Overexpression of DJ-1 reduces oxidative stress and attenuates hypoxia/reoxygenation injury in NRK-52E cells exposed to high glucose

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Abstract. Patients with diabetes are more vulnerable to renal ischemia/reperfusion (I/R) injury, which is implicated in hyperglycemia-induced oxidative stress. We previously reported that the hyperglycemia-induced inhibition of DJ-1, a novel oncogene that exhibits potent antioxidant activity, is implicated in the severity of myocardial I/R injury. In the present study, we aimed to explore the role of DJ-1 in hypoxia/reoxygenation (H/R) injury in renal cells exposed to high glucose (HG). For this purpose, NRK-52E cells were exposed to HG (30 mM) for 48 h and then exposed to hypoxia for 4 h and reoxygenation for 2 h, which significantly decreased cell viability and superoxide dismutase (SOD) activity, and increased the malondialdehyde (MDA) content, accompanied by a decrease in DJ-1 protein expression. The overexpression of DJ-1 by transfection with a DJ-1 overexpression plasmid exerted protective effects against HG-induced H/R injury, as evidenced by increased CCK-8 levels and SOD activity, the decreased release of lactate dehydrogenase (LDH) and the decreased MDA content, and increased nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and heme oxygenase-1 (HO-1) expression. Similar effects were observed following treatment with the antioxidant, N-acetylcysteine. These results suggest that the overexpression of DJ-1 reduces oxidative stress and attenuates H/R injury in NRK-52E cells exposed to HG.

Introduction

Diabetes mellitus (DM) has gained increasing attention globally. Diabetic nephropathy (DN) is one of the most seriousdiabetic microvascular complications in DM, which leads to end-stage renal failure, seriously threatening the lives of patients (1). Recent studies have shown that oxidative stress plays an important role in the progression of DN (2,3). Ischemic injury often occurs in patients with diabetes during the peri-operative period. Diabetic nephropathy with ischemia/reperfusion (I/R) injury is also closely related to oxidative stress (4,5). However, the underlying mechanisms responsible for the adverse effects caused by oxidative stress on renal injury induced by hyperglycemia with I/R insults have not yet been completely elucidated.

Currently, several hypotheses have emerged regarding DJ-1, which is also known as Park 7. DJ-1 was originally described as an oncogene (6). It is a multifunctional protein related to Parkinson's disease, neurodegeneration and oxidative stress (7,8). Several lines of evidence have demonstrated the antioxidative function of DJ-1 in various disease models both *in vitro* and *in vivo* (9-12). In a study on hypertensive nephropathy, the physiological role of DJ-1 was shown to be associated with reactive oxygen species (ROS) in primary renal tubular epithelial cells (13). Another study demonstrated a compensatory increase in DJ-1 expression in the renal cortex against increased oxidative stress of the hyperglycemic milieu (14). Thus, it is suggested that DJ-1 may be effective against oxidative stress and it is increasingly considered as an important target for DN therapy (15-17).

The protective role of DJ-1 has been indicated in many organs and tissues, such as the heart, brain, liver, kidneys and pancreas. It has been shown that DJ-1 exerts neuroprotective effects against ischemic damage to the spinal cord through its antioxidant functions (18). The overexpression of DJ-1 may participate in a protective strategy against I/R injury-induced oxidative stress in rat heart-derived H9c2 cells (19). We previously demonstrated that the hyperglycemia-induced inhibition of DJ-1 expression was implicated in the severity of myocardial I/R injury (20). However, the potential mechanisms of action of DJ-1 in renal cells exposed to high glucose (HG) and hypoxia/reoxygenation (H/R) injury have not yet been fully clarified. Moreover, DJ-1 can regulate the expression of various antioxidant genes, including nuclear factor (erythroid-derived 2)-like 2 (Nrf2) (21,22) and heme oxygenase-1 (HO-1), enhancing the antioxidant ability of cells (23).

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In the present study, we hypothesized that the overexpression of DJ-1 reduced oxidative stress and attenuated H/R injury in rat proximal tubular epithelial (NRK-52E) cells exposed to HG. As the antioxidant, N-acetylcysteine (NAC), has been shown to protect the kidneys against I/R injury by regulating the Nrf2 signaling pathway (24), we therefore, also examined the effects of NAC and compared them to those of DJ-1.

