

Research Article

Melatonin attenuates acute kidney ischemia/reperfusion injury in diabetic rats by activation of the SIRT1/Nrf2/HO-1 signaling pathway

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Background and aims: Diabetic kidney is more sensitive to ischemia/reperfusion (I/R) injury, which is associated with increased oxidative stress and impaired nuclear factor erythroid 2-related factor 2 (Nrf2)/heme oxygenase-1 (HO-1) signaling. Melatonin, a hormone that is secreted with the rhythm of the light/dark cycle, has antioxidative effects in reducing acute kidney injury (AKI). However, the molecular mechanism of melatonin protection against kidney I/R injury in the state of diabetes is still unknown. In the present study, we hypothesized that melatonin attenuates renal I/R injury in diabetes by activating silent information regulator 2 associated protein 1 (SIRT1) expression and Nrf2/HO-1 signaling. **Methods:** Control or streptozotocin (STZ)-induced Type 1 diabetic rats were treated with or without melatonin for 4 weeks. Renal I/R injury was achieved by clamping both left and right renal pedicles for 30 min followed by reperfusion for 48 h. **Results:** Diabetic rats that were treated with melatonin undergoing I/R injury prevented renal injury from I/R, in aspects of the histopathological score, cell apoptosis, and oxidative stress in kidney, accompanied with decreased expressions of SIRT1, Nrf2, and HO-1 as compared with those in control rats. All these alterations were attenuated or prevented by melatonin treatment; but these beneficial effects of melatonin were abolished by selective inhibition of SIRT1 with EX527. **Conclusion:** These findings suggest melatonin could attenuate renal I/R injury in diabetes, possibly through improving SIRT1/Nrf2/HO-1 signaling.

Introduction

Acute kidney injury (AKI) is a global public health problem that affects millions of people, and it has become increasingly prevalent in recent years [1]. Several risk factors such as age, race, genetic factors, hypertension, and diabetes are associated with AKI [1]. Diabetes is associated with a variety of metabolic disorders, such as hypoxia, overproduction of reactive oxygen species (ROS), mitochondrial dysfunction, and inflammation [2]. Moreover, diabetes is the major cause of chronic kidney disease in most developed countries [3]. Diabetic nephropathy (DN) is one of the serious organ complications of diabetes, and DN is the leading cause of end-stage renal disease (ESKD) in the world [2,4]. In diabetic kidney tissue, hyperglycemia can promote the production of ROS and increase the level of oxidative stress [5]. Animal model studies in rats confirmed that diabetic rats had increased vulnerability to renal ischemia/reperfusion (I/R) compared with normal rats [6,7]. However, the underlying mechanisms by which hyperglycemia adversely affects renal I/R in diabetes has remained elusive.

Melatonin is mainly produced by the pineal gland and it acts as a natural antioxidant and free radical scavenger [8–10]. Melatonin not only acts on ROS, reactive nitrogen species, and free radicals but also up-regulates antioxidant enzymes and down-regulates pro-oxidant enzymes [11–13]. Endogenous

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and exogenous melatonin can reduce diabetes-related metabolic disorders by regulating insulin secretion and scavenging ROS [14]. Chronic melatonin treatment reduces renal damage by restricting lipid oxidation and NO production in STZ-induced diabetic rats exposed to renal I/R [15]. In rat kidney transplantation model, melatonin protects kidney from I/R injury by down-regulating the expression of NF- κ Bp65, iNOS, and caspase-3 [16], while in the rat model of renal warm I/R, the melatonin signaling phosphorylated Akt, inhibited GSK-3 β and VDAC [17]. Melatonin showed neuroprotective effects by activating nuclear factor erythroid 2-related factor 2 (Nrf2)/ARE pathway and increasing levels of antioxidant enzymes heme oxygenase-1 (HO-1) and NQO1 expression [18]. Although melatonin has both pro- and anti-inflammatory activities [19,20], the known protective effects of melatonin in I/R injury is primarily via its antioxidative stress rather than the pro-inflammatory cytokines [21,22].

Silent Information Regulator 2 Associated Protein 1 (SIRT1) is a deacetylase regulating the processes of aging, cancer, glucose metabolism, and energy homeostasis [23–25]. It is well described that SIRT1 reduces oxidative stress, inflammation stimuli, cell senescence, and apoptosis [26–32]. In addition, a study suggested that SIRT1 protects kidneys from renal damage in a melatonin-dependent manner in rats with severe burn-induced AKI rat model [33].

Nrf2 is a major transcriptional regulator of antioxidant proteins [34]. After cell injury, Nrf2 translocates to the nucleus and promotes the expression of HO-1 [35]. Several studies confirmed that SIRT1 could promote the activation of Nrf2, including increasing its nuclear accumulation, DNA binding activity, and transcriptional activity, and up-regulating the expression of HO-1 [36–38]. However, whether SIRT1 can attenuate renal I/R injury by activating the Nrf2/HO-1 signaling pathway in diabetes has not been reported. In the present study, we determined that melatonin activates the Nrf2/HO-1 signaling pathway by up-regulating the expression of SIRT1, thereby reducing diabetic renal I/R injury.

Materials and methods

Animals and reagents

Male adult Sprague–Dawley rats (250 \pm 10 g, 6–8 weeks of age) were purchased from Beijing HFK Bioscience Co., Ltd. (Beijing, China). All rats were housed at 22–24°C, a 12-h light/dark cycle with free access to standard rat chow and water. The experimental protocols were in accordance with the principles of Animal Care of Wuhan University (Wuhan, China), and approved by the Ethics Committee of Renmin Hospital of Wuhan University. Streptozotocin (STZ) and melatonin were purchased from Sigma–Aldrich (St. Louis, MO, U.S.A.). EX527 was purchased from Selleck Chemicals (TX, U.S.A.). Primary antibodies against SIRT1 and HO-1 were purchased from Abcam, Inc. (Cambridge, U.K.). Primary antibodies against Nrf2 were purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX, U.S.A.). Primary antibodies against GAPDH and Lamin B were purchased from Cell Signaling Technology, Inc. (MA, U.S.A.). Second antibodies were purchased from LI-COR Biosciences (IRDye 800CW; LI-COR Corporate, Lincoln, NE, U.S.A.).

