the glomerular filtration rate (GFR), excessive deposition of extracellular matrix (ECM), thickening of the glomerular basement membrane (GBM) and hypertrophy, which ultimately result in glomerulosclerosis and tubulointerstitial fibrosis [2, 3]. Many researchers are convinced that microinflammation and fibrosis play central roles in the development of DN and are the common pathways for progression to ESRD [4, 5]. The various proinflammatory molecules and pathways implicated in DN include proinflammatory cytokines, chemokines, adhesion molecules, Toll-like receptors (TLRs), transcription factors and nuclear receptors. These molecules lead to activation of fibroblasts, which then initiate the inflammatory response and sustain the fibrotic process, suggesting that inflammatory signalling pathways are amplified by a positive feedback loop involving fibrosis [3, 6].

High mobility group box 1 (HMGB1), a structural chromatin-binding nuclear protein existing in almost all eukaryotic cells, can be actively secreted or passively released into extracellular regions by inflammatory or necrotic cells under specific conditions [7]. Extracellular HMGB1 acts as a pluripotent cytokine in many pathophysiological processes such as infection, injury, inflammation, obesity and insulin resistance. There are several major types of HMGB1 receptors, but receptor for advanced glycation end products (RAGE) and TLRs, mainly TLR2 and TLR4, are particularly important. By binding to the receptor, HMGB1 activates nuclear factor Kappa B (NF- κ B), leading NF- κ B translocation from the cytoplasm to the nucleus where NF- κ B induces the transcription of a cascade of proinflammatory cytokines and chemokines. The mitogen-activated protein kinase (MAPK) signalling pathways, including extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 MAPK, are a series of parallel cascades of serine/threonine kinases that are activated by extracellular HMGB1, which also results in the inflammatory response. It is worth mentioning that these inflammatory molecules, in turn, promote additional release of HMGB-1 from cells, resulting in a vicious cycle [8-11]. GA, also known as Glycyrrhizin, is a triterpenoid triterpene glycoside that is naturally extracted from the roots of licorice plants. The anti-inflammatory, anti-diabetic, antioxidant, anti-tumour properties of GA have been reported by researchers worldwide [12]. GA ameliorates advanced glycation end-product (AGE)-induced endothelial dysfunction by inhibiting TGF- β 1 and RAGE/NF- κ B pathway activation in human umbilical vein endothelial cells [13]. GA treatment markedly inhibits NLRP3 inflammasome activation, diet-induced adipose tissue inflammation and IL-1 β and caspase-1 production in white adipose tissue in ex vivo cultures [14]. GA protects the retinal vascular barrier and prevents retinal neuropathy by inhibiting the HMGB1-related inflammatory response and inactivating ERK(1/2) and NF- κ B in the diabetic retina (DR) [15, 16]. Several recent studies [17, 18] have indicated that HMGB1 is activated in type 2 diabetes mellitus patients and high glucose-induced mesangial cells and that HMGB1 promotes AGE-induced expression of TGF- β and connective tissue growth factor (CTGF) in renal tubular epithelial cells. Thus, we investigated whether the GA treatment could attenuate diabetic kidney injury by regulating HMGB1 and related inflammatory signalling. In the present study, our aim was to explore the protective role of GA on renal injury and the effects of GA on the HMGB1-related inflammatory signalling pathways in STZ-induced diabetic rats.

Materials and Methods

Animal models

All animal procedures and experimental methods were approved by the Institutional Animal Care and Use Committee of the Nanjing Medical University. Male Sprague-Dawley rats, aged 8 weeks (180-200 g), were fasted overnight, and 60 mg/kg streptozotocin (STZ) (Sigma Aldrich, St. Louis, MO, USA) in 0.1 M citric buffer, pH 4.5, was singly injected intraperitoneally. Equal volumes of the citric buffer were injected into the normal control rats (n=10). Fasting glucose concentrations above 300 mg/dl were considered to indicate diabetes. The rats were randomly divided into three groups: Control, Diabetic and Diabetic+GA. The rats in the Diabetic+GA group were treated intragastrically with GA at a dose of 150 mg/kg/day (GA, Santa Cruz Biotechnology, Inc, Santa Cruz, CA), while the rats in the Control and Diabetic groups were administered intragastrically with saline for 8 weeks. We monitored the blood glucose levels and body weights each week during the experiment.