Materials and methods

Materials. The following materials were used: NRK-52E cells (American Tissue Type Culture Collection, Manassas, VA, USA); Dulbecco's modified Eagle's medium (DMEM; HyClone, Logan, UT, USA); phosphate-buffered saline (PBS; Gino Biological Medical Technology Co., Ltd., Hangzhou, China); fetal bovine serum (FBS; Gibco, Carlsbad, CA, USA); penicillin and streptomycin (Beyotime Institute of Biotechnology, Haimen, China); 0.25% Trypsin with 0.02% ethylenediaminetetraacetic acid (EDTA) (Gino Biological Medical Technology Co., Ltd.); D-Glucose (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China); NAC (Sigma, St. Louis, MO, USA); the empty vector plasmid and pEX-2-EGFP-DJ-1 (GenePharma, Suzhou, China); Attractene transfection reagent (Qiagen, Valencia, CA, USA). The following kits were also used: the cell counting kit-8 (CCK-8; Dojindo, Kumamoto, Japan); lactate dehydrogenase (LDH) kit; superoxide dismutase (SOD) and malondialdehyde (MDA) kit (both from Nanjing Jiancheng Bioengineering Institute, Nanjing, China); the BCA protein assay kit; nuclear and cytoplasmic protein extraction kit (both from Beyotime Institute of Biotechnology). The antibodies used are listed as follows: rabbit anti-rat DJ-1 monoclonal antibody (#5933; Cell Signaling Technology, Danvers, MA, USA); rabbit anti-rat Nrf2 (sc-722) and HO-1 (sc-10789) polyclonal antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) rabbit anti-rat β-actin polyclonal antibody (GB13001; Wuhan Goodbio Technology Co., Ltd., Wuhan, China) and rabbit anti-rat Lamin B1 polyclonal antibody (BA1228; Boster, Wuhan, China). All other chemicals were obtained from commercial sources and were of highest grade available.

Cell culture. Rat proximal tubular epithelial (NRK-52E) cells were maintained in low-glucose DMEM medium (the concentration of glucose was 5.5 mM), which was supplemented with 10% FBS, 100 U/ml penicillin and 0.1 mg/ml streptomycin, and the medium was replaced every 24 h. The cells were subcultured using 0.25% trypsin with 0.02% EDTA after being washed with PBS twice and allowed to grow up to 70-80% confluency.

The NRK-52E cells were inoculated in 6-well plates with low-glucose DMEM medium synchronously. When the cells grew to the appropriate density, they were incubated in low-glucose DMEM medium without FBS for 24 h, pre-treated for 2 h with NAC (1 mM), incubated with low glucose concentrations (LG; final concentration, 5.5 mM) and HG (final concentration, 30 mM) for various periods of time, srespectively, and then exposed to hypoxia (5% CO_2, 1% O_2 and 94% N_2) for 4 h, then to reoxygenation (5% CO_2, 21% O_2 and 74% N_2) for 2 h.

Plasmid transfection. The day prior to transfection, the cells were seeded in 6-well plates at 30-50% confluence containing 1% serum, 100 U/ml penicillin and 0.1 mg/ml streptomycin. The cells were 40-80% confluent on the day of transfection and were transfected with the plasmids according to the manufacturer's instructions. The empty vector plasmid and pEX-2-EGFP-DJ-1 (4 μg) were transfected into the cells without FBS and removed after 12 h with fresh low-glucose and HG medium added respectively, and then exposed to hypoxia for 4 h, then to reoxygenation for 2 h.

Determination of cell viability and LDH assay. The cell suspension (100 μl) was inoculated in 96-well plates, and the supernatant was collected for the LDH toxicity emitting experiment. According to the instructions of the manufacturer of the LDH kit, the absorbance was measured at 450 nm using a microplate reader (Victor3 1420-050; Perkin Elmer, Waltham, MA, USA). The medium was removed and the cells were washed twice with PBS. The fresh medium and 10 μl CCK-8 solution were added to each well followed by incubation for 2 h in 37˚C and 5% CO_2. The absorbance was then measured at 450 nm using a microplate reader (Victor3 1420-050; Perkin Elmer) and the cell viability was calculated.

Detection of oxidative stress. The NRK-52E cells were washed 3 times with PBS and sonicated using an ultrasonic crusher (FB120220; Thermo Fisher Scientific, Waltham, MA, USA). The cells were examined to determine the contents of SOD and MDA using the respective kits (from Nanjing Jiancheng Bioengineering Institute) according to the manufacturer's instructions. Cellular protein was measured using the BCA protein assay kit. The MDA content in the NRK-52E cells was measured by the colorimetric method (MDA kit; Nanjing Jiancheng Bioengineering Institute). The absorbance (530 nm) was measured using a microplate reader (Victor3 1420-050; Perkin Elmer) and the results were expressed in nmol of MDA/mg protein. SOD activity in the NRK-52E cells was measured at an optical density at 450 nm according to the WST-1 method (SOD kit; Nanjing Jiancheng Bioengineering Institute). The results were expressed as U/mg protein and 1 unit of enzyme is defined as the enzyme activity that inhibits the autoxidation of pyrogallol by 50%.