Induction of diabetes

Type 1 diabetes was induced by a single intraperitoneal injection of STZ solution dissolved in 0.1 M citrate buffer (pH 4.5) at a dose of 60 mg/kg body weight, as previously described [39]. Normal rats were given a single intraperitoneal of the same equal volume citrate buffer. Three days after STZ injection, tail vein blood glucose levels were measured with a One Touch Ultra Glucose meter (Johnson & Johnson, New Brunswick, NJ, U.S.A.). Only those rats with fasting blood glucose level \geq 16.7 mM were considered as diabetic [39].

Renal I/R injury model

Animals were intraperitoneally anesthetized by pentobarbital sodium (60 mg/kg body weight) and then placed on a homeothermic pad to maintain a core body temperature of 37°C. Kidneys were exposed by abdominal midline incisions, and both left and right renal pedicles were clamped for 30 min to induce ischemia. After ischemia, the clamps were released for 48 h reperfusion. The same procedure was performed in the nondiabetic control animals without the bilateral clamping process. The abdominal wall wounds were closed and rats intraperitoneally received 1 ml warm saline. All rats were killed by cervical dislocation after 48 h of reperfusion. Plasma and kidneys samples were collected and stored at –80°C for further analysis.

Experimental protocol

At 4 weeks of diabetes, both diabetic and nondiabetic control rats were randomly allocated into six groups of 6–8 rats each: (i) nondiabetic rats sham-operated group (NS); (ii) nondiabetic rats I/R group (NI/R); (iii) diabetic rats sham-operated group (DS); (iv) diabetic rats I/R group (DI/R); (v) diabetic rats I/R+melatonin group; and (vi) diabetic rats I/R+melatonin+EX527 group. Melatonin was intraperitoneally injected daily after 3 days of STZ treatment for 4

weeks before renal I/R injury model (10 mg/kg, dissolved in 1% ethanol) [40]. EX527 was intraperitoneally injected daily for 3 days before renal I/R injury and once injected at 20 min before reperfusion (5 mg/kg, 1% DMSO diluted in sterile saline) [41].

Renal function and histology

Blood urea nitrogen (BUN) and serum creatinine (Scr) were measured by using commercial kits (Jiancheng Biotech, Nanjing, China) to detect renal function. Kidney tissues were cut into sections and fixed with 4% formaldehyde for 24 h, dehydrated and embedded in paraffin following routine protocols. After embedding in paraffin, 4- μ m-thick sections were stained with Hematoxylin at room temperature for 3 min and Eosin for 60 s using light microscopy.

Histopathological changes were evaluated by the degree of tubular injury graded from 0 to 4, according to tubular epithelial cell swelling, interstitial expansion, intertubular hemorrhaging, brush border loss, vacuolar degeneration, necrotic tubules, cast formation, and desquamation. Each sample was quantitated by five randomly selected fields with the following criteria: 0, no damage; 1, <25%; 2, 25–50%; 3, 50–75%; 4, >75%. Histological sections were evaluated in a blinding manner by two examiners [42].

Apoptosis assay

Terminal-deoxynucleotidyl Transferase Mediated Nick End Labeling (TUNEL) was used to detect kidney tissue apoptosis using an *in situ* cell death detection kit (Roche Diagnostics, Mannheim, Germany). Briefly, paraffin sections routinely underwent deparaffinization and rehydration, and then the slides were treated with 20 mg/l of proteinase K at 37°C for 15–25 min. The slides were then washed in PBS, the mass concentration of 3 g/l hydrogen peroxide/methanol was used to block endogenous peroxidase activity for 30 min at room temperature. The slides were then washed in PBS and then added to the TUNEL reaction mixture for 60 min in a humidified atmosphere at 37°C in the dark. The steps including washing in PBS, adding converter-POD, and incubating at 37°C for 30 min were then performed. Then, the slides were washed in PBS, and Diaminobenzidine (DAB) staining was performed. In addition, Hematoxylin was selected for re-staining. Finally, dehydration and transparent treatment were performed. TUNEL-positive cells were stained brown within the nucleus of apoptotic cells. Cell counting was performed by using five randomly selected fields, and the apoptosis index was calculated as the percentage of positive cells to total cells.

Measurement of oxidative stress

The level of malondialdehyde (MDA) and superoxide dismutase (SOD) from the homogenized kidney tissue was measured by using commercial kits respectively (Jiancheng Biotech, Nanjing, China), according to the manufacturer's instructions.

Western blot analysis

Cytoplasmic and nuclear proteins were extracted from the renal tissues using a nuclear extraction kit (Beyotime Institute of Biotechnology, Haimen, China) according to the manufacturer's instructions. The expressions of SIRT1, Nrf2, and HO-1 were examined by Western blot. GAPDH was used as the internal loading control of cytoplasmic protein. Lamin B was used as the internal loading control of nuclear protein. Protein content was determined by BCA protein assay kit (Beyotime Institute of Biotechnology, China). Protein samples were separated by electrophoresis on SDS/PAGE and transferred to PVDF membranes (Millipore, Billerica, MA, U.S.A.). Each membrane was blocked with 5% nonfat milk and incubated overnight at 4°C with the appropriate primary antibodies (1:1000 dilution, anti-SIRT1 and anti-HO-1 antibody, 1:500 dilution, anti-Nrf2 antibody), respectively followed by incubation with suitable secondary antibodies for 1 h at room temperature. Immune complexes were detected by using an Odyssey fluorescence-imaging scanner and band densities were quantitated using Odyssey software v3.0.29 imaging analysis system (both from LI-COR Biosciences, Lincoln, NE, U.S.A.).