The blood from the vena cava was drawn for measurements of blood glucose, TNF- α , IL-6 and IL-1 β after the 8-week treatment. Rat kidneys tissues were preserved at -80°C to determine the levels of TNF- α , IL-6, IL-1 β , MCP-1, ICAM-1, TGF- β 1 and Col-IV gene expression. The right renal tissues were fixed with 10% buffered formalin for HE staining and immunohistochemistry. Blood glucose was measured with the Fast Blood Glucose Monitoring System (Breeze 2, Bayer Healthcare, Mishawaka, IN, USA). Urinary albumin was measured using immunonephelometry (DCA 2000, Bayer, Leverkusen, Germany). Urinary creatinine was determined using an enzyme method (Kyowa Medex Co., Ltd, Tokyo, Japan). The urinary albumin/creatinine ratio (UACR) was calculated as albumin (mg)/creatinine (g).

Enzyme-linked immunosorbent assay (ELISA) kits

The serum TNF- α , IL-1 β and IL-6 levels were assessed by ELISA using matched antibody pairs per the manufacturer's instructions (Bender MedSystems GmbH).

HE staining and immunohistochemistry of kidney sections

Rat kidneys were fixed in 10% buffered formalin, embedded in paraffin and sliced into 4- μm -thick transverse sections. The slides were then stained with haematoxylin-eosin (HE). The expression of CD14 in the kidney was examined by immunohistochemistry. Briefly, the slides were washed with phosphate-buffered saline (PBS), permeabilized for 30 min with 0.25% Tx-100 in PBS, incubated with rabbit anti-CD14 antibody (1:1000, Sigma Aldrich, St. Louis, MO, USA), washed with PBS, incubated with peroxidase-labelled goat secondary antibody against rabbit (1:2000, Santa Cruz Biotechnology, CA, USA), visualized with DAB and quantified by Image-Pro Plus (Media Cybernetics, Inc, USA).

Real-time PCR

Total RNA from the renal tissues was extracted using a standard Trizol RNA isolation method (Invitrogen). Complementary DNA (cDNA) was obtained by reverse transcription from 1 μg of total RNA using a PrimeScript RT reagent Kit (Takara, Japan) and stored at -80°C until real-time PCR. Real-time PCR was performed in a StepOnePlus™ Real-Time PCR System (Applied Biosystems), using the SYBR Premix Ex Taq (Takara Bio). The results were normalized against β -actin gene expression. The PCR primers for TNF- α , IL-6, IL-1 β , MCP-1, ICAM-1, TGF- β 1 and Col-IV are listed in Table 1.

Western Blotting

The kidney tissues were washed twice with cold PBS and lysed in an ice-cold cell lysis buffer supplemented with a Protease Inhibitor Cocktail (Roche, Basel, Switzerland) to extract the total protein. The total protein lysates were dissolved in the gel loading buffer and boiled for 5 min. The nuclear and cytoplasmic proteins of the fresh

Table 1. Specific primer pairs used in the present study

Gene	Sequence
TNF- α F	GTCTGTGCCTCAGCCTCTTC
TNF- α R	TGGAAGTATGAGAGGGAGC
IL-6 F	TCCAGTTGCCCTTCTTGGGAC
IL-6 R	GTACTCCAGAAGACCAGAGG
IL-1 β F	CACCTCTCAAGCAGACACAGA
IL-1 β R	ACGGGTTCATGGTGAAGTC
ICAM-1 F	AGGTATCCATCCATCCACA
ICAM-1 R	GCCACAGTTCTCAAAGCACA
MCP-1 R	TGAGGTGGTTGTGGAAAAGA
TGF- β 1 F	GCCAGATCCTGTCCAACTAA
TGF- β 1 R	GCCAGATCCTGTCCAACTAA
Collagen IVF	GCCCTACGTTAGCAGATGTACC
Collagen IVR	TATAAATGGACTGGCTCGGAAT
ACTIN F	CACCCGCGGTACAACCTTC
ACTIN R	CCCATACCCACCATCACACC