Western blot analysis. The cells were washed 3 times with PBS and trypsinized after almost completely covering the bottom of the wells. Cell suspensions were centrifuged for 5 min at 1,000 rpm, after which the supernatant was discarded. Total cellular proteins were extracted using RIPA lysis buffer (Beyotime Institute of Biotechnology). Nuclear and cytoplasmic proteins were extracted according to the manufacturer's instructions (nuclear and cytoplasmic protein extraction kit; Beyotime Institute of Biotechnology). An equal amount of protein was loaded onto sodium dodecyl sulfate (SDS) polyacrylamide gels, electrophoresed and transferred to PVDF membranes. To prevent non-specific background binding of the antibody, the membranes were blocked by using 5% BSA and incubated for 2 h at room temperature under agitation. The membranes were then incubated with the specific rabbit anti-rat DJ-1 (1:1,000), Nrf2 (1:200), HO-1 (1:200), β-actin (1:2,000) and Lamin B1 (1:200) antibodies overnight at 4˚C. After repeated washing, the membranes were incubated with the corresponding goat anti-rabbit horseradish peroxidase-conjugated secondary

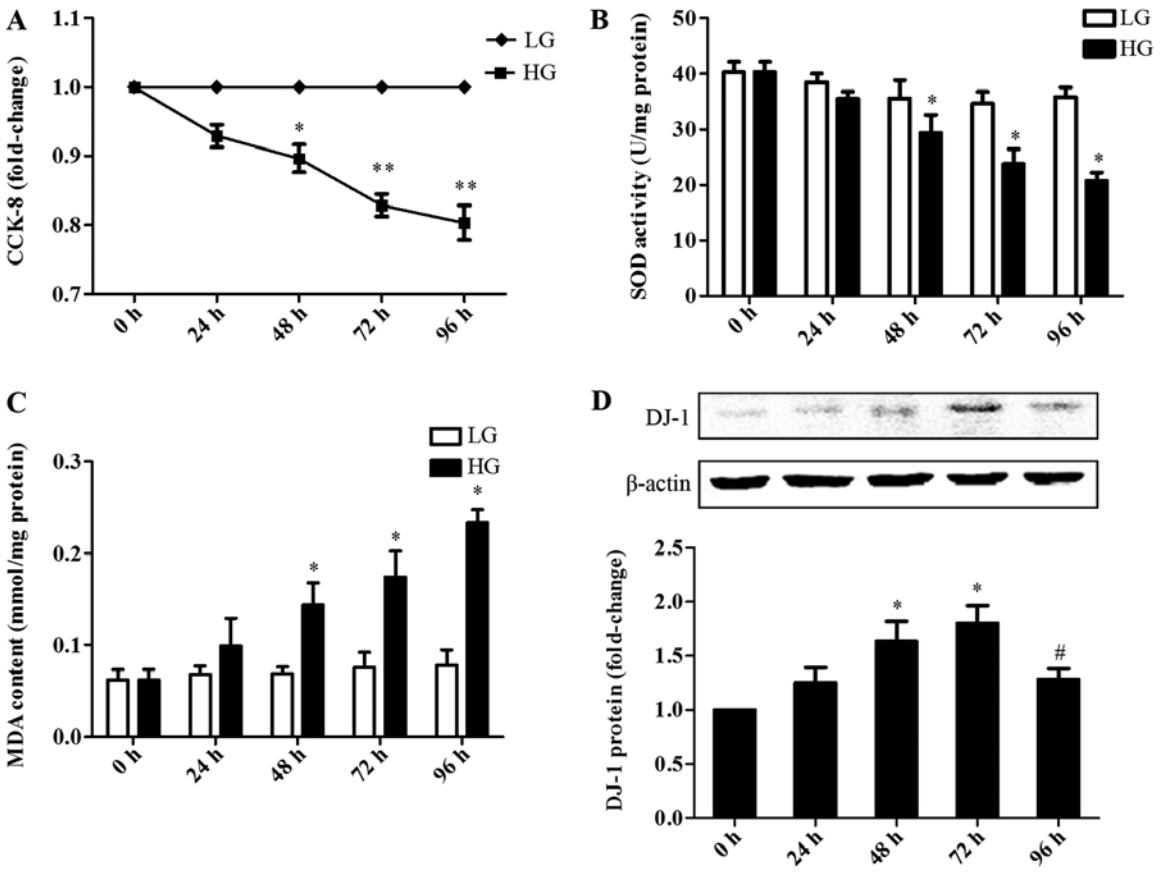


Figure 1. Effects of high glucose (HG) on the viability of, and oxidative stress and DJ-1 expression in NRK-52E cells over time. NRK-52E cells were exposed to HG and we then examined (A) cell viability by CCK-8 assay; (B) SOD activity; (C) the MDA content; (D) The relative protein level of DJ-1 was measured by western blot analysis. β-actin was used for normalization of the data. Data are expressed as the means ± SEM. *P<0.05, **P<0.01 compared with the 0-h group; #P<0.05 compared with the 72-h HG group. Results shown are representative of at least 3 independent experiments.

antibody (1:10,000; IRDye 800CW, LI-COR Biosciences, Lincoln, NE, USA) for 1 h at room temperature. The intensity of the identified bands accomplished with chemiluminescence was detected on an Odyssey two-color infrared laser imaging system (Li-Cor, Lincoln, NE USA) and densitometry was carried out using Odyssey software.