Statistical analysis

All data were expressed as the mean \pm S.E.M. and analyzed using GraphPad Prism software version 6.0 (GraphPad Software, Inc., La Jolla, CA, U.S.A.). The statistical significance of differences amongst control and diabetic rats were evaluated by one-way ANOVA or two-way ANOVA followed by a Bonferroni's post hoc test. *P*-values <0.05 were considered to be statistically significant.

Table 1. Fasting blood glucose levels and body weight of nondiabetic and diabetic rats after 4 weeks

	NS	NI/R	DS	DI/R	DI/R+melatonin	DI/R+melatonin+EX527
Fasting blood glucose (mM)	5.88 ± 0.88	7.11 ± 1.61	25.51 ± 2.73*	27.19 ± 2.38*	24.45 ± 2.27*	26.21 ± 1.90*
Body weight (g)	345.69 ± 13.01	354.5 ± 9.98	206.06 ± 9.28*	213.76 ± 12.56*	221.12 ± 10.63*	209.32 ± 13.29*

The data are expressed as means ± S.E.M. ($n=6-8$ per group). NS and DS: nondiabetic and diabetic rats were subjected to sham operation. NI/R and DI/R: nondiabetic and diabetic rats were subjected to 30 min bilateral renal pedicle ligation and followed by 48 h reperfusion. DI/R+melatonin group: diabetic rats subjected to I/R surgery were treated with melatonin (10 mg/kg, ip daily) for 4 weeks after 3 days of STZ treatment. DI/R+melatonin+EX527 group: diabetic rats subjected to I/R operation were treated with melatonin (10 mg/kg, ip daily) for 4 weeks after 3 days of STZ treatment, and treated with EX527 for 3 days before renal I/R injury model and once injected at 20 min before reperfusion (5 mg/kg, ip daily).

* $P<0.05$ compared with NS group and NI/R group.

Results

Characteristics of control and diabetic rats before I/R modeling

At the end of the present study, the diabetic rats showed obvious characteristic systems of diabetes including hyperglycemia, polydipsia, polyphagia, and weight loss. Compared with the age-matched nondiabetic rats, the blood glucose of diabetic rats was significantly increased, and their body weight was significantly reduced (Table 1). Melatonin treatment had no significant effects on blood glucose and body weight in diabetic rats (Table 1).

Diabetic rats exhibit aggravated ischemia AKI-induced kidney injury on histopathology and apoptosis

We compared the susceptibility of diabetic and nondiabetic rats with I/R. The tubular injury score was used to evaluate the severity of kidney injury. Pathological changes were observed in renal tubules, including tubular epithelial cell swelling, brush border loss, interstitial expansion, intertubular hemorrhaging, vacuolar degeneration, necrotic tubules, cast formation, and desquamation in the NI/R group and diabetic groups (Figure 1A,C). A significant aggravating tissue damage was also observed in DI/R group (Figure 1A,C). When compared with the sham group, the NI/R group and diabetic groups exhibited a significant increase in histopathological scoring individually ($P<0.05$; Figure 1A,C). Moreover, a TUNEL assay was performed to identify the apoptotic cells in renal tissues. The TUNEL-positive cells were diminished in both NS and DS groups (Figure 1B). After I/R for 48 h, the apoptosis index of DI/R group was significantly higher than NI/R group ($P<0.05$; Figure 1D).

Diabetic rats exhibit aggravated kidney dysfunction and increased oxidative stress in the kidney after ischemia AKI

Diabetic I/R injury aggravated renal damage and oxidative stress [43]. Compared with the NS and DS groups, the levels of the BUN and SCr were significantly increased 48 h post I/R in both the NI/R and DI/R groups ($P<0.05$; Figure 2A,B). The damage evoked by AKI was further increased in the DI/R group, demonstrated by higher levels of the BUN and Scr than NI/R group ($P<0.05$; Figure 2A,B). In addition, as an indicator of antioxidant, SOD levels were significantly decreased in the NI/R and DI/R groups as compared with the NS and DS groups, respectively ($P<0.05$; Figure 2C). Meanwhile, the SOD level in DI/R group was lower than the NI/R group ($P<0.05$; Figure 2C). In contrast, the level of MDA, used as a measure of the level of oxidative stress, was significantly increased in the NI/R and DI/R groups, compared with those in the NS and DS groups, respectively ($P<0.05$; Figure 2D). When compared with NI/R group, the MDA level was significantly higher in DI/R group ($P<0.05$; Figure 2D).

Protein expression of SIRT1, Nrf2, and HO-1 in renal tissues

The previous research showed that the expression of SIRT1 is significantly reduced in DKI [44]. Similarly, the protein expression of SIRT1 was significantly diminished in DS group compared with NS group ($P<0.05$; Figure 3A,C). Renal SIRT1 expression was increased in NI/R group compared with NS group ($P<0.05$; Figure 3A,C). Renal SIRT1 expression was decreased in DI/R group compared with DS group ($P<0.05$; Figure 3A,C). Furthermore, the expression of SIRT1 was decreased in DI/R group compared with NI/R group ($P<0.05$; Figure 3A,C). The Nrf2/HO-1 pathway plays an important role in antioxidant reaction [45,46]. We detected the expression of Nrf2 and HO-1 in all groups. The results revealed that Nrf2 and HO-1 expression was diminished in DS and DI/R groups, compared with NS group, respectively. A significant increase in Nrf2 and HO-1 were observed in the NI/R group, when compared with