kidney samples were extracted using the Thermo Scientific™ NE-PER Nuclear and Cytoplasmic Extraction Kit (Pierce Biotechnology, Rockford, USA). The kidney tissue preparation and extraction protocols were performed per the manufacturer's instructions. Forty milligrams of kidney tissue was cut into small pieces, washed with PBS, centrifuged for 5 min at 500 g, and carefully removed, and the supernatant was discarded, leaving the cell pellet as dry as possible. The tissue was then homogenized using a tissue grinder with CERI, and the extraction was performed using the CERI and NER reagent in the kit; the volume ratio of the CER I:CER II:NER reagents was maintained at 200:11:100 μ L. The extracts were stored at -80°C until use. The protein concentration was determined using the BCA method. The sample proteins were then separated by 10% sodium dodecyl sulfate polyacrylamide electrophoresis, transferred to a PVDF membranes, blocked with 7.5% non-fat milk and incubated overnight at 4°C in diluted antibodies against RAGE, TLR-4, HMGB1, ICAM, Col-IV, the p65 subunit of NF- κ B (NF- κ B p65), β -actin (Biotechnology, Santa Cruz, CA), extracellular signal-regulated kinase (ERK), p-ERK (Thr202/Tyr204), ERK, anti-p-JNK (Thr183/Tyr185), JNK, p-p38 MAPK (Tyr322), p38 MAPK (Cell Signaling Technologies, Danvers, MA) or Histone H3 (Enogene Biotech. Co., Ltd). The membranes were then incubated with the appropriate secondary antibodies for one hour at room temperature. Proteins were visualized by enhanced chemiluminescence (ECL Millipore) and quantified by densitometry (Quantity One, Bio-Rad, Hercules, CA, USA).

Statistical analysis

Data are reported as the mean $\pm$ standard deviation. Comparisons of 2 groups were performed with unpaired T tests, and multiple group comparisons were performed by one-way ANOVA followed by post hoc individual comparisons with the SNK test. Differences were considered statically significant when the p value was under 0.05. All statistical analyses were performing with SPSS v18.0 (SPSS Inc, Chicago, IL).

Results

Metabolic anomalies and the urine albumin creatinine ratio (UACR)

We investigated the changes in body weight, blood glucose and the UACR in all rats among the 3 groups. The body weight of the diabetic rat group was lower than the control rat group, while diabetic rat blood glucose level and UACR were higher. The 8-week GA treatment did not change the weight loss and hyperglycaemia (Fig. 1 a and b) but significantly reduced the UACR levels of the diabetic rats (Fig. 1c). The results suggested that the HMGB1 inhibitor reduced urinary albumin excretion regardless of blood glucose and body weight.

Kidney morphology

HE staining of the kidney sections showed that the diabetic rats contained more noticeable renal lesions, including glomerular hypertrophy, increased glomerular mesangial cells, increased mesangial matrix and injury, increased cell injury and increased necrosis

Fig. 1. The changes in body weight, blood glucose and the UACR in the rats a: body weight change among the 3 groups; b: blood glucose level; c: the UACR levels for the 3 groups. Data are expressed as the mean $\pm$ SEM of each group. *P<0.05 vs the control group; #P<0.05 vs the diabetic group.

Fig. 2. H&E staining of the kidney sections from the 3 groups ($\times 200$): a: normal control rats; b: diabetic rats; c: diabetic rats with the GA treatment.

Fig. 3. The expression of CD14 in rat renal tissues (immunohistochemical staining) ($\times 200$). a1-c1: CD14 expression in the glomeruli of the control, diabetic and diabetic rats with the GA treatment. a2-c2: CD14 expression in the tubular among the 3 groups. d: Integral optical density (IOD) value of CD14 in the glomerulus. e: IOD value of CD14 in the renal tubular. n=6 per group. * $P < 0.05$ vs the control group, # $P < 0.05$ vs the diabetic group.

in the renal tubules and glomeruli, than the control group. The GA treatment significantly alleviated renal pathological damage in the kidneys of diabetic rats (Fig. 2).

