

Honokiol protects against renal ischemia/reperfusion injury via the suppression of oxidative stress, iNOS, inflammation and STAT3 in rats

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Abstract. Honokiol is the predominant active ingredient in the commonly used traditional Chinese medicine, Magnolia, which has been confirmed in previous studies to exhibit anti-oxidation, antimicrobial, antitumor and other pharmacological effects. However, its effects on renal ischemia/reperfusion injury (IRI) remain to be elucidated. The present study aimed to examine the effects of honokiol on renal IRI, and to investigate its potential protective mechanisms in the heart. Male adult Wistar albino rats were induced into a renal IRI model. Subsequently, the levels of serum creatinine, blood urea nitrogen (BUN), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), and the levels of serum nitrite and the kidney nitrite were examined in the IRI group. The levels of oxidative stress, inducible nitric oxide synthase (iNOS), inflammatory factors and caspase-3 were evaluated using a series of commercially available kits. The levels of phosphorylated signal transducer and activator of transcription 3 (p-STAT3) and the protein expression levels of STAT3 were determined using western blotting. Pretreatment with honokiol significantly reduced the levels of serum creatinine, BUN, ALT, AST and ALP, and the level of nitrite in the kidney of the IRI group, compared with the control group. The levels of malondialdehyde, the activity of myeloperoxidase, and the gene expression and activity of iNOS were reduced in the IRI rats, compared with the sham-operated rats, whereas the levels of superoxide dismutase and catalase were increased following treatment with honokiol in the IRI rats. In addition, the expression levels of tumor necrosis factor- α and interleukin-6 in the IRI rats were increased by honokiol. Treatment with honokiol suppressed the protein expression levels of p-STAT3 and caspase-3 in

the IRI rats. These findings indicated that honokiol protects against renal IRI via the suppression of oxidative stress, iNOS, inflammation and STAT3 in the rat.

Introduction

Investigations in previous years have shown that the predominant cause of acute renal failure is closely associated with renal ischemia/reperfusion injury (IRI), as ischemia-reperfusion can lead to renal vasoconstriction, tubular obstruction, anti-leakage of glomerular filtrate and decreased glomerular filtration rate, leading to impaired renal function, and conditions of shock, heart failure and requirement for kidney transplant. This is often accompanied by renal ischemia and reperfusion, which affect treatment outcomes (1,2). Therefore, reducing or avoiding IRI is one of the areas in renal protection, which has received significant attention.

The generation of excessive reactive oxygen species disrupts normal redox homeostasis of renal tissue, causing a state of oxidative stress in renal tissues (1). Previous studies have demonstrated that, in sustained diabetes, states of hyperglycemia and oxidative stress *in vivo*, and non-enzymatic glycation (glycosylation) reactions are evident, which are important in the pathogenesis of diabetes and nephropathy (3-6). Jin *et al* reported that C-type natriuretic peptide ameliorates IRI-induced acute kidney injury through the inhibition of oxidative stress (7).

The physiological concentration of nitric oxide (NO) is involved in the functional regulation of several vital organisms under normal circumstances, and the pathological induction of renal IRI leads to a significant increase in NO synthesis (8). NO at high concentrations reacts rapidly in the peroxide microenvironment at the site of injury to produce peroxynitrite ion (ONOO⁻), directly or indirectly leading to the damage of target cells and tissues (9). Inflammatory reactions can markedly promote IRI (10). Certain reports have suggested that IRI is a process of inflammation, although this is debated, and reflects the importance of IRI in inflammatory reactions (11). A cascade network, comprising reactive oxygen species, a substantial number of NO generated by inducible nitric oxide synthase (iNOS) and inflammatory reactions is an important mechanism of IRI (12). A previous study demonstrated that inhibiting the expression of lipopolysaccharide-induced iNOS synthase and inflammation reduces the content of NO in rats

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with acute myocardial ischemia (13). A previous study demonstrated that the development of IRI is characterized by the activation of the signal transducer and activator of transcription (STAT) pathway, which is involved in signaling associated with various cytokines and growth factors (14).

Magnolia officinalis is widely used in traditional Chinese and in Japanese Kampo medicines, which are used clinically to treat bacterial infections, inflammation and gastrointestinal diseases (15). Since 1973, two of the predominant active compounds in *Magnolia officinalis* have been isolated, magnolol and its isomer honokiol (Fig. 1), and have been investigated in several studies (16-19). Previous studies have demonstrated that honokiol has pharmacological functions, including central muscle relaxation, central nervous system inhibition, anti-inflammatory, antibacterial, anti-ulcer, anti-oxidative and anticancer properties, and hormone regulation (20-23). Therefore, the present study aimed to evaluate the protective effects of honokiol against IRI and examine its possible mechanism.