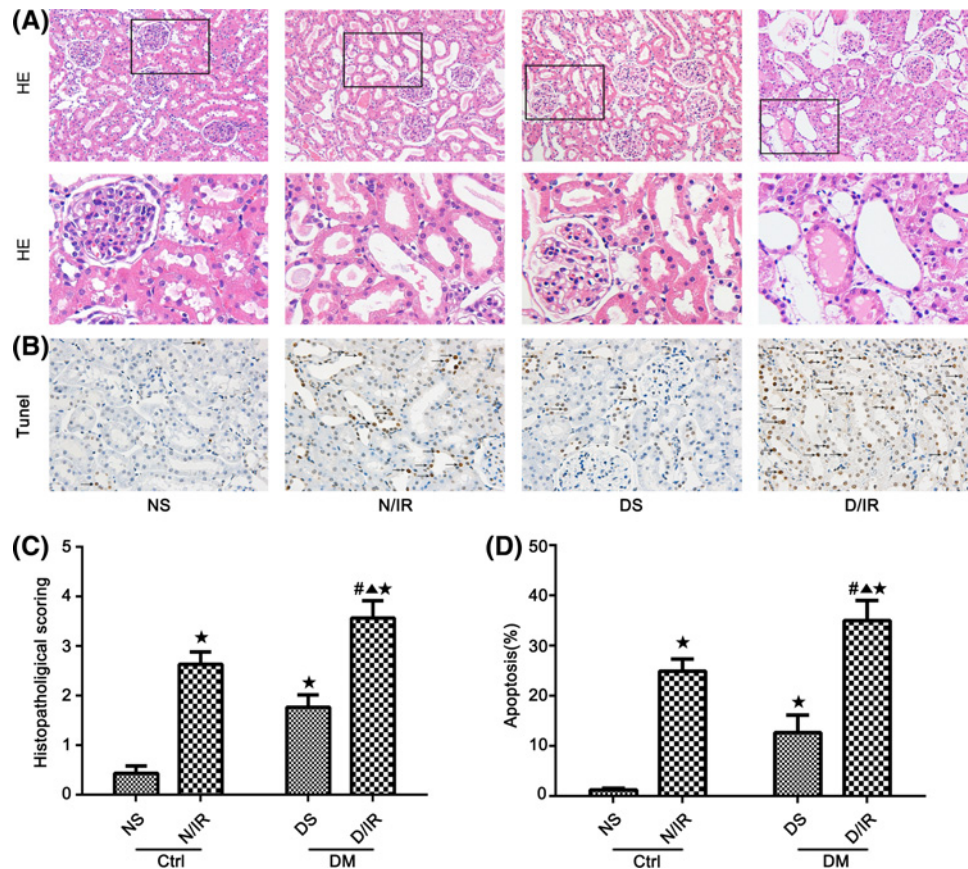


Figure 1. A diabetic model of adult male SD rats was induced using STZ

All rats were subjected to sham surgery or bilateral renal I/R injury. (A) Renal Hematoxylin and Eosin staining. Magnification: 200 \times . The lower panels are the magnified images of the boxed areas in the upper panels. (B) TUNEL staining. Magnification: 400 \times . (C) Histopathological scoring. (D) TUNEL assay apoptosis%. Data are presented as the mean \pm S.E.M. ($n=6$ per group). $\star P<0.05$ compared with NS group; $\blacktriangle P<0.05$ compared with NI/R group; $\# P<0.05$ compared with DS group. Abbreviations: Ctrl, control; DM, diabetes mellitus; HE, Hematoxylin and Eosin.

NS group. Renal expression of Nrf2 and HO-1 were both decreased in DI/R group compared with NI/R group and DS group respectively ($P<0.05$; Figure 3A,B,D).

Effects of melatonin on histopathology and apoptosis

Ischemia induces NO synthase in tubule cells. Subsequently, ROS cause renal tubule cell injury via oxidation of proteins, peroxidation of lipids, damage to DNA, and induction of apoptosis [47]. After I/R occurred, C3 deposited in renal and injured tubular epithelial cells, subsequently led to apoptosis due to the lack of the decay accelerating factor (DAF) and membrane cofactor protein (MCP) which inhibit complement activation of C3/4 level that is located in glomerular, rather tubular [48,49]. Melatonin exhibited anti-apoptosis in STZ-induced diabetic renal injury [50], as well as in I/R injury in the model of experimental kidney transplantation [16]. However, whether melatonin remains anti-apoptotic and how it affects the histopathology in the kidney is still unknown. Interestingly, the pathological changes in renal tubules, histopathological scoring, and apoptotic index were significantly increased in DI/R group and DI/R+melatonin+EX527 group, as compared with DS group ($P<0.05$; Figure 4A–D), while melatonin pre-treatment markedly ameliorated the histology score and apoptosis in DI/R+melatonin group, compared with the DI/R group and DI/R+melatonin+EX527 group ($P<0.05$; Figure 4C,D). Pre-treatment with EX527 in DI/R+melatonin+EX527 group abolished the positive effects elicited by melatonin ($P<0.05$; Figure 4C,D). There was no statistically significant difference in histopathological scoring and apoptotic index between DI/R group and DI/R+melatonin+EX527 group ($P>0.05$; Figure 4C,D).