CD14 expression in rat renal tissue

CD14 is regarded as a cell surface marker of macrophages. In this study, we examined the number of CD14-positive cells in the glomeruli and renal tubules by immunohistochemistry. CD14 expression in the glomeruli (Fig. 3a1-c1) and renal tubules (Fig. 3a2-c2) was upregulated in the diabetes group and significantly downregulated by the GA treatment (Fig. 3d-e).

TNF- α , IL-6 and IL-1 β levels in rat serum and renal tissue

The protein levels of TNF- α , IL-6 and IL-1 β in the serum and the levels of gene expression in renal tissue were significantly increased in the diabetic rats compared with the controls, while the GA treatment significantly suppressed the TNF- α , IL-6, and IL-1 β levels in the kidneys and serum (Fig. 4).

Changes in ICAM-1, MCP-1, TGF- β 1 in rat renal tissue

ICAM-1, an adhesion factor, plays a key role in promoting macrophage infiltration into glomeruli, causing adhesion, and the transformation of monocytes into macrophages. We demonstrated that the increased gene and protein expression levels of ICAM-1 in the kidneys of the diabetic rats were remarkably inhibited by the GA treatment (Fig. 5 a and e).

MCP-1 can recruit mononuclear macrophages to the glomerulus or tubulointerstitium. In this study, we found that MCP-1 gene expression in the kidneys was significantly increased in diabetic rats compared with the control group. The GA treatment significantly reduced MCP-1 expression in diabetes (Fig. 5 b).

TGF- β 1, a potent fibrogenic factor, stimulates the synthesis of extracellular matrix such

Fig. 4. The levels of TNF-α, IL-6 and IL-1β in the rats from the 3 groups a-c: serum levels of TNF-α, IL-6 and IL-1β by ELISA. d-f: mRNA level of TNF-α, IL-6 and IL-1β in the renal tissues by real-time PCR. n=6 per group. *P<0.05 vs the control group, #P<0.05 vs the diabetic group.

Fig. 5. ICAM-1, MCP-1, TGF-β1 and Col-IV expression in the renal tissue. a-d: ICAM-1, MCP-1, TGF-β1 and Collagen IV mRNA expression in the renal tissue by real-time PCR. e and f: ICAM-1 and Collagen IV protein expression in the rat kidneys. Data for the indicated groups are normalized to β-actin or GAPDH; n=6 per group. *P<0.05 vs the control group, #P<0.05 vs the diabetic group.

as collagen. It is considered to be the strongest cytokine involved in glomerulosclerosis in diabetic nephropathy [19]. We found that the TGF-β1 level in diabetic rats was significantly higher than in the controls. HMGB1 inhibitor GA significantly decreased the expression of TGF-β1 (Fig. 5 c).

Expression levels of HMGB1, HMGB1 receptors RAGE and TLR-4, and Col-IV in rat renal tissue

Type IV collagen (Col-IV), the main framework of GBM, is predominant in kidney fibrosis [20]. Our results showed that the protein expression levels of HMGB1, RAGE and

TLR-4 in the kidneys were greatly upregulated in the diabetic rats. Interestingly, the HMGB1 inhibitor GA significantly downregulated the levels of RAGE, TLR-4 and HMGB1 (Fig. 6 a-d). Meanwhile, the increased gene and protein expression levels of Col-IV in the diabetic rats were significantly suppressed by the HMGB1 inhibitor GA (Fig. 5 d and f).

Phosphorylation of ERK, JNK and p38 MAPK and the nuclear and cytoplasmic NF- κ B p65 expressions levels in rat renal tissue by western blot

Our present results demonstrate that the phosphorylation levels of ERK, JNK and p38 MAPK are enhanced in diabetic rats compared to control rats. The GA treatment significantly decreased p-ERK and p-p38 MAPK, while the level of p-JNK was not affected by the GA treatment (Fig. 7 a-d).