Materials and methods

Reagents and kits. Serum creatinine, blood urea nitrogen (BUN) and nitrite commercial kits were purchased from BioAssay Systems (Hayward, CA, USA). Malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), myeloperoxidase (MPO), tumor necrosis factor- α (TNF- α) and interleukin (IL)-6 commercial kits were purchased from Jiancheng Bioengineering Institute (Nanjing, China). TRIzol was purchased from Invitrogen (Thermo Fisher Scientific, Inc., Waltham, MA, USA). SYBR Green I dye was purchased from Qiagen GmbH (Hilden, Germany). The BIOER Linegene-3320 system was purchased from Hangzhou Bioer Technology, (Hangzhou, China). The Bicinchoninic Acid (BCA) assay kit was purchased from Thermo Fisher Scientific, Inc.

Animals. Male adult Wistar albino rats, weighing 250-300 g, were provided by the Animal Experimental Center of the Navy General Hospital of Chinese PLA and maintained at a room temperature of $23\pm 2^{\circ}\text{C}$, with a 12 h light-dark cycle, and were allowed 1 week to acclimatize to the conditions with free access to water and food. The present study was approved by the Experimental Animal Research Committee of the Navy General Hospital of Chinese PLA (Beijing, China) and the ethics committee of the Navy General Hospital of Chinese PLA (Beijing, China).

Induction of renal IRI and experimental protocol. The Wistar rats were used to perform the renal ischemia/reperfusion surgery, as described previously (24,25). Briefly, the rats were anesthetized by intraperitoneal (i.p.) injection of 10 mg/kg xylazine (Jiangsu Biological Technology, Co., Ltd. Jiangsu, China) and 100 mg/kg of ketamine hydrochloride (Sigma-Aldrich, St. Louis, MO, USA). The site of surgery (abdomen) was shaved and swabbed with betadine solution (Beyotime Institute of Biotechnology, Jiangsu, China) and ethanol. Particular care was taken to avoid damage to the organs throughout. A single medial incision was made, the kidney vasculature was exposed and rats were subjected to renal IRI injury by placing a clamp on the vessels for 45 min. In the sham operation group, surgery

was performed, but the kidneys were not clamped. After 45 min, the clamp was removed and blood flow perfused into the kidneys. At this stage, the animals exhibiting insufficient restoration of blood flow or with vessel damage were excluded from the experiment.

The rats were randomly allocated into four groups: (1) Sham group (n=10), in which the normal rats received physiological saline (i.p.); (2) sham+honokiol group (Sham+Hon; n=10), in which normal rats received 5 mg/kg, i.p. honokiol; (3) IRI group (IRI; n=10), in which the IRI model rats received physiological saline (i.p.); (4) IRI+honokiol group (IRI+Hon; n=10), in which the IRI model rats received 5 mg/kg, i.p. honokiol.

Assessment of renal function. Rats were sacrificed by decapitation and the blood samples were collected from the rats at the room temperature. The samples were centrifuged at $12,000 \times g$ for 10 min, following which the supernatants were collected. The levels of serum creatinine and BUN in the samples were measured using commercially available kits, according to the manufacturer's protocol (BioAssay Systems). Nitrite levels were measured using a colorimetric assay kit, according to the manufacturer's protocol (BioAssay Systems).

Assessment of hepatic function. Rats were anesthetized with ketamine (75 mg/kg; Sigma-Aldrich) and xylazine (5 mg/kg; Sigma-Aldrich) and blood samples were collected from the eye socket at room temperature. The samples were centrifuged at $12,000 g$ for 10 min, following which the supernatants were collected. The levels of alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured using an autoanalyzer (Pars Azmun, Karaj, Iran).

Assessment of oxidative stress. Rats were sacrificed by decapitation and the areas of ischemia in the renal tissues were collected at room temperature and homogenised using liquid nitrogen and lysed in radioimmunoprecipitation assay bugger (Jiancheng Bioengineering Institute). The samples were centrifuged at $12,000 \times g$ for 10 min, following which the supernatants were collected. The levels of MDA and SOD and the activities of CAT and MPO in the renal ischemic zone of the tissues were measured using colorimetric assay kits (Jiancheng Bioengineering Institute), according to the manufacturer's protocol.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis of iNOS. The ischemic zone tissue samples were collected at room temperature and homogenised using liquid nitrogen and lysed in radioimmunoprecipitation assay bugger (Jiancheng Bioengineering Institute). The samples were centrifuged at $12,000 g$ for 10 min, following which the supernatants were collected. Total RNA ($1 \mu\text{g}$) was isolated from the supernatants using TRIzol, according to the manufacturer's protocol (Invitrogen; Thermo Fisher Scientific, Inc.). cDNA was obtained following the RT reaction using SYBR Green I dye (Qiagen GmbH). qPCR amplifications were performed using a BIOER Linegene-3320 system (Hangzhou Bioer Technology). The sequences of the primers used were as follows: iNOS, forward 5'-AGTGATGGCAAGCACGAC TTC-3' and reverse 5'-TCTGTCACTCGCTCACCACGG-3';