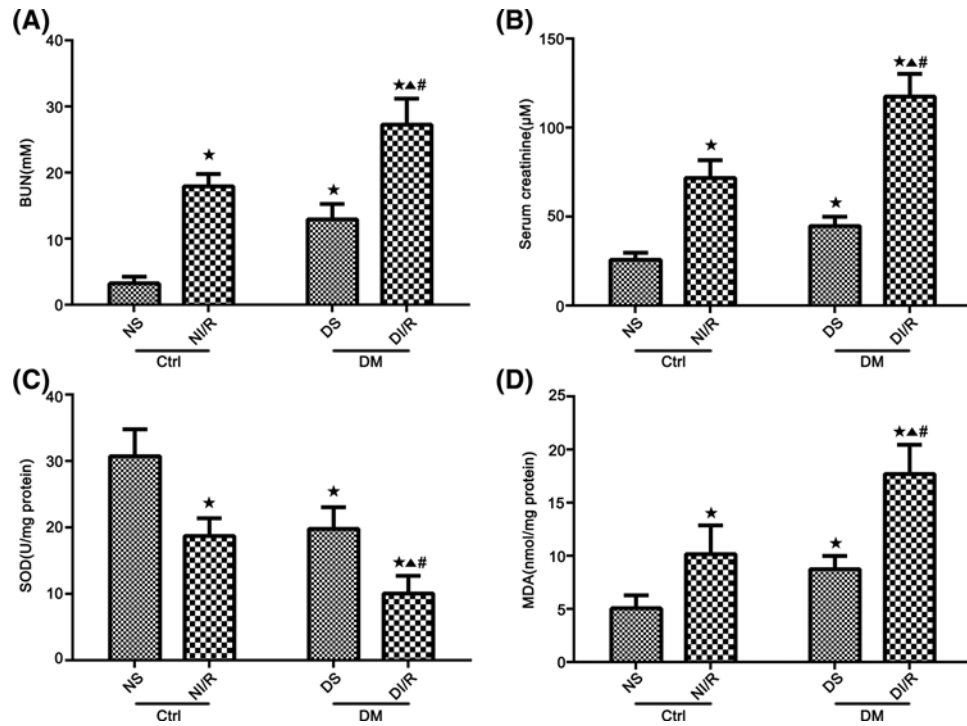


Figure 2. Serum BUN, creatinine, and renal tissues SOD and MDA levels in the different treatment groups (A) BUN. (B) Scr. (C) Kidney SOD contents. (D) MDA activity. Data are presented as the mean ± S.E.M. ($n=6$ per group). ★ $P<0.05$ compared with NS group; ▲ $P<0.05$ compared with NI/R group; # $P<0.05$ compared with DS group. Abbreviations: Ctrl, control; DM, diabetes mellitus.

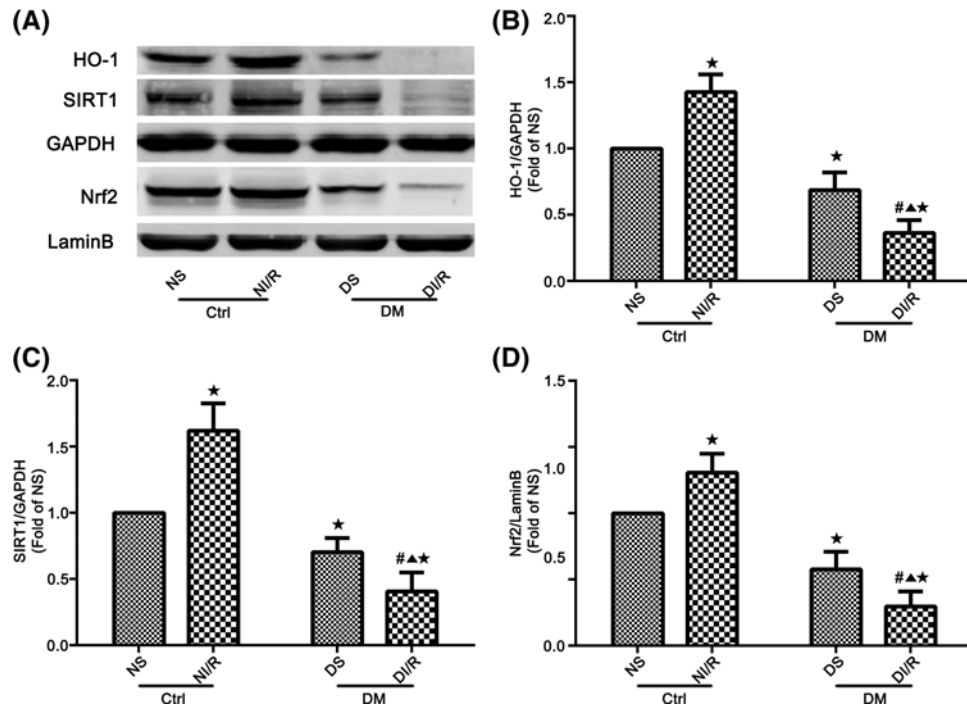


Figure 3. Expression of SIRT1, Nrf2, and HO-1 in renal tissues by Western blot analysis (A) Western blotting showed protein levels of SIRT1, Nrf2, and HO-1. (B–D) Quantitation of Western blot data from (A). Data are presented as the mean ± S.E.M. ($n=6$ per group). ★ $P<0.05$ compared with NS group; ▲ $P<0.05$ compared with NI/R group; # $P<0.05$ compared with DS group. Abbreviations: Ctrl, control; DM, diabetes mellitus.