Statistical analysis. All data are presented as the means ± SEM. All statistical analyses were performed using GraphPad Prism 5.0 software (GraphPad Software, La Jolla, CA, USA). Comparisons between multiple groups were evaluated by one-way analysis of variance (ANOVA) and comparisons between 2 groups by the Student's unpaired t-test. A value of P<0.05 was considered to indicate a statistically significant difference.

Results

Effects of HG on cell viability, oxidative stress and DJ-1 expression in NRK-52E cells over time. We first examined the effects of HG on cell viability and oxidative injury in NRK-52E cells over time. Both cell viability measured by CCK-8 assay and the antioxidant activity measured by SOD activity assay in the NRK-52E cells were significantly decreased following 48 h of exposure to HG, with a decreasing tendency continuing 72-96 h after initial the HG exposure (Fig. 1A and B). By contrast, the MDA content was significantly increased at 48 h, and further increased 72-96 h following exposure to HG (Fig. 1C).

To investigate the role of DJ-1 in NRK-52E cells exposed to HG, we also measured the protein expression of DJ-1. DJ-1 expression did not significantly increase until 48 h, a peak increase occurred at 72 h, and DJ-1 expression was reduced to basal levels within 96 h following exposure to HG (Fig. 1D). Thus, we selected the duration of exposure to HG to be 48 h for our subsequent experiments. The osmotic control, mannitol, exerted no effects on cell viability, injury and DJ-1 expression (data not shown).

Effects of H/R on cell viability, oxidative stress and DJ-1 expression in NRK-52E cells exposed to HG. As the diabetic kidneys are more vulnerable to I/R injury (25,26), we thus wished to examine the effects of H/R on NRK-52E cells following exposure to HG. H/R significantly decreased the levels of CCK-8 and SOD activity as compared to the LG control or HG control groups not subjected to H/R (Fig. 2A and B). Following exposure to HG for 48 h, H/R further decreased the levels of CCK-8 and SOD activity (P<0.05, LG + H/R vs. HG + H/R). By contrast, the MDA content in the HG control group was much higher than that in the LG control group. H/R further increased the MDA content as compared with that in the LG control or HG control groups not subjected to H/R (Fig. 2C). Of note,