and β -actin, forward 5'-AAGGGACTTCCTGTAACAATGCA-3' and reverse 5'-CTGGAACGGTGAAGGTGACA-3'. The cycling conditions were as follows: 5 min at 95°C, 40 cycles of 30 sec at 95°C, 45 sec at 60°C, and 30 sec at 72°C, followed by a cycle of 10 min at 72°C. The expression levels was quantified by Ct value: $Ct = -1/\lg(1+Ex) * \lg X0 + \lg N/\lg(1+Ex)$ (26,27).

Assessment of iNOS activity. The ischemic zone tissue samples were collected at room temperature and homogenised using liquid nitrogen and lysed in radioimmunoprecipitation assay bugger (Jiancheng Bioengineering Institute). The samples were centrifuged at 12,000 g for 10 min and the supernatants were collected. The renal ischemic zone tissues were homogenized and nuclear proteins were quantified using a BCA assay (Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. The supernatant was incubated with 0.6 ml reaction buffer (containing 210 mM sucrose, 40 mM NaCl, 2 mM EGTA and 30 mM HEPES; Beyotime Institute of Biotechnology) and 1 mmol/l ethylene glycol tetraacetic acid (Amresco LLC, Solon, OH, USA) at room temperature for 30 min. The activity of iNOS was then determined at 530 nm using a TECH M200 microplate reader (Tecan Group, Ltd., Männedorf, Switzerland).

Assessment of inflammatory cytokines. The ischemic zone tissue samples were collected at room temperature and homogenised using liquid nitrogen and lysed in radioimmunoprecipitation assay buffer (Jiancheng Bioengineering Institute). The samples were centrifuged at 12,000 g for 10 min and the supernatants were collected. The levels of TNF- α and IL-6 in the were measured using a colorimetric assay kit, according to the manufacturer's protocol (Jiancheng Bioengineering Institute).

Western blot analysis of the protein expression of STAT3. The ischemic zone tissue samples were collected at room temperature. The samples were centrifuged at 12,000 g for 10 min and the supernatants were collected. The renal ischemic zone tissues were homogenized, and nuclear proteins were extracted using a BCA assay (Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. The proteins (50 μ g) were electrophoresed on 12% SDS-polyacrylamide gels (Jiangsu Biological Technology, Co., Ltd.) and transferred into nitrocellulose membranes (Abcam, Cambridge, UK) at 4°C for 2 h. The membranes were blocked with Tris-buffered saline-0.05% Tween 20 (TBST) containing 5% skim milk powder for 1 h at room temperature. Following blocking, the membranes were incubated with rabbit polyclonal anti-phosphorylated (p-)STAT3 (sc-135649; 1:1,500; Santa Cruz Biotechnology, Inc., Dallas, TX, USA), rabbit polyclonal anti-STAT3 (sc-7179; 1:1,000; Santa Cruz Biotechnology, Inc.) and rabbit polyclonal anti- β -actin (sc-7210; 1:500; Santa Cruz Biotechnology, Inc.) overnight at 4°C with agitation. Subsequently, the membranes were washed with TBST for 1 h at room temperature and incubated with goat anti-rabbit IgG-PerCP-Cy5.5 secondary antibody (sc-45101; 1:1,000; Santa Cruz Biotechnology, Inc.). Finally, the membranes were visualized using an enhanced chemiluminescence system (Pierce Biotechnology, Rockford, IL, USA). Bands were exposed in a ChemiDoc-It TS2 imager

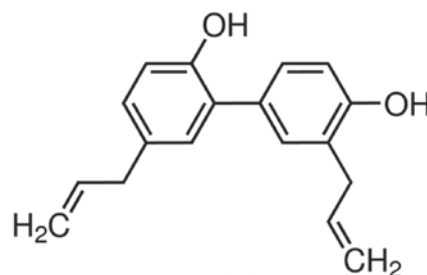


Figure 1. Chemical structure of honokiol.

