Chinese Journal of Integrative Medicine
Available online at link.springer.com/journal/11655
Journal homepage: www.cjim.cn/zxyjhen/zxyjhen/ch/index.aspx
E-mail: cjim_en@cjim.cn

Original Article

Protective Effects and Mechanisms of Shenhua Tablet (肾华片) on Toll-Like Receptors in Rat Model of Renal Ischemia-Reperfusion Injury*

LI Qing-ping, WEI Ri-bao, YANG Xi, ZHENG Xiao-yong, SU Ting-yu, HUANG Meng-jie, YIN Zhong, and CHEN Xiang-mei

ABSTRACT **Objective:** To investigate the protective effects and potential mechanisms of Shenhua Tablet (肾华片, SHT) on the toll-like receptors (TLRs)-mediated signaling pathways in a rat model of kidney ischemia-reperfusion injury (IRI). **Methods:** Sixty male Wistar rats were randomly divided into 5 groups: sham surgery, model control, astragaloside (150 mg·kg^{-1}·d^{-1}), low- and high-dose SHT (1.5 and 3.0 g·kg^{-1}·d^{-1}, repectively) groups. One week after drug treatment, rats underwent surgery to establish the IRI models. At 24 h and 72 h after the modeling, serum creatinine (Scr) and blood urea nitrogen (BUN) were analyzed; pathological damage were scored after periodic acid-Schiff staining. TLR2, TLR4 and myeloid differentiation factor 88 (MyD88) protein and mRNA expressions were detected by inmmunohistochemistry, Western blot and qPCR. Tumor necrosis factor α (TNF-α) and interleukin-6 (IL-6) protein expressions were detected by enzyme linked immunosorbent assay. **Results:** Compared with the sham group, the model group exhibited severe change in renal function (Scr: 189.42±21.50, P<0.05), pathological damage (damage score: 4.50±0.55, P<0.05), and the expression levels of TLR2, TLR4, MyD88, TNF-α, IL-6 were significantly higher than other groups. Meanwhile, the levels of TLRs in model group showed upward tendency from 24 to 72 h, unparalleled with pathological and functional changes. The aforementioned parameters were alleviated to a certain extent, and, in addition to TLRs, presented the obvious downward trending from the 24 to 72 h after the intervention in the SHT and astragaloside groups relative to the model (P<0.05); in particular, the most significant mitigation of these changes was observed in the SHT-H group (P<0.05). **Conclusions:** TLRs may be an important spot to treat and research in acute kidney injury. SHT could effectively mitigate renal injuries and promote recovery of IRI injuries through suppression of degeneration induced by up-regulation of TLR2 and TLR4 expression levels in the MyD88-dependent signaling pathway and exhibit some dose dependence.

KEYWORDS Shenhua Tablet, Chinese medicine, kidney injury, toll-like receptors, myeloid differentiation factor 88, ischemia-reperfusion injury

Acute kidney injury (AKI) mediated by renal ischemia-reperfusion injury (IRI) often takes place in clinical works. As the major cause of AKI, renal IRI is associated with high morbidity.[1,2] The exact molecular mechanisms underlying renal IRI is not fully researched clearly, point of view on research involve inflammation, oxidative stress, necrosis, and so on.[3] However, the important role of the immune-inflammatory response to IRI has been widely understood. In particular, many studies have examined the relationship between IRI and toll-like receptors (TLRs), which are inflammatory receptors activating the innate immune response and onset of necrosis in the pathogenesis of renal IRI.[4-8] These investigations have provided approaches to clinical interventions in AKI.

Shenhua Tablet (肾华片, SHT) is a traditional Chinese drug that has been used clinically for many years in People's Liberation Army (PLA) General Hospital. It has won a national invention patent (ZL No. 03146504.8). Our previous studies have demonstrated that hundreds IgA nephropathy patients

*Supported by the National Sciences Foundation of China (Nos. 81273968, 81471027 and 81401160), Ministerial Project of the National Working Commission on Aging (No. QLB2014W002), and the Four Hundred Project of Chinese People's Liberation Army (PLA) General Hospital (No. YS201408)
Department of Nephrology, Chinese PLA General Hospital, Chinese PLA Institute of Nephrology, State Key Laboratory of Kidney Diseases, National Clinical Research Center for Kidney Diseases, Beijing Key Laboratory of Kidney Disease Research, Beijing (100853), China
Correspondence to: Prof. WEI Ri-bao, Tel: 86-10-55499133, Fax: 86-10-88626068, E-mail: wrbbj2006@126.com
DOI: 10.1007/s11655-017-2756-6

Chin J Integr Med 2019 Jan;25(1):37-44

obtained clinically proteinuria reduction, protection of renal function and alleviation of glomerulosclerosis by receiving treatment of SHT.[9,10] Furthermore, SHT can produce effect of remission in rats renal IRI though increasing reduced levels of Na^+-K^+-ATPase.[11] This study will utilized a rat kidney model of IRI to further investigate the association between the extent of renal tissue damage and the immune-inflammatory response as well as the protective mechanisms of SHT for renal IRI, particularly with respect to the effects of SHT on the regulation mechanisms of the inflammatory receptors TLR2 and TLR4.