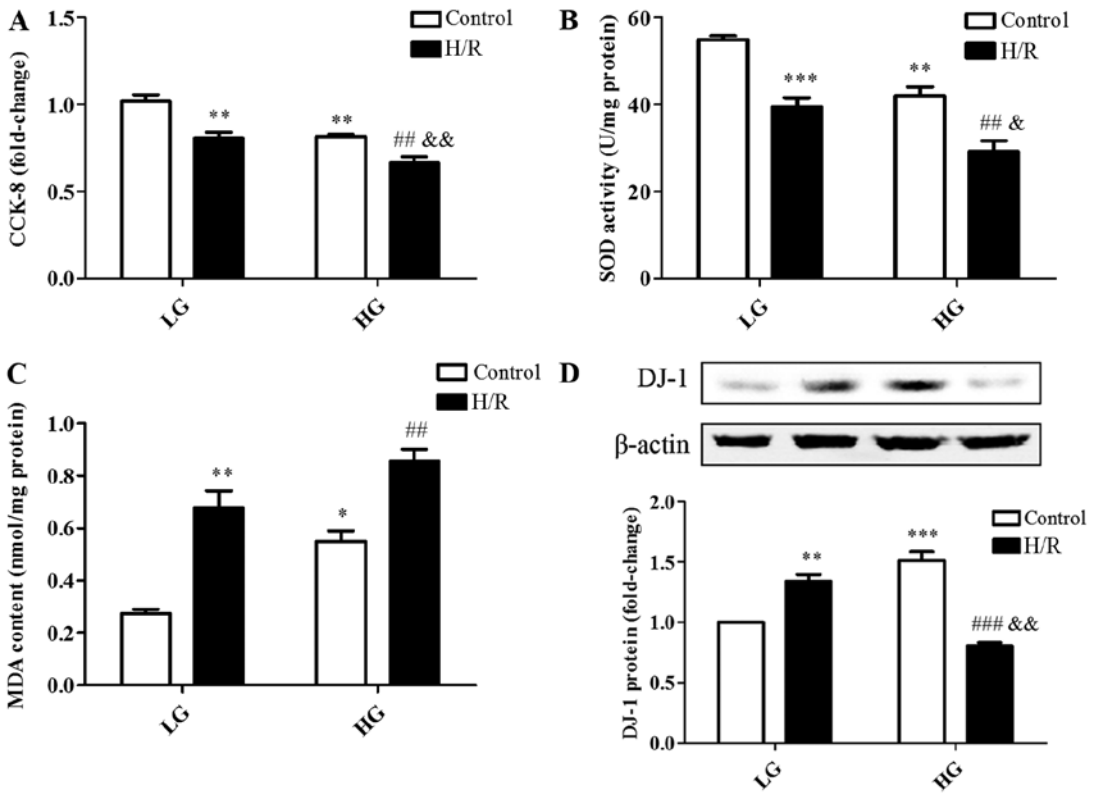


Figure 2. Effects of hypoxia/reoxygenation (H/R) on the viability of, and oxidative stress and DJ-1 expression in NRK-52E cells exposed to high glucose (HG). NRK-52E cells were exposed to HG and H/R and we then examined (A) cell viability by CCK-8 assay; (B) SOD activity; (C) the MDA content; (D) the relative protein level of DJ-1 was measured by western blot analysis. β-actin was used for normalization of the data. Data are expressed as the means ± SEM. *P<0.05, **P<0.01, ***P<0.001 compared with the LG control group; $^{\#\#}$P<0.01 compared with the HG control group; $^{\&}$P<0.05, $^{\&\&}$P<0.01 compared with the LG + H/R group. Results shown are representative of at least 3 independent experiments.

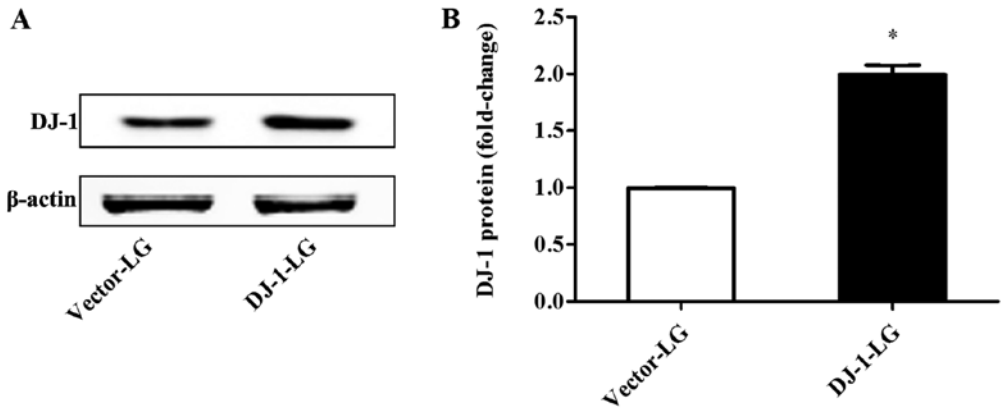


Figure 3. Overexpression of DJ-1 in the NRK-52E cells. (A) Western blot analysis was used to measure the protein expression of DJ-1 in cells transfected with pEX-2-EGFP-DJ-1. β-actin was used for normalization of the data. (B) Quantitative analysis of the relative protein level of DJ-1. Data are expressed as the means ± SEM. *P<0.05 compared with the vector-LG group (emtpy vector group). Results shown are representative of at least 3 independent experiments.

H/R significantly increased DJ-1 protein expression in the LG control group, but decreased DJ-1 protein expression in the NRK-52E cells following exposure to HG for 48 h (Fig. 2D). Thus, the decreased expression of DJ-1 may be involved in the vulnerability to renal I/R injury in diabetes.

Overexpression of DJ-1 in NRK-52E cells. To confirm the role of DJ-1 in H/R injury in renal cells, we transfected the NRK-52E cells with a DJ-1 overexpression vector (pEX-2-EGFP-DJ-1; DJ-1 group) or an empty vector plasmid as the negative control group (vector group). The protein expression of DJ-1 was significantly increased following transfection with the overexpressio plasmid, as compared with that in the vector group (Fig. 3).

Effects of DJ-1 overexpression and NAC on H/R injury in NRK-52E cells following exposure to HG. To examine the molecular mechanisms of action of DJ-1 under HG and H/R conditions, we also treated the cells with the antioxidant, NAC. Both DJ-1 overexpression and NAC had no significant effects