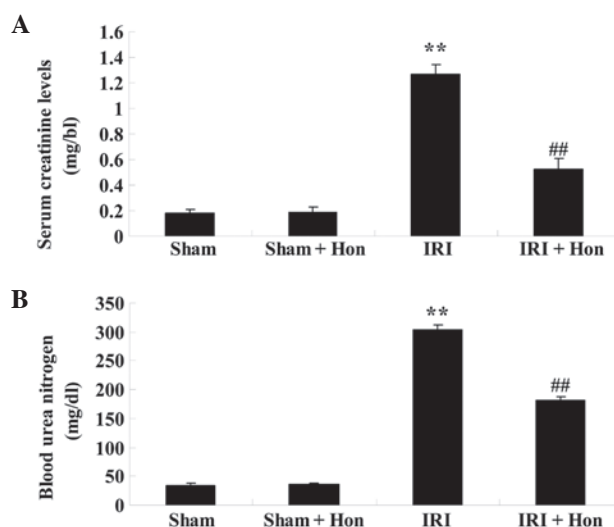


Figure 2. Effect of honokiol on renal function. Effect of honokiol on the expression of (A) serum creatinine and (B) blood urea nitrogen. Data are presented as the mean \pm standard error of the mean. ** $P < 0.01$, compared with the Sham group; ## $P < 0.01$, compared with the IRI group. Hon, honokiol treatment (5 mg/kg); Sham, sham surgery; IRI, ischemia/reperfusion injury.

Imager (UVP, LLC, Upland, CA, USA) and analyzed using Image J version 2 software (National Institutes of Health, Bethesda, MD, USA).

Assessment of caspase-3 activity. The ischemic zone tissue samples were collected at room temperature. The samples were centrifuged at 12,000 g for 10 min and the supernatants were collected. The renal ischemic zone tissues were homogenized and nuclear proteins were extracted using a BCA assay (Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. Equal quantities of protein (30 μ g) and Ac-LEHD-pNA (Beyotime Institute of Biotechnology) were incubated at 37°C for 2 h in the dark, following which the activity of caspase-3 was measured using a SpectraMax M2 Microplate Autoreader (Bio-Tek Instruments Inc., Winooski, VT, USA) at an absorbance of 405 nm.

Statistical analysis. All statistical data are presented as the mean \pm standard error of the mean, and statistical analyses were performed using SPSS software, version 17.0 (SPSS, Inc., Chicago, IL, USA). Statistical analysis was conducted with three or more groups using one-way analysis of variance and Dunnett's test. $P < 0.05$ was considered to indicate a statistically significant difference.

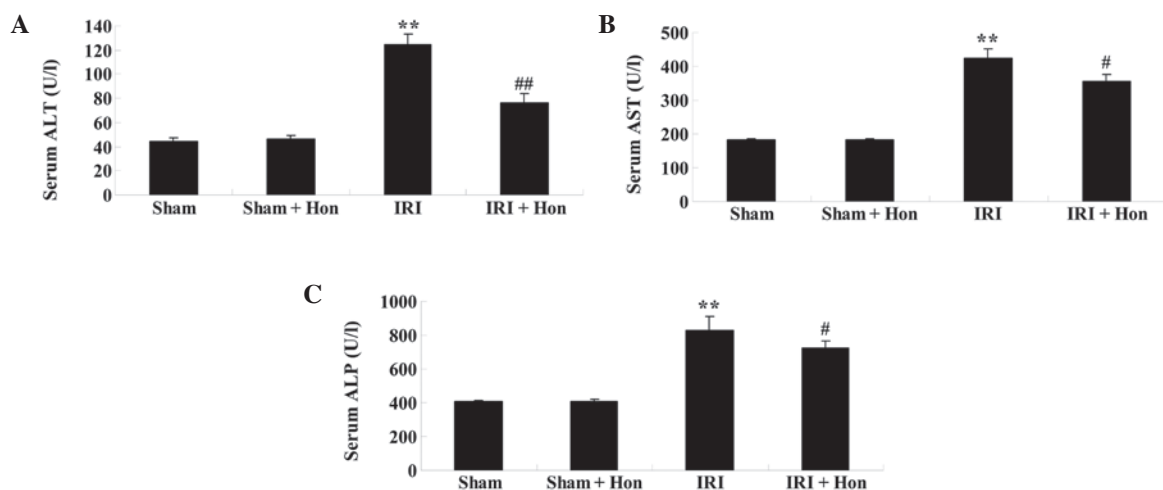


Figure 3. Effect of honokiol on hepatic function. Effect of honokiol on the serum levels of (A) ALT, (B) AST and (C) ALP. Data are presented as the mean \pm standard error of the mean. ** P <0.01, compared with Sham group; ## P <0.01, compared with IRI group. Sham, sham surgery; Hon, honokiol treatment (5 mg/kg); IRI, ischemia/reperfusion-injury group. ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase.