METHODS

Drugs and Reagents Preparation

SHT (0.6 g/tablet, with each tablet having 3.095 g of crude drug, composed of *Radix Astragali*, *Rhizoma Atractylodis Macrocephalae*, *Rhizoma Curcumae*, *Fructus Ligustri Lucidi*, *Flos Lonicerae*, *Radix Paeoniae Alba* and *Rhizoma Sparganii*) were supplied by the SZYY Group Pharmaceutical Limited, China (lot No. 13122011). Astragaloside was provided by Shaanxi Sciphar Biotechnology, Co., Ltd., China. TLR2, TLR4, and myeloid differentiation factor 88 (MyD88) quantitative polymerase chain reaction (qPCR) kits were purchased from QuantoBio (China). The TLR2, MyD88 polyclonal antibody (rabbit anti-mouse) and TLR4 monoclonal antibody (mouse anti-rat) were bought from Abcam (HKSP, N.T. Hong Kong, China). Sodium citrate buffer (50 mmol/L, pH 4.5) was prepared for dilution. A Roche C-8000 automatic biochemical analyzer (USA), the microscopic image acquisition and analysis system (Model OlympusIX51 and FV1000, Japan), an ABI 7900HT high-throughput real-time fluorescence qPCR system (USA) were also utilized in this study.

Animal Model of IRI

A total of 60 adult male Wistar rats (6–8 months of age, with weights of 180–200 g) were purchased from Vital River Laboratory Animal Technology Co., Ltd., China, with certificate No. SCXK (Beijing) 2014-0008. After 1 week of acclimation in a specific pathogen-free animal room of the Experimental Animal Center of the PLA General Hospital, all animal experiments were conducted in accordance with the protocols and recommendations approved by the Ethics Committee for Animal Experimentation of PLA General Hospital and the National Institutes of Health Guide to the Care and Use of Laboratory Animals. The rats were randomly allocated to 5 groups by a random number table (12 in each group): sham, model, astragaloside, low- and high-dose SHT (SHT-L and SHT-H) groups. Drugs were administered by gavage for 7 days prior to surgery; in particular, 150 $mg \cdot kg^{-1} \cdot d^{-1}$ astragaloside, 1.5 and 3.0 $g \cdot kg^{-1} \cdot d^{-1}$ SHTs (3 and 6 times clinical dosage, respectively) were dissolved (1–1.5 mL/d) and fed to the rats of the astragaloside, SHT-L, and SHT-H groups respectively, and an equal volume of CMC-Na was put to use by gavage to the rats of the sham and model groups.

The IRI model was established in the model, astragaloside, SHT-L, and SHT-H groups, using methods detailed in the previously published literature.[12,13] In brief, firstly the left kidney of rats were resected, and then the contralateral renal pedicle was clipped by a noninvasive vascular clamp, with clamp removed after 30 min. The sham group went under a dissection of the renal vessels and a left nephrectomy.

After the operation, mental status, activity, food feeding, weight, and the occurrence of mortality were observed for each rat. At postoperative 24 and 72 h, 6 randomly selected rats from each of groups were sacrificed. Blood was drawn from the abdominal aorta for evaluations of renal function and levels of inflammatory markers. In addition, renal tissue specimens were preserved for light microscopy, and immunohistochemistry analyses. Portions of these tissue specimens were preserved at –80 ℃ for Western blot and qPCR assays.

Biochemical Indicator

An automatic biochemical analyzer was utilized to measure serum creatinine (Scr) and blood urea nitrogen (BUN) levels in samples of blood at postoperative 24 and 72 h.

Renal Pathological Examination

Periodic acid-Schiff (PAS) staining was applied to obtain light microscopic observations of the histopathological changes in renal tissue samples of rats of each experimental group. Histopathological observations were scored by two pathologists in accordance with criteria described in extant literature.[7,14] In particular, the extent of injury was based on observations of tubular dilatation, casts formation, brush border loss, degeneration and necrosis of epithelial cells. A 5-point marking system was used: 0 (normal tissue without damage),

1 (≤10%), 2 (11%–25%), 3 (26%–45%), 4 (46%–75%), and 5 (≥76%). Each section was assessed by at least 10 randomly selected, non-overlapping fields of view (at a magnification of ×200).