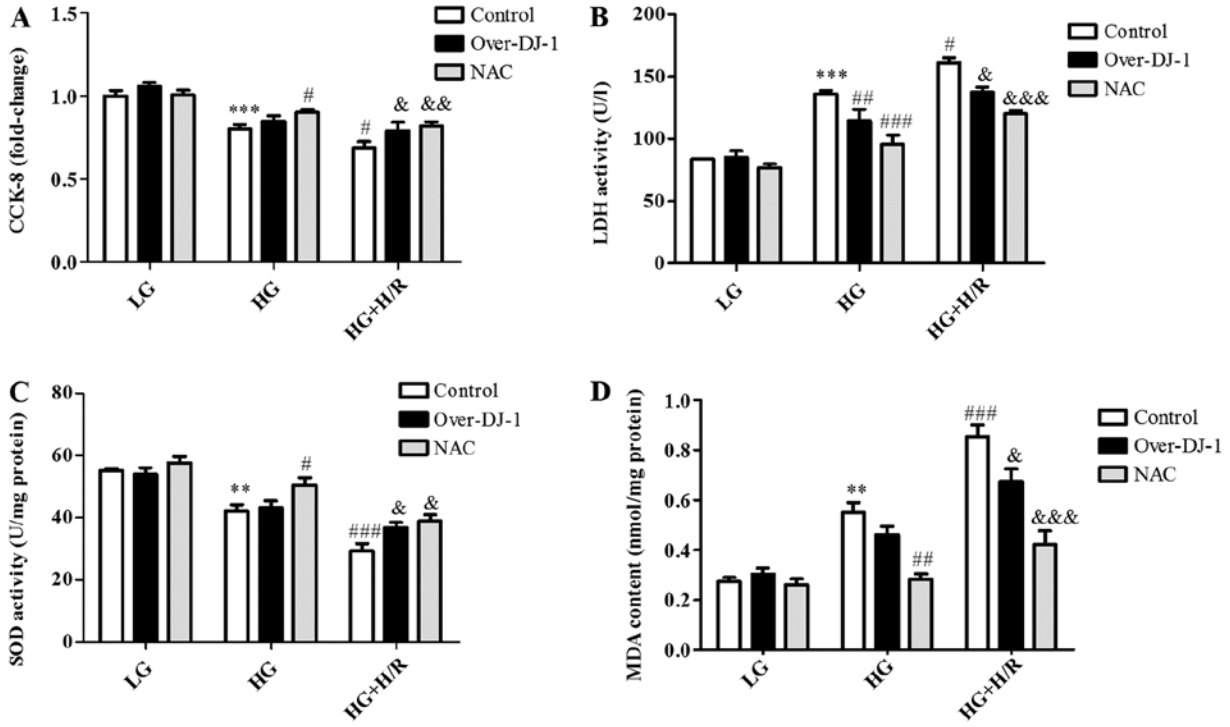


Figure 4. Effects of DJ-1 overexpression and NAC on H/R injury in NRK-52E cells following exposure to high glucose (HG). NRK-52E cells transfected with pEX-2-EGFP-DJ-1 or pre-treated of NAC were exposed to HG and hypoxia/reoxygenation (H/R) and we then measured (A) cell viability by CCK-8 assay; (B) LDH toxicity; (C) SOD activity; (D) the MDA content. Data are expressed as the means ± SEM. **P<0.01, ***P<0.001 compared with the LG control group; $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 compared with the HG control group; $^{\&}$P<0.05, $^{\&\&}$P<0.01, $^{\&\&\&}$P<0.001 compared with the HG + H/R control group. Results shown are representative of at least 3 independent experiments.

on CCK-8, LDH release, MDA content and SOD activity in the NRK-52 cells under LG conditions (Fig. 4). Exposure to HG significantly decreased the levels of CCK-8 and SOD activity, but significantly increased LDH release and the MDA content as compared with that in the LG group. All these changes were further intensified by H/R. However, DJ-1 overexpression and treatment with the antioxidant, NAC, significantly attenuated or reversed the increase in LDH release and the MDA content, and elevated the levels of CCK-8 and SOD activity.

Effects of DJ-1 overexpression and NAC on the protein expression of Nrf2 and HO-1. After the NRK-52E cells were transfected with pEX-2-EGFP-DJ-1, the protein expression levels of DJ-1, Nrf2 and HO-1 were significantly increased as compared with the corresponding control group transfected with the empty vector (Fig. 5A, C and E). In addition, treatment with NAC attenuated the HG-induced increase in DJ-1, Nrf2 and HO-1 expression, and attenuated the decrease in the protein expression of DJ-1 and Nrf2 following exposure to HG and H/R (Fig. 1B, D and F).

Discussion

The present observations provide several pieces of important evidence supporting the protective effects of DJ-1 against oxidative stress in response to HG and H/R injury in renal cells. Firstly, it was demonstrated that HG decreased cell viability and SOD activity, but increased the MDA content, with DJ-1 protein expression increasing compensatively within 72 h of exposure to HG. Furthermore, H/R injury markedly decreased cell viability and increased oxidative stress in the cells exposed to HG, but decreased DJ-1 protein expressoin. Finally, the overexpression of DJ-1 exerted antioxidant effects against HG and H/R injury, which were similar to the effects of NAC, promoting the protein expression of Nrf2 and HO-1. In short, the overexpression of DJ-1 reduced oxidative stress and attenuated H/R injury in NRK-52E cells exposed to HG.

It has been suggested that increased oxidative stress is recognized as the key factor in the pathogenesis and progression of DN (27-29). It has been indicated that HG enhances oxidative stress in renal cell injury (30). As an end-product of lipid peroxidation, MDA is widely used to detect the influence of oxidative stress on the mitochondria respiratory chain (31), while SOD is an antioxidant, protecting cells against oxidative stress. In the present study, the viability of NRK-2E cells was significantly decreased in a time-dependent manner following exposure to HG. A significant increase in MDA content and a decrease in SOD activity induced by HG were also observed. These results demonstrate that the antioxidant activity was weakened in parallel with the severity of NRK-52E cell injury. The results mentioned above indicate that the model of renal injury in oxidative stress was successfully established, which may be considered as an early-stage cell model of DN.