In addition, we found that NF- κ B p65 in the cytoplasm was significantly downregulated in the diabetic group and significantly upregulated by the GA treatment, while the expression of NF- κ B p65 in the nucleus was significantly enhanced in the diabetic group compared with the control group; moreover, the GA treatment significantly inhibited NF- κ B p65 translocation to the nucleus. (Fig. 7 e and f).

Discussion

In the current study, we demonstrated that the protein expression levels of HMGB1 and its receptors, RAGE and TLR4, were significantly upregulated in the kidneys of diabetic rats, with an accompanying increase in the UACR level, Col-IV expression and kidney pathological damage. Furthermore, we found that HMGB1 inhibitor GA markedly downregulated the renal expression levels of HMGB1, RAGE and TLR4, decreased the levels of the UACR, and

Fig. 6. The expression levels of HMGB1, RAGE and TLR-4 in the rat kidneys. a: Western blot showing HMGB1, RAGE and TLR-4 production. Quantitative analysis of HMGB1 (b), RAGE (c), and TLR-4 (d). n=6 per group. *P<0.05 vs the control group, #P<0.05 vs the diabetic group.

Fig. 7. Western blot analysis of the expression levels of ERK, JNK, p38 MAPK and NF-κB p65 in the kidneys a: Activation of the ERK, JNK, p38 MAPK, and NF-κB p65 proteins in the rat kidney. b-f: Quantitative analysis of ERK, JNK, p38 MAPK, cytoplasmic NF-κB p65 and nuclear NF-κB p65 among the three groups. n=6 per group. *P<0.05 vs the control group, #P<0.05 vs the diabetic group.

suppressed renal Col-IV expression and pathological injury, while body weight and blood glucose were not changed. In addition, our results indicated that the GA treatment alleviated kidney injury by suppressing (1) serum TNF- α , IL-1 β and IL-6; (2) CD14-positive cells infiltration into the glomerulus and tubule; (3) the renal gene levels of TNF- α , IL-1 β , IL-6, MCP-1, ICAM-1, and TGF- β 1 and the protein expression level of ICAM; (4) NF-κB translocation from the cytoplasm to the nucleus; and (5) activation of ERK and p38 MAPK. Taken together, these findings suggest that HMGB1 inhibitor GA ameliorates kidney injury in STZ-induced diabetic rats independent of blood glucose and body weight.

As a potential inflammatory cytokine, HMGB1 plays multiple roles in the pathogenesis of renal diseases. Lin *et al.* [21] reports that HMGB1 is highly expressed in the nucleus and cytoplasm in the proximal and distal tubules of DN patients but poorly expressed in DM non-nephropathy patients; however, TLR-4 is predominantly expressed in the tubules and peritubular capillaries of tissues from DN patients, but little staining by immunohistochemistry is evident in tissues from DM non-nephropathy subjects. Kim *et al.* [22] has shown that HMGB1 is expressed in renal glomerular cells and tubular epithelial cells in STZ-induced diabetic rats and that expression of RAGE in the kidneys is also increased. Recent studies have demonstrated that high glucose and AGE significantly upregulate the HMGB1 mRNA levels in mesangial cells and tubular epithelial cells and that an HMGB1 inhibitor reduces HMGB1 mRNA expression [17, 18]. In this study, we found that both HMGB1 and its receptors, RAGE and TLR-4, were highly expressed in the kidneys of diabetic rats and that the GA treatment markedly inhibited the expression of HMGB1, RAGE and TLR-4, reduced urinary albumin excretion, and alleviated kidney pathologic damage. These results show that the renoprotective effects of HMGB1 inhibitor GA may come from its suppression of HMGB1, RAGE and TLR-4.

Inflammatory cytokines, including TNF- α , IL-1 β and IL-6, stimulate macrophages and damage renal cells to produce a spectrum of chemokines, adhesion molecules, TGF- β , ROS, angiotensin II, nuclear factors and so on. In turn, these factors promote macrophage recruitment and upregulate renal adhesion molecules [23]. These factors activate ECM-producing cells resulting in the accumulation of ECM and, ultimately, renal fibrogenesis.