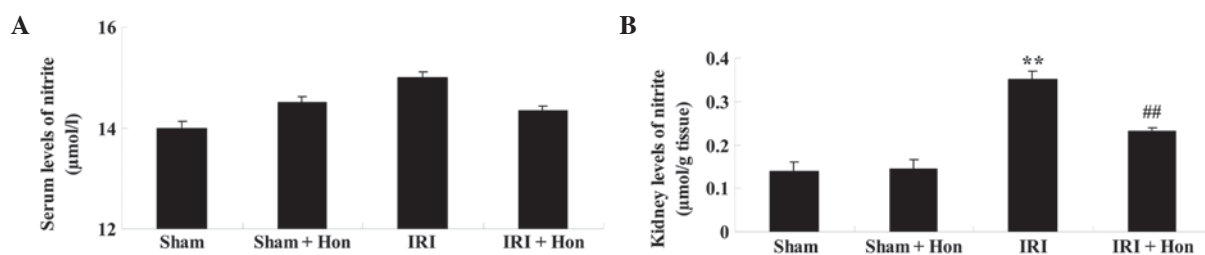


Figure 4. Effect of honokiol on serum and kidney levels of nitrite. Effect of honokiol on the levels of nitrite in the (A) serum and (B) kidney. Data are presented as the mean \pm standard error of the mean. ** P <0.01, compared with the Sham group; ## P <0.01, compared with the IRI group. Sham, sham surgery; Hon, honokiol treatment (5 mg/kg); IRI, ischemia/reperfusion-injury.

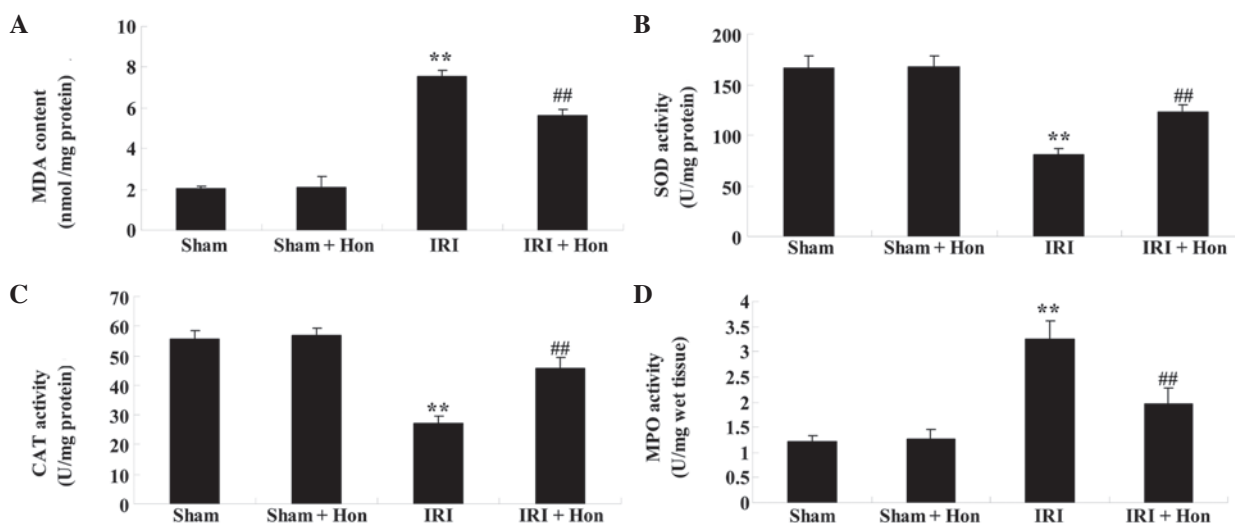


Figure 5. Effect of honokiol on oxidative stress. Effect of honokiol on the (A) serum levels of MDA and activities of (B) SOD, (C) CAT and (D) MPO. Data are presented as the mean \pm standard error of the mean. ** P <0.01, compared with the Sham group; ## P <0.01, compared with the IRI group. Sham, sham surgery; Hon, honokiol treatment (5 mg/kg); IRI, ischemia/reperfusion-injury MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase; MPO, myeloperoxidase.

Results

Effect of honokiol on renal function. The levels of serum creatinine and BUN in the IRI group were significantly increased,

compared with those in the sham group (Fig. 2A and B). The elevation in the levels of serum creatinine and BUN in the IRI rats were reduced significantly with honokiol pretreatment in the IRI+Hon group, compared with the IRI group (Fig. 2A and B).