DJ-1 is an ubiquitous cytoprotective protein, and acts as an antioxidant to scavenge ROS in various cells. It has been shown that DJ-1 protects the morphology and function of the mitochondria and protects against cell injury (32). With the increase in blood glucose levels, the content of DJ-1 has been shown to increase in pancreatic β-cells, to inhibit the production of ROS (33). The antioxidant function of DJ-1

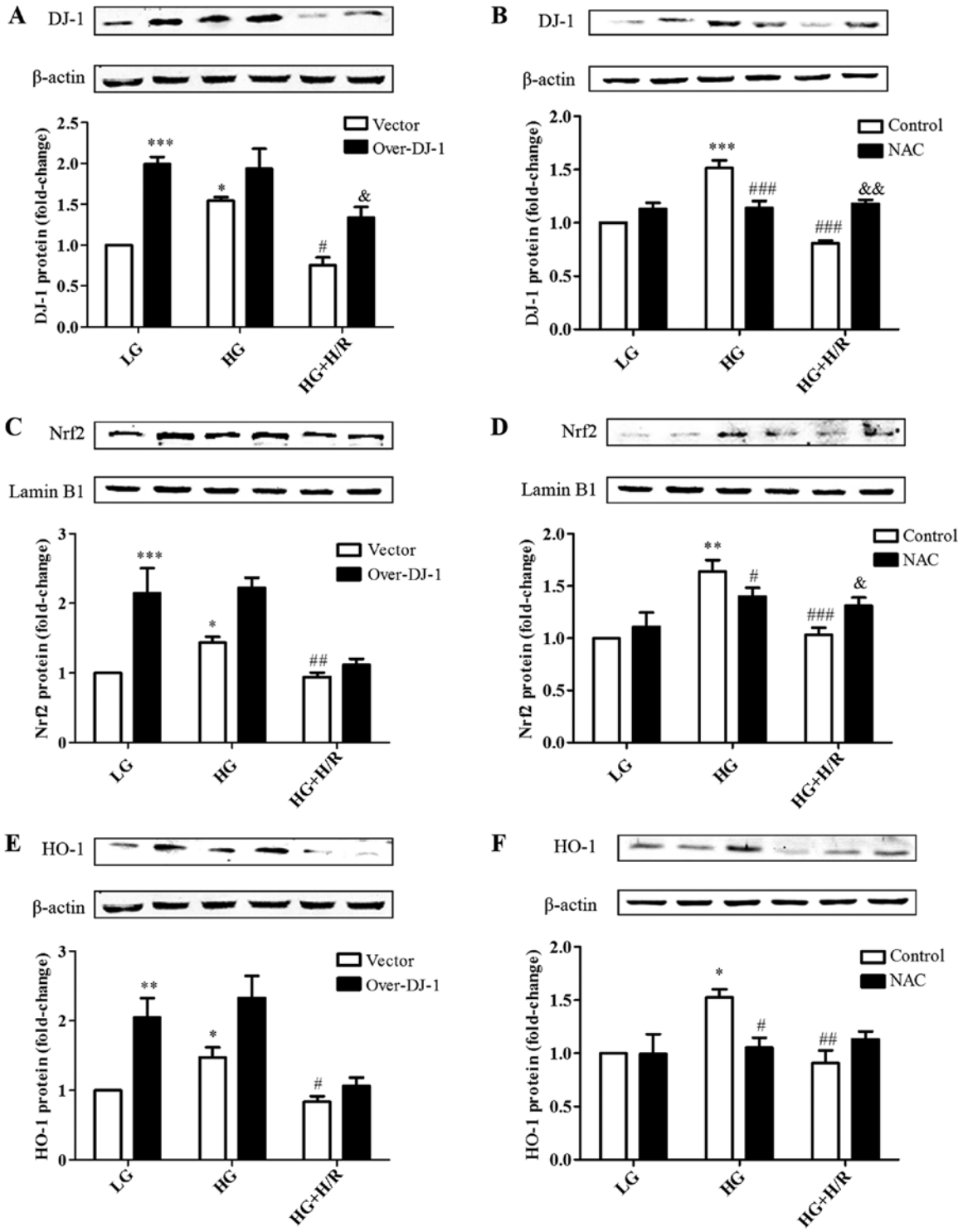


Figure 5. Effects of DJ-1 overexpression and NAC on the protein expression of nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and heme oxygenase-1 (HO-1). (A, C and E) Western blot analysis results of DJ-1, Nrf2 and HO-1 protein expression in NRK-52E cells transfected with pEX-2-EGFP-DJ-1 and exposed to high glucose (HG) and hypoxia/reoxygenation (H/R); (B, D and F) western blot analysis results of DJ-1, Nrf2 and HO-1 protein in cells pre-treated with NAC and exposed to HG and H/R. β-actin was used for normalization of the total protein DJ-1 and HO-1 levels. Lamin B1 was used for normalization of the nuclear protein Nrf2. Data are expressed as the means ± SEM. *P<0.05, **P<0.01, ***P<0.001 compared with the LG vector (empty vector) control group; $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 compared with HG vector control group; $^{\&}$P<0.05, $^{\&\&}$P<0.01 compared with HG + H/R vector control group. Results shown are representative of at least 3 independent experiments.