Immunohistochemistry Analysis

Paraffin sections of renal tissue were stained with using the avidin-biotin complex (ABC) method to examine TLR2, TLR4, and MyD88 expressions. These sections were manually analyzed with an Olympus IX51 biomedical image analysis system. A total of 10 fields of view were randomly chosen at high magnification (200×) for the study. The positively stained cells were counted in each field, and the intensity of positive expression was assessed by calculating the average number of positively stained cells per field.

Western Bloting

Western blotting was used to assess TLR2, TLR4, and MyD88 expression levels in renal tissue. For each sample, approximately 100 μL renal tissue was added into 300 μL radio immunoprecipitation (RIPA) tissue lysis buffer. This tissue was homogenized by sonication and then centrifuged at 12,000 r/min for 30 min at 4 ℃. The protein concentration of the resulting supernatant of this solution was assessed using a bicinchoninic acid (BCA) protein assay kit. Protein from tissue samples was then separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) with a 10% polyacrylamide gel, then transferred to a membrane. The proteins on the membrane were subjected to Ponceau staining and blocked with 5% non-fat dry milk for 1 h of room temperature. Primary antibodies were added to the blocking solution to 1:1000 ratio, and the membrane was incubated overnight at 4 ℃. Subsequently, the membrane was rinsed with Tris-HCl, NaCl, Tween-20 (TBST) and incubated with secondary antibodies for 1 h at room temperature. The membrane was washed with TBST, and signals to this membrane were detected via chemiluminescence, using β-actin as an internal reference. The Quantity One software package was used to analyze the TLR2/β-actin, TLR4/β-actin, and MYD88/β-actin grayscale signal ratios from the resulting film.

qPCR Test

TRIzol reagent was used for total RNA extraction from approximately 200 μg of cryopreserved rat renal tissue. Samples were assessed using a NanoDrop 2000C spectrophotometer and by agarose gel electrophoresis (150 V, 20 min); these qualities controls assessments indicated that RNA extraction was successful in all samples. The sequences of the primers used for qPCR amplification are presented in Table 1. The amplification conditions were 40 cycles of 95 ℃ for 5 min, 95 ℃ for 15 s, 58 ℃ for 15 s, and 60 ℃ for 15 s. An ABI7900HT (Applied Biosystems, USA) thermocycler was utilized for the qPCR amplifications. The mRNA expression level of each target gene was calculated with the $2^{-\triangle\triangle Ct}$ method, using GAPDH mRNA as the reference to standardization of the results from various samples.[15]

Enzyme Linked Immunosorbent Assay Examination

Tumor necrosis factor α (TNF-α) and interleukin-6 (IL-6) expression levels were detected by enzyme linked immunosorbent assay (ELISA). TNF-α and IL-6 levels were measured by double antibody sandwich method kit. The operation was performed according to kit, measured absorbance in the 450 nm wavelength with an enzyme-labeled instrument (optical density value, OD), a standard curve to calculated the concentrations of TNF-α and IL-6.

Statistical Analysis

The SPSS (version 18.0) software package was utilized for statistical analysis. All data were expressed as mean ± standard deviation ($\bar{x} \pm s$).

Table 1. Sequences of Primers for qPCR Reaction System

mRNA	Primer	Sequence	Primer length	PCR product length	Tm	GC (%)
My-D88	F	GAGCAGTGTCCCACAGACAA	20	98	59.89	55.00
	R	AGTAGCAGATGAAGGCGTCG	20	98	59.9	55.00
TLR4	F	ATGCCTCTCTTGCATCTGGC	20	94	60.47	55.00
	R	GAAGTACCTCTATGCAGGGATTCA	24	94	59.65	45.83
TLR2	F	GAGGTCTCCAGGTCAAATCTCA	22	97	59.17	50.00
	R	GCAGAATGGCCTTCCCTTGA	20	97	60.32	55.00

Chin J Integr Med 2019 Jan;25(1):37-44

The Kolmogorov-Smirnov test was used to assess normality. Groups were compared using one-way analysis of variance (ANOVA), and Levene's test was used to examine the homogeneity of variances. The Student-Newman-Keuls (SNK)-*q* test was applied to compare pairs of groups. $P<0.05$ was regarded as statistically significant.

RESULTS

Effects of SHT on Renal Function and Biochemical Indicators in Blood

In the sham group, the postoperative Scr level was steady ($P>0.05$). In the model group, Scr and BUN levels at 24 h were significantly higher than the sham group ($P<0.05$). At the same time, each of these targets was lower in the astragaloside, SHT-L, and SHT-H groups, respectively ($P<0.05$). The reduction of indices of renal function from 24 to 72 h was significantly greater in the SHT-H group than in the SHT-L or astragaloside groups ($P<0.05$); however, the astragaloside and SHT-L groups did not differ significantly compared with each other (Figure 1).