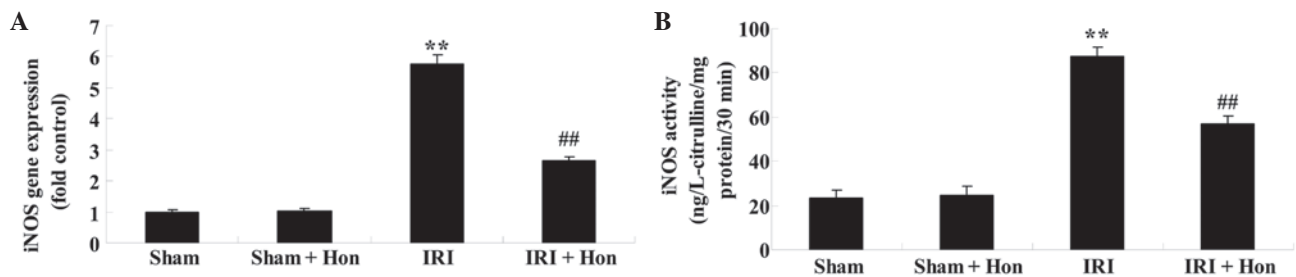


Figure 6. Effect of honokiol on the levels of iNOS. Effect of honokiol on the (A) gene expression of iNOS and the (B) activity of iNOS activity. Data are presented as the mean \pm standard error of the mean. ** $P < 0.01$, compared with the Sham group; ## $P < 0.01$, compared with the IRI group. Sham, sham surgery; Hon, honokiol treatment (5 mg/kg); IRI, ischemia/reperfusion-injury; iNOS, inducible nitric oxide synthase.

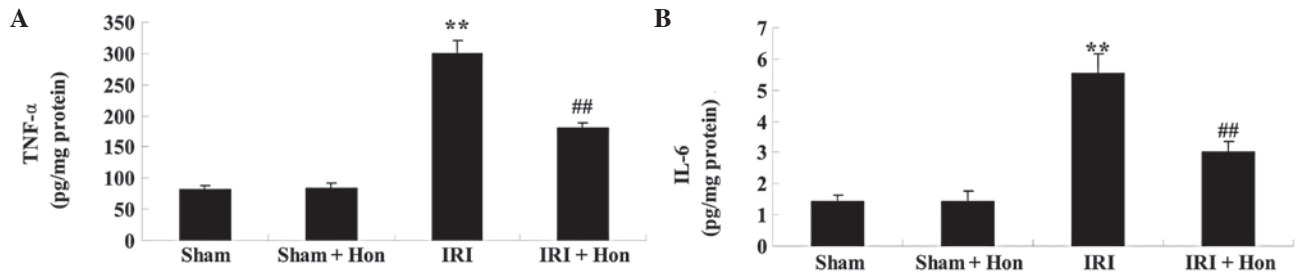


Figure 7. Effect of honokiol on inflammatory cytokines. Effect of honokiol on the levels of (A) TNF- α and (B) IL-6. Data are presented as the mean \pm standard error of the mean. ** $P < 0.01$, compared with the Sham group; ## $P < 0.01$, compared with the IRI group. Sham, sham surgery; Hon, honokiol treatment (5 mg/kg); IRI, ischemia/reperfusion-injury; TNF- α , tumor necrosis factor- α ; IL-6, interleukin-6.

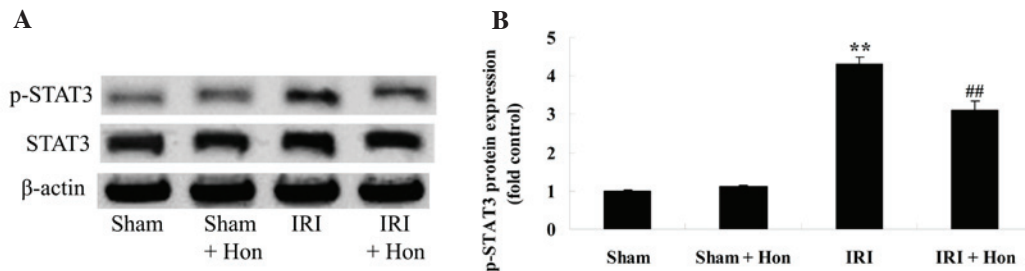


Figure 8. Effect of honokiol on the protein expression of STAT3. (A) Effect of honokiol on the protein expression of STAT3 was determined using western blot analysis (B) Statistical analysis of the quantification of the protein expression levels of STAT3. Data are presented as the mean \pm standard error of the mean. ** $P < 0.01$, compared with the Sham group; ## $P < 0.01$, compared with the IRI group. Sham, sham surgery; Hon, honokiol treatment (5 mg/kg); IRI, ischemia/reperfusion-injury; STAT3, signal transducer and activator of transcription 3; p-, phosphorylated.

Effect of honokiol on hepatic function. As shown in Fig. 3A-C, the serum levels of ALT, AST and ALP were elevated following IRI, compared with the respective levels in the sham-operated group. The administration of honokiol prior to IRI was observed to significantly reduce the serum levels of ALT, AST and ALP, compared with the levels in the IRI group (Fig. 3A-C).