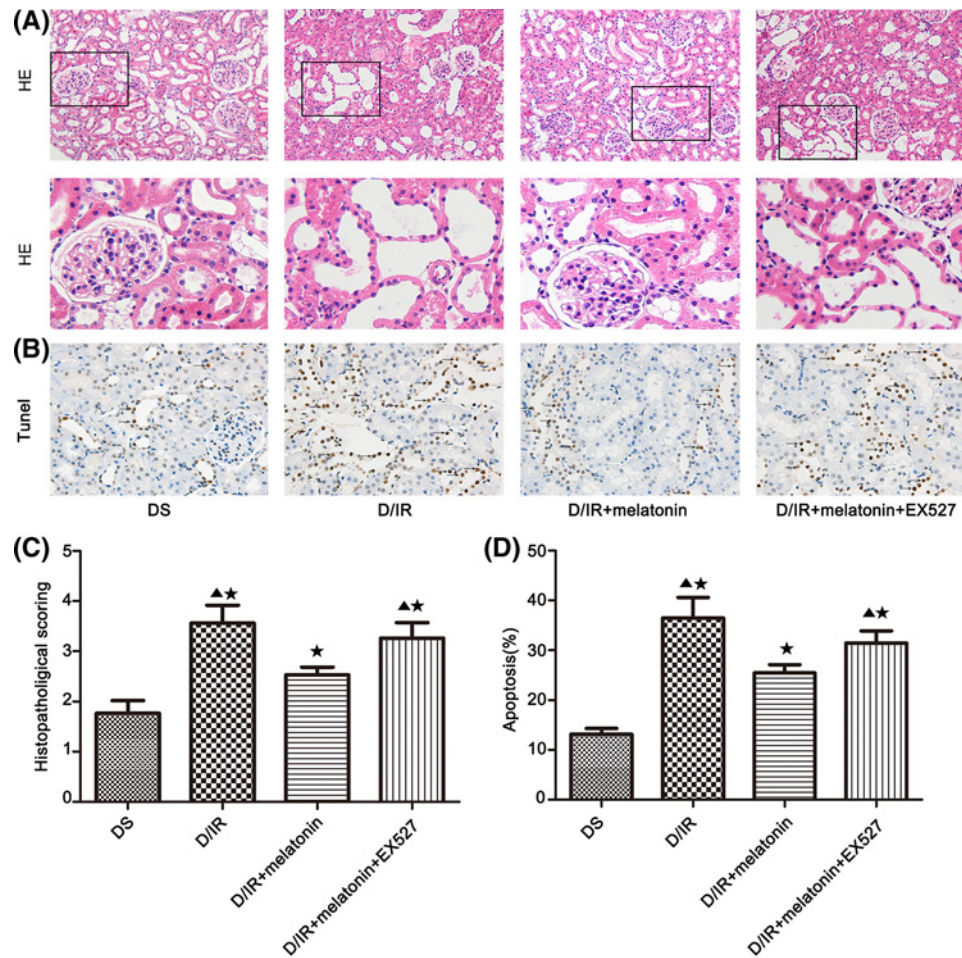


Figure 4. Melatonin pre-treatment markedly ameliorated the histology score and apoptosis after bilateral renal I/R injury in diabetic rats

(A) Renal HE staining. Magnification: 200 \times . The lower panels are the magnified images of the boxed areas in the upper panels. (B) TUNEL staining. Magnification: 400 \times . (C) Histopathological scoring. (D) TUNEL assay apoptosis%. Data are presented as the mean \pm S.E.M. ($n=6$ per group). $\star P<0.05$ compared with DS group; $\blacktriangle P<0.05$ compared with DI/R+melatonin group. Abbreviation: HE, Hematoxylin and Eosin.

Melatonin attenuates kidney dysfunction and oxidative stress in the kidney after ischemia AKI

Both diabetes or I/R injury aggravated kidney dysfunction and oxidative stress that could be reversed by melatonin treatment [50,51]. In our study, melatonin significantly decreased the BUN and Scr in DI/R+melatonin group compared with DI/R group and DI/R+melatonin+EX527 group ($P<0.05$; Figure 5A,B). EX527 pre-treatment abolished the positive effects on BUN and Scr elicited by melatonin ($P<0.05$; Figure 5A,B). We next examined the effect of melatonin on SOD and MDA levels. Compared with DI/R group and DI/R+melatonin+EX527 group, SOD activity was significantly increased in DI/R+melatonin, while the effect was abolished by EX527 in DI/R+melatonin+EX527 group ($P<0.05$; Figure 5C). The MDA production in the melatonin-treated group was significantly decreased, compared with DI/R group and DI/R+melatonin+EX527 group ($P<0.05$; Figure 5D). Meanwhile, the melatonin-induced reduction in MDA level was abolished by EX527 in DI/R+melatonin+EX527 group ($P<0.05$; Figure 5D).

Effects of melatonin on protein expression of SIRT1, Nrf2, and HO-1 in renal tissues

The activation of SIRT1 was observed in AKI [52]. We detected SIRT1 in all groups and found that protein expression of SIRT1 was significantly reduced in the DI/R group compared with the DS group ($P<0.05$; Figure 6A,C). Melatonin

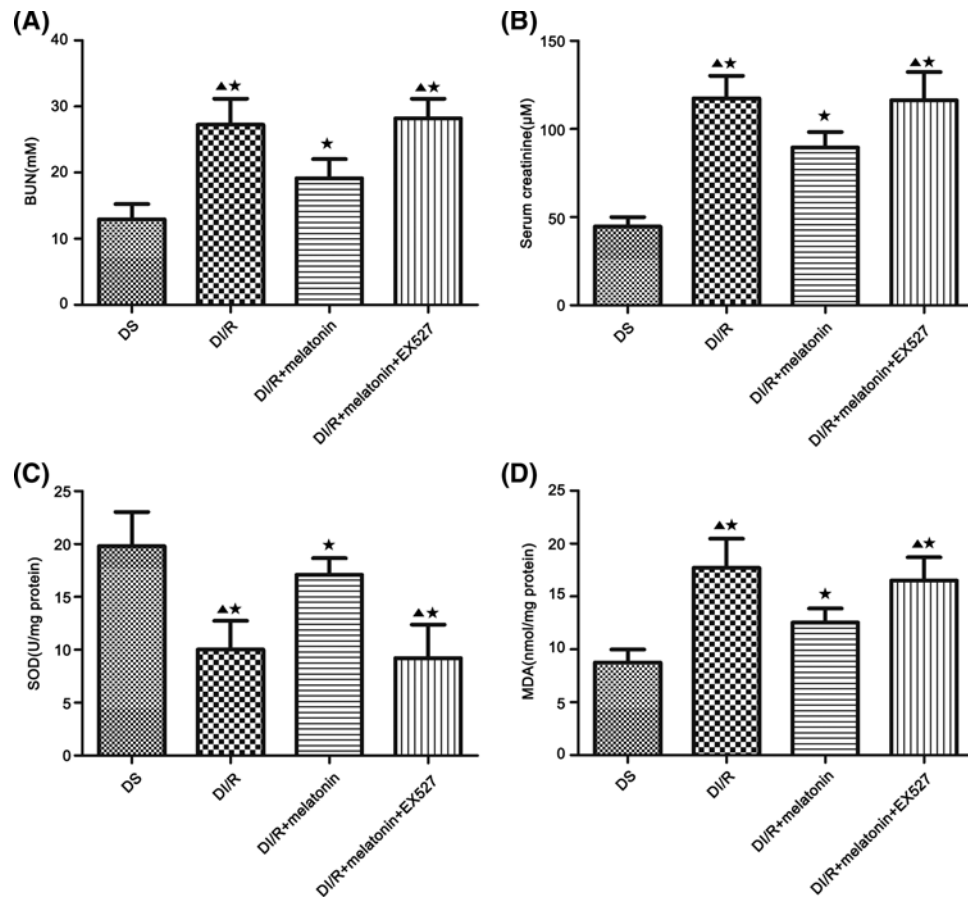


Figure 5. Melatonin pre-treatment attenuates kidney dysfunction and oxidative stress after I/R injury in diabetic rats (A) BUN. (B) Scr. (C) Kidney SOD contents. (D) MDA activity. Data are presented as the mean \pm S.E.M. ($n=6$ per group). $\star P<0.05$ compared with DS group; $\blacktriangle P<0.05$ compared with DI/R+melatonin group.