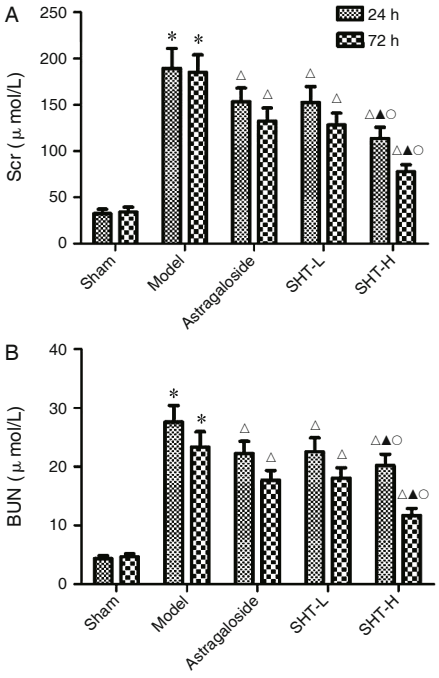

Figure 1. Comparison of Scr (A) and BUN (B) Levels in Rats among Groups

Notes: $^{*}P<0.05$ vs. shame group at the same time; $^{\triangle}P<0.05$ *vs*. model group at the same time; $^{\blacktriangle}P<0.05$ *vs*. astragaloside groups at the same time; $^{\bigcirc}P<0.05$ *vs*. SHT-L group at the same time; the same below

SHT Reduces Tubular Injury to Rats with IRI and Improves Renal Pathological Scores

There were no decided morphological changes in the tissues of the sham group after IRI. In comparison to the sham group, the most serious pathological damage was presented in the model group at 24 and 72 h after surgery ($P<0.05$), which included widespread tubular epithelial vacuolization, brush border losing, a large number of protein casts of dilated tubules, and tubular architecture lesion. The astragaloside, SHT-L and SHT-H groups damaged lighter than the model group ($P<0.05$). At 72 h after the surgery, the damage reduced in each group, and the pathological improvement were significant in the three drug intervention groups as compared with the model group ($P<0.05$). The SHT-H group had the best effects of restoring and, through the scores, the situation in each group can be found in Table 2 and Figure 2 ($P<0.05$).

Table 2. Comparison of Tubular Damage Score among Groups (Score, $\bar{x} \pm s$)

Group	*n*	Tubular damage score	
		24 h	72 h
Sham	6	0.33 ± 0.52	0.50 ± 0.55
Model	6	$4.50 \pm 0.55^{*}$	$4.00 \pm 0.63^{*}$
Astragaloside	6	$3.83 \pm 0.75^{\triangle}$	$2.83 \pm 0.75^{\triangle}$
SHT-L	6	$3.50 \pm 0.55^{\triangle}$	$2.67 \pm 0.52^{\triangle}$
SHT-H	6	$2.83 \pm 0.75^{\triangle\blacktriangle\bigcirc}$	$2.00 \pm 0.63^{\triangle\blacktriangle\bigcirc}$

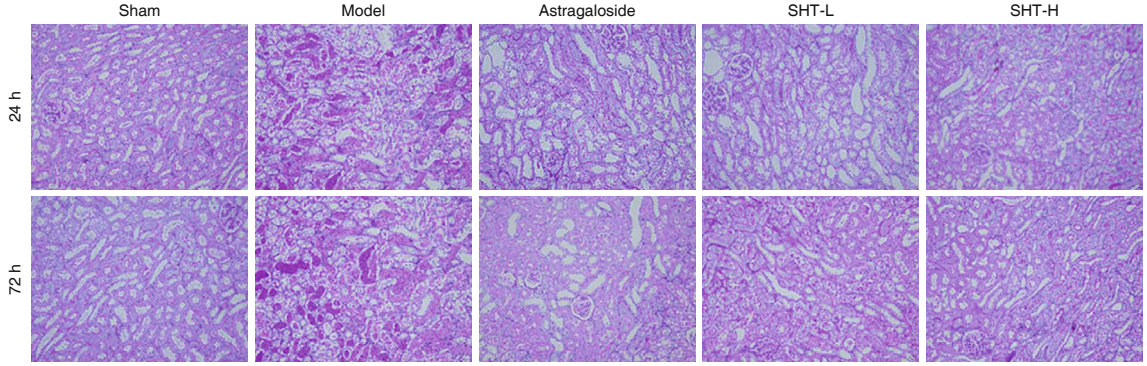

Figure 2. Periodic Acid-Schiff-Stained Tissues of Rat Kidney (× 200)