maintains the integrity and physiological characteristics of the mitochondria, which is a prerequisite for glucose-stimulated insulin secretion. However, the mechanisms responsible for the protective effects of DJ-1 on renal tubular epithelial cells under HG conditions remain unclear. Our data demonstrated that the protein expression of DJ-1 was compensatively increased in a time-dependent manner and was significantly enhanced until the 72-h time period, but was decreased at the later time period of 96 h in the NRK-52E cells exposed to HG. Based on our results of the examination of cell injury and oxidative stress,

we hypothesized that DJ-1 protein expression may be compensatively increased due to its antioxidant function in renal cells during the earlier time periods, but it is decreased following its depletion over a longer periods of time, and may thus be insufficient to protect against more severe oxidative stress. It can be considered that DJ-1 plays an important role in oxidative stress induced by HG in renal cells.

In the clinical peri-operative period, I/R injury occurs quite frequently, producing redundant ROS, leading to a multi-organ oxidative stress, including that in the kidneys (34). It has been shown that in rat renal I/R injury, the disrusption of mitochondrial metabolism and oxidative stress, results in ventricular function disorder, and leads to cardiac and renal injury (35). High glucose levels have been shown to highly associated with a a lower tolerance to ischemia, and an increased severity of renal I/R injury (36,37). The present study demonstrated that the viability of renal cells in response to H/R decreased, and the oxidative stress level increased, while the cells exposed to HG and H/R exhibited more severe oxidative stress. It is inferred that in the peri-operative period, non-diabetic patients may suffer from oxidative stress injury induced by I/R; however, patients with diabetes may suffer more severely, and this matter requires more attention. We have already reported that cardiac DJ-1 expression was downregulated in hyperglycemia-induced I/R injury (20). However, there is little evidence of the association between I/R injury and DJ-1 in DN. In the present study, compared with the LG group, the NRK-52E cells in the LG + H/R group exhibited a much higher protein expression of DJ-1. However, compared with the HG group, the HG + H/R group exhibited a much lower protein expression of DJ-1. All the above-mentioned data indicated that HG or H/R led to oxidative stress injury, which caused a compensatory increase in the protein expression of DJ-1 during the early stage. However, in the HG + H/R group, DJ-1 expression could not be maintained at higher, compensatory levels but was decreased, leading to more severe oxidative stress. This evidence proves DJ-1 to be vitally important for diabetic renal I/R injury in the peri-operative period.

Oxidative stress is involved in cellular injury in kidney, and overexpression of antioxidant proteins and treatment with antioxidants prevents renal damage. To further assess the cytoprotective function of DJ-1, we examined the overexpression of DJ-1 in H/R exposed to HG compared with the antioxidant NAC. Plasmid-derived overexpression of DJ-1 showed the antioxidative effect on high glucose and H/R injury. Interestingly, NAC played the similar role of DJ-1 overexpression in protecting cells against oxidative stress and increased DJ-1 expression during H/R injury exposed to HG. These results suggest that overexpression of DJ-1 can be partially explained by its inhibitory effects on oxidative stress induced by HG and H/R, yet weaker than NAC treatment.

Several enzymes and signaling pathways are related the antioxidative functions of DJ-1. It has been shown that renal I/R leads to increased apoptosis and oxidative stress in renal tubular epithelial cells, accompanied by the increased protein expression of Nrf2 and HO-1 (38). The activation of the DJ-1/Nrf2 pathway was found to exert a significant protective effect against oxidative stress (21,39). The overexpression of DJ-1 reduces the ubiquitination of Nrf2 to stabilize the Nrf2 protein level, whereas the knockdown of DJ-1 decreases the stability of Nrf2 protein (40). Our results revealed that the overexpression of DJ-1 promoted the expression of Nrf2 and HO-1 following exposure to HG and H/R injury. To gain insight into the potential mechanisms responsible for the protective effects of DJ-1 against H/R injury, we further evaluated the role of the DJ-1/Nrf2 pathway by treating the cells with NAC. We observed a significant increase in the protein expression levels of DJ-1, Nrf2 and HO-1 in the cells in the HG + H/R group treated with NAC. All the above-mentioned findings indicate that the upregulation of DJ-1 may attenuate the progression of diabetic renal I/R injury, and is associated with a significant increase in the expression levels of Nrf2 and HO-1.

In conclusion, the findings of the present study demonstrates that the overexpression of DJ-1 reduces oxidative stress and attenuates H/R injury in NRK-52E rat proximal tubular epithelial cells exposed to HG.

Acknowledgements

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