Honokiol decreases the levels of nitrite in the kidney. As shown in Fig. 4A, the level of serum nitrite in the IRI group was higher, compared with the levels in the Sham group, Sham+Hon group and IRI+Hon group. However, the differences identified between the groups were not statistically significant (Fig. 4A). The level of nitrite in the kidney of the IRI group was increased significantly, compared with that in the sham group (Fig. 4B). Honokiol administration prior to IRI led to a decrease in kidney nitrite levels, compared with the IRI group (Fig. 4B).

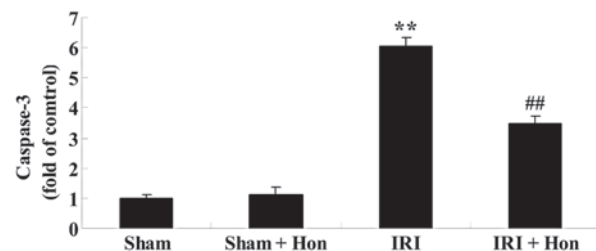


Figure 9. Effect of honokiol on the levels of caspase-3. Data are presented as the mean \pm standard error of the mean. ** $P < 0.01$, compared with the Sham group; ## $P < 0.01$, compared with the IRI group. Sham, sham surgery; Hon, honokiol treatment (5 mg/kg); IRI, ischemia/reperfusion-injury.

Honokiol reduces levels of oxidative stress. IRI caused a significant increase in the serum level of MDA and activity of MPO, compared with the sham group, and honokiol treatment decreased the levels of MDA and activity of MPO,

compared with the IRI group (Fig. 5A). The activities of SOD and CAT decreased in the IRI group, compared with the sham group (Fig. 5B and C). However, the administration of honokiol significantly increased the activities of SOD and CAT, compared with the IRI group (Fig. 5B and C). Honokiol treatment decreased the activity of MPO, compared with the IRI group (Fig. 5D).

Honokiol reduces the expression and activity of iNOS. The expression and activity levels of iNOS in the IRI group were significantly augmented, compared with those in the Sham group, exhibiting significant increases (Fig. 6A and B). By contrast, treatment with honokiol prior to IRI caused a significant reduction in the expression and activity of iNOS, compared with the IRI group (Fig. 6A and B).

Honokiol reduces levels of inflammatory cytokines. The levels of TNF- α and IL-6 in the IRI group increased significantly, compared with those in the Sham group (Fig. 7A and B). These elevated levels of TNF- α and IL-6 in the IRI rats were reduced significantly by honokiol pretreatment (Fig. 7A and B).

Honokiol decreases the protein expression of STAT3. In order to elucidate the mechanistic basis of the effects of honokiol in the IRI rats, the protein expression levels of STAT3 were measured using western blotting. The results suggested that the protein expression of p-STAT3 was promoted in the IRI group, compared with the Sham group (Fig. 8A and B). The increased protein expression of p-STAT3 by IRI was reversed in the rats pretreated with honokiol (Fig. 8A and B).

Honokiol reduces levels of caspase-3. The results of the present study showed that increased activity of caspase-3 was observed in the IRI group, compared with the Sham group (Fig. 9). In addition, honokiol administration prior to IRI significantly reduced the levels of caspase-3 levels, compared with the levels observed in the IRI group (Fig. 9).

Discussion

The kidney is an organ with a high level of perfusion, and is particularly sensitive to ischemia and reperfusion. When ischemia persists for a certain duration, following which perfusion recovers, organizational structural and functional recovery may be impaired and kidney dysfunction and structural damage may be aggravated, which is known as IRI (28). Following shock, heart failure, cardiopulmonary bypass or kidney transplantation, IRI usually affects the treatment outcome, particularly in kidney transplant recipients, in which IRI is one of the causes of surgical failure. At present, the pathogenesis of IRI remains to be fully elucidated. In the present study, honokiol pretreatment reduced the levels of serum creatinine, BUN, ALT, AST and ALP, and the levels of nitrite in the kidneys of IRI rats. Therefore, honokiol may be a potential drug for treatment following IRI.

Oxidative stress is one of the important pathogenetic mechanisms of IRI. In ischemia, a high level of ATP is decomposed, leading to the accumulation of hypoxanthine, recovery of blood perfusion causes the generation of a substantial number of reactive oxygen free radicals, and peroxy radicals

and their degradation products cause tissue damage by lipid peroxidation of mitochondria and lipid membranes (29). In the present study, honokiol treatment depressed serum levels of MDA and MPO, and increased the activities of SOD and CAT in the IRI rats. Hsu *et al* reported that honokiol protected against heatstroke in diabetic rats through reducing inflammation and oxidative stress (30).