administration significantly increased SIRT1 expression in DI/R+melatonin group compared with the DI/R group, whereas EX527 eliminated this effect in the DI/R+melatonin+EX527 group ($P<0.05$; Figure 6A,C). There was no significant difference in the expression of SIRT1 between the DI/R group and the DI/R+melatonin+EX527 group ($P>0.05$; Figure 6A,C). The protective effects of melatonin in I/R injury are relative to antioxidative stress rather than the pro-inflammatory cytokines [21,22]. Meanwhile melatonin attenuates cisplatin-induced nephrotoxicity by increasing the expression of Nrf2 and HO-1 [53]. We then examined the protein expression of Nrf2 and HO-1 after AKI, and the expression of Nrf2 and HO-1 was decreased in the DI/R group compared with the DS group ($P<0.05$; Figure 6A,B,D). The levels of Nrf2 and HO-1 in DI/R+melatonin group were significantly increased compared with the DI/R group, respectively ($P<0.05$; Figure 6A,B,D). However, the use of EX527 in the DI/R+melatonin+EX527 group abolished the increased expression of Nrf2 and HO-1 compared with DI/R+melatonin group ($P<0.05$; Figure 6A,B,D). There was no significant difference in the expression of Nrf2 and HO-1 between the DI/R group and the DI/R+melatonin+EX527 group ($P>0.05$; Figure 6A,B,D).

Discussion

Diabetes is a potential risk factor for increasing AKI and mortality/morbidity of AKI [54]. One of the major risk factors for AKI is the I/R injury. Ischemia-induced AKI after kidney transplant surgery, secondary to I/R injury, is a major factor affecting both short-term and long-term grafts and patient survival [55]. Previous studies have shown that increased diabetic kidney I/R susceptibility correlates with oxidative stress and nitrification stress [56]. A recent study showed that diabetic rats undergoing I/R can increase apoptosis, BUN, and Scr levels, and the decreased tolerance to I/R in diabetic rats may be associated with increased pro-inflammatory cytokines [7]. Consistent with previous studies, we found that diabetes aggravated renal I/R injury through the pathological changes in renal tubules.

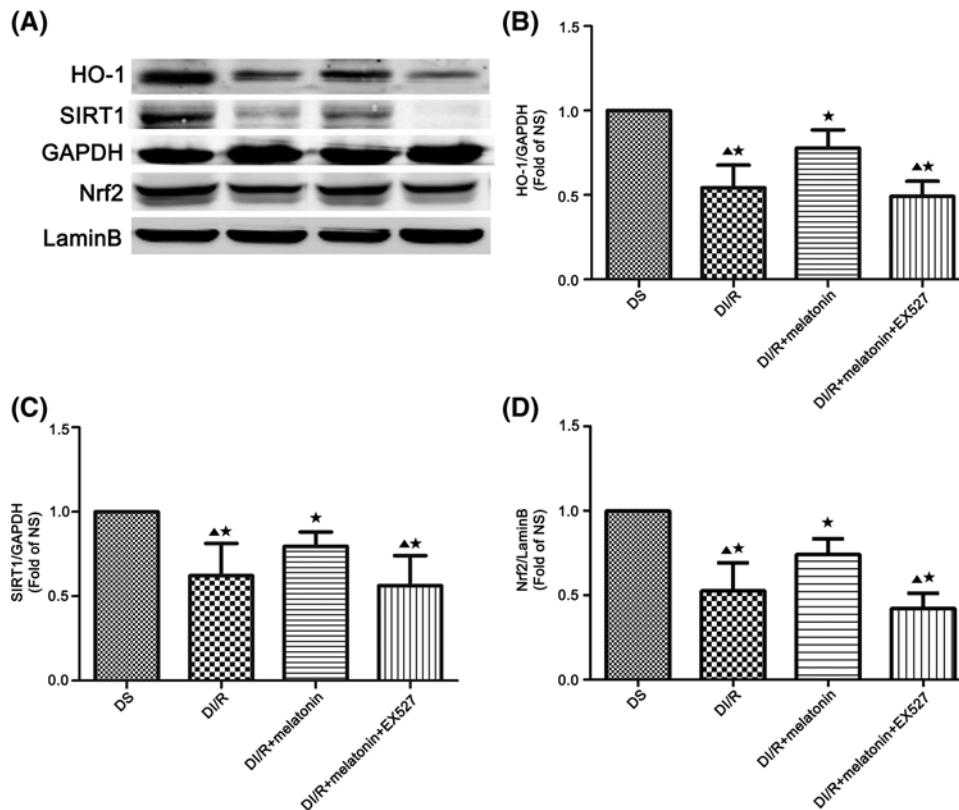


Figure 6. Melatonin pre-treatment up-regulates SIRT1, Nrf2, and HO-1 expression after kidney I/R injury in diabetic rats
 (A) Western blotting showed protein levels of SIRT1, Nrf2, and HO-1. (B–D) Quantitation of Western blot data from (A). Data are presented as the mean \pm S.E.M. ($n=6$ per group). ★ $P<0.05$ compared with DS group; ▲ $P<0.05$ compared with DI/R+melatonin group.