CD14, a surface hallmark of macrophages, is a co-receptor for several TLRs [24]. Signalling through CD14 and TLRs leads to the innate immune response, which induces NF- κ B activation and proinflammatory molecule production [25]. In the present study, we showed that CD14 expression in the glomerulus and tubule was significantly upregulated in the diabetic rats and was downregulated by the GA treatment in the diabetic rats. In addition, we found that increased levels of TNF- α , IL-1 β and IL-6 in the serum and renal tissue in the diabetic rats were also decreased by the HMGB1 inhibitor GA treatment. Meanwhile, increased MCP-1, ICAM-1 and TGF- β 1 mRNA and ICAM protein levels in the kidney tissues of the diabetic rats were remarkably reduced after 8 weeks of the GA treatment. These data collectively indicated that suppressing HMGB1 could improve DN by inhibiting macrophage infiltration and decreasing production of proinflammatory cytokines, adhesion molecules, chemokines and profibrotic factor TGF- β 1.

A major element that is implicated in DN-associated inflammation is NF- κ B, which is regarded as a key nexus in the regulation of inflammatory molecules and signalling pathways [3]. Initiation of inflammatory reactors by HMGB1 is mediated through activation of the NF- κ B signalling pathway [8]. In high glucose-induced mesangial cells, HMGB1 elevation significantly enhances the levels of NF- κ B p65 in the cytosol and nucleus and increases gene expression of TNF- α , IL-1 β and IL-6, whereas knockdown of HMGB1 decreases high glucose-induced NF- κ B activation, TNF- α , IL-1 β and IL-6 mRNA [17]. In the retinas of diabetic rats, the protein level of NF- κ B is increased, and the GA treatment attenuates activation of NF- κ B [26]. In this study, we found that the GA treatment significantly inhibited NF- κ B translocation from the cytoplasm to the nucleus in the kidneys of the diabetic rats. Based on previous studies and our present results, we may reasonably conclude that suppression of NF- κ B by the HMGB1 inhibitor has a beneficial effect on preventing DN progression.

ERK, JNK and p38 MAPK, which are strongly activated in human DN, are crucial in cellular hypertrophy, kidney fibrosis, and renal cytokine-mediated inflammation [27-29]. The ERK1/2 pathway is activated under high glucose in mesangial cells, which is followed by the complicated synthesis of TGF- β 1 [30]. In biopsy samples of human DN, JNK activation correlates with interstitial macrophage accumulation, kidney injury molecule-1 expression, interstitial fibrosis, and loss of renal function [31]. p38 MAPK, followed by the activation of NF- κ B, participates in intracellular signal transduction and production of cytokines and chemokines [32]. In rat macrophages or neuroblastoma cells, HMGB1 binding to RAGE and TLR4 activates MAPK pathways and results in NF- κ B activation and nuclear translocation [8]. Liang *et al.* [33] have shown that HMGB1 binding to RAGE enhances inflammatory responses of human bronchial epithelial cells by activating p38 MAPK and ERK1/2. Chen *et al.* [34] have demonstrated that the HMGB1 inhibitor prevents neointimal hyperplasia in the rat carotid artery injury model via deactivation of the p38 MAPK/NF- κ B signalling pathway. In the present study, we also found that the phosphorylation levels of ERK, JNK and p38 MAPK were enhanced in the kidneys of diabetic rats. Importantly, our results demonstrated that the HMGB1 inhibitor GA significantly suppressed the phosphorylation of ERK and p38 MAPK in the kidneys of the diabetic rats and that JNK phosphorylation was not affected. To our knowledge, this is the first study to demonstrate that the HMGB1 inhibitor ameliorates kidney injury by inhibiting ERK/p38MAPK and the NF- κ B-related inflammatory response in diabetic rats.

Conclusion

Our findings indicate that the HMGB1 inhibitor GA improves renal injury and inflammatory responses in STZ-induced diabetic rats, and the improvement might be directly related to the suppression of HMGB1 receptor-mediated phosphorylation of components in the ERK, p38 MAPK, and NF- κ B signalling pathways. Therefore, blockage of HMGB1 might represent a novel therapeutic strategy for diabetic kidney injury in the future.

Disclosure Statement

All authors claim no conflicts of interest.

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