NO in the body is generated by NOS, and the level of NO is closely associated with the level and activity of NOS. In normal kidney tissues, endothelial NOS is predominantly expressed in the renal blood vessels and capillaries; neuronal NOS is predominantly expressed in the juxtaglomerular macula densa; and iNOS is expressed in the renal medullary thick ascending limb, proximal tubule, distal convoluted tubule and interlobular arteries, arcuate arteries and blood vessels, and other areas of the glomerulus (31). Under a pathophysiological state, iNOS exhibits high levels of expression in mesangial cells, epithelial cells, smooth muscle cells and renal tubular epithelial cells, and there are high levels of infiltrated inflammatory cells, including in the glomeruli and renal interstitium (32). The present study showed that honokiol significantly decreased the gene expression and activity of iNOS in the IRI rats. Previous studies have suggested that honokiol prevents the inflammatory response and the expression of iNOS in human osteoarthritis chondrocytes (33), and the level of iNOS is attenuated by honokiol in septic rats (34).

Inflammatory reactions, including a series of complex pathological processes, develop and interconnect with each other, which can be roughly divided into the following four processes: Leukocyte activation, chemotaxis, leukocyte-endothelial cell adhesion and migration. In IRI, polymorphonuclear cells within the kidney or proximal tubule, activating factors, including bradykinin, histamine, leukotrienes and platelet-activating factors, which are generated by mesangial cells, pro-inflammatory cytokines, including TNF- α , IL-1 β , IL-6, and monocyte chemoattractant protein-1, macrophage inflammatory protein-2, interferon-inducible protein 10 and other chemokines, lead to leukocyte activation and subsequent chemotaxis to the site of injury (35). Subsequently, following interaction with vascular endothelial cells, the leukocytes roll and then adhere closely to the skin layer in the endoderm of blood vessels, migrating into the skin layer and ultimately penetrating the endoderm to reach the extravascular tissue where it exerts its effects (36). Inflammatory cells directly damage cells following reperfusion by the release of oxidase and hydrolytic enzymes, and adhered neutrophils block the capillary bed, which further increases the circulatory disorder. The present study showed that honokiol significantly reduced the levels of TNF- α and IL-6 in the IRI rats. Chiang *et al* demonstrated that honokiol protects against eccentric exercise-induced skeletal muscle damage by inhibiting oxidative stress and inflammation in rats (37), and Munroe *et al* indicated that the anti-inflammatory effects of honokiol decreased the levels of TNF- α and IL-6 in a mouse model of allergic asthma (38).

The STAT pathway is important in cytokine signaling, and STAT transcription factors exist in the cytoplasm during the resting state, which can be activated by cytokines, growth factors, and reactive oxygen species, as intracellular signal transduction proteins and transcription factors. Once

phosphorylated by Janus kinase, STATs form homologous or heterologous dimers, which are then translocated into the nucleus and combine with DNA, initiating gene transcription. Ishikawa *et al* reported that honokiol induces cell cycle arrest and apoptosis through inhibiting the DNA binding of nuclear factor- κ B and STAT3 (39). Previous studies have suggested that the STAT signaling pathway and IRI have a specific association. IRI can produce large quantities of reactive oxygen species, and when it exceeds the processing ability of antioxidant enzymes, reactive oxygen species accumulate, leading to excessive oxidative stress and cell damage, and the direct activation of STAT3 (40). In conditions of hypoxia without reperfusion, ATP may not be sufficient to make STAT3 fully phosphorylated; however, following reperfusion, ATP levels rise again, and STAT3 can be activated to a maximum degree by phosphorylation (41). In the present study, honokiol suppressed the protein expression of p-STAT3 in the IRI rats. Yu *et al* suggested that honokiol exerts pro-apoptotic effects on transformed Barrett's cells through inhibition of STAT3 (42). Luan *et al* reported that honokiol induces cell cycle arrest and apoptosis through inhibiting the DNA binding of nuclear factor- κ B and STAT3 (43). In the present study, honokiol administration significantly decreased the levels of caspase-3 in the IRI rats. Weng *et al* suggested that honokiol attenuates the severity of acute pancreatitis and lung injury through suppression of apoptosis and caspase-3 activity (44). Honokiol also inhibits the activation of caspase-3 and caspase-9 in H₂O₂-induced apoptosis in human lens epithelial cells (45).

In conclusion, the present study demonstrated the protect effect of honokiol on renal IRI through the suppression of oxidative stress, iNOS, inflammation and STAT3 in the rats. These results may be of potential clinical relevance, and the protective effect of honokiol as a clinical therapeutic strategy may be of value in the future.

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