Meanwhile, diabetes dramatically increased I/R-induced cell apoptosis [6]. As known previously, I/R induced kidney dysfunction and on this basis, diabetes remarkably exacerbated the damage in kidney function reflected in the higher levels of BUN and Scr. In addition, the change of SOD, an indicator of antioxidant, was lower in both NI/R and DI/R groups, especially in DI/R group, and MDA, as an indicator of oxidative stress that was higher in both NI/R and DI/R groups, especially in DI/R group, we speculated that all the above effects are caused by enhanced oxidative stress and diabetes further aggravated it on the basis of I/R injury. Our findings are consistent with previous studies [6,7].

Previous studies have verified that SIRT1, an NAD⁺-dependent histone deacetylase, plays a positive role in type 2 diabetes mellitus (T2DM) with the function of anti-oxidation and anti-inflammation [57]. Meanwhile the activation of SIRT1 promoted Nrf2 nuclear translocation and anti-oxidation in the situation of diabetic MI/R injury [58]. And activating Nrf2/HO-1 pathway alleviates renal I/R injury in diabetic rats [59]. Our results showed that, in the condition of diabetic AKI, the expression of SIRT1 was decreased, and Nrf2/HO-1 pathway was inhibited. However, the Nrf2/HO-1 pathway was activated by melatonin through activating SIRT1.

Melatonin is anti-inflammatory, antioxidative, and reduces endoplasmic reticulum stress in the AKI [60–62]. The previous study has reported that melatonin reduces kidney injury by reducing lipid oxidation and NO production in STZ-induced diabetic rats [15]. In our research, we found melatonin ameliorated the histopathological scoring, alleviated apoptotic index, reduced the levels of BUN and Scr, decreased MDA, and increased SOD in DI/R+melatonin group, while this effect was eliminated by EX527, a SIRT1 inhibitor. However, the mechanism of melatonin treatment to reduce AKI in diabetes has not been reported yet. In the present study, we provided evidence that diabetic rats exhibited enhanced kidney histological damage, increased kidney apoptosis, aggravated kidney dysfunction, and elevated oxidative stress after AKI injury. However, melatonin administration attenuated these unfavorable results. Previous studies have shown that the melatonin protects the function of kidney and brain by effecting SIRT1, as well as in diabetic heart [33,63–65]. Our study showed that treatment with melatonin, restores the expression of SIRT1 that could further enhance expression of Nrf2 and HO-1, compared with the DI/R group. While EX527 reversed the

changes of Nrf2 and HO-1 induced by SIRT1, suggesting melatonin could protect kidney against I/R injury in diabetes by up-regulating the expression of the SIRT1 protein, which then consequently activates Nrf2 and induce HO-1 expression.

A study has reported that activating SIRT1 could stabilize the transcription factor Nrf2 by its deacetylation [66]. Up-regulating the SIRT1-Nrf2 signaling pathway can reduce oxidative stress and inflammation [67]. Another study in experimental traumatic brain injury reported that melatonin can affect the expression of NRF2 and HO-1, but its mechanism needs further investigation [68]. However, a recent study confirmed that melatonin attenuates lipopolysaccharide-induced oxidative stress in rat brain by activating the SIRT1/Nrf2 signaling pathway [69]. In our study, we found that diabetes aggravate kidney apoptosis, kidney dysfunction, and oxidative stress after AKI injury. Melatonin reduced the kidney damage caused by diabetes and I/R injury, however, EX527 abolished the protective effect of melatonin. We further investigated how melatonin and EX527 influence the diabetic kidney that underwent I/R injury and found that melatonin could change the amount of Nrf2 and HO-1 through influencing the expression of SIRT1, at the same time EX527 blocking the activation of Nrf2/HO-1 pathway by inhibiting the SIRT1 expression. According to our findings, we suggest that melatonin protects diabetes kidney by activating SIRT1/Nrf2/HO-1 pathway.

Conclusion

Taken together, our findings indicate that hyperglycemia-induced oxidative stress is involved in impaired SIRT1/Nrf2/HO-1 signaling and ischemia AKI in diabetes. Inhibition of oxidative stress with melatonin attenuates ischemia AKI in diabetes by improving the SIRT1/Nrf2/HO-1 signaling. Melatonin attenuates apoptosis and oxidative stress in diabetes ischemia AKI through activation of the SIRT1/Nrf2/HO-1 pathway. Moreover, its beneficial effects on heart and brain in diabetes may make melatonin a potential therapeutic drug especially under the condition of I/R. In addition, SIRT1/Nrf2/HO-1 pathway could be a new target in decreasing the oxidative stress in diabetic I/R injury.

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Competing interests

The authors declare that there are no competing interests associated with the manuscript.

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Author contribution

S.S. designed and executed the experiments, analyzed data, and drafted the paper. Z.X. designed the study and proofread the paper. C.T. and S.L. analyzed data, and drafted the paper. K.W. executed the experiments and analyzed the data.

Abbreviations

AKI, acute kidney injury; Akt, Protein Kinase B; ARE, antioxidant response elements; BUN, blood urea nitrogen; DI/R, diabetic rats ischemia/reperfusion; DKI, diabetic kidney injury; DN, diabetic nephropathy; DS, diabetic rats sham-operated; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GSK-3 β , Glycogen synthase kinase-3 beta; HO-1, heme oxygenase-1; I/R, ischemia/reperfusion; iNOS, nitric oxide synthase; MDA, malondialdehyde; NF-kBp65, nuclear factor-kappa B p65; NI/R, non-diabetic rats ischemia/reperfusion; NQO1, NADPH Quinone oxidoreductase 1; Nrf2, nuclear factor erythroid 2-related factor 2; NS, nondiabetic rats sham-operated; POD, peroxidase; ROS, reactive oxygen species; Scr, serum creatinine; SIRT1, silent information regulator 2 associated protein 1; SOD, superoxide dismutase; STZ, streptozotocin; TUNEL, terminal-deoxynucleotidyl transferase mediated nick end labeling; VDAC, Voltage-dependent anion channel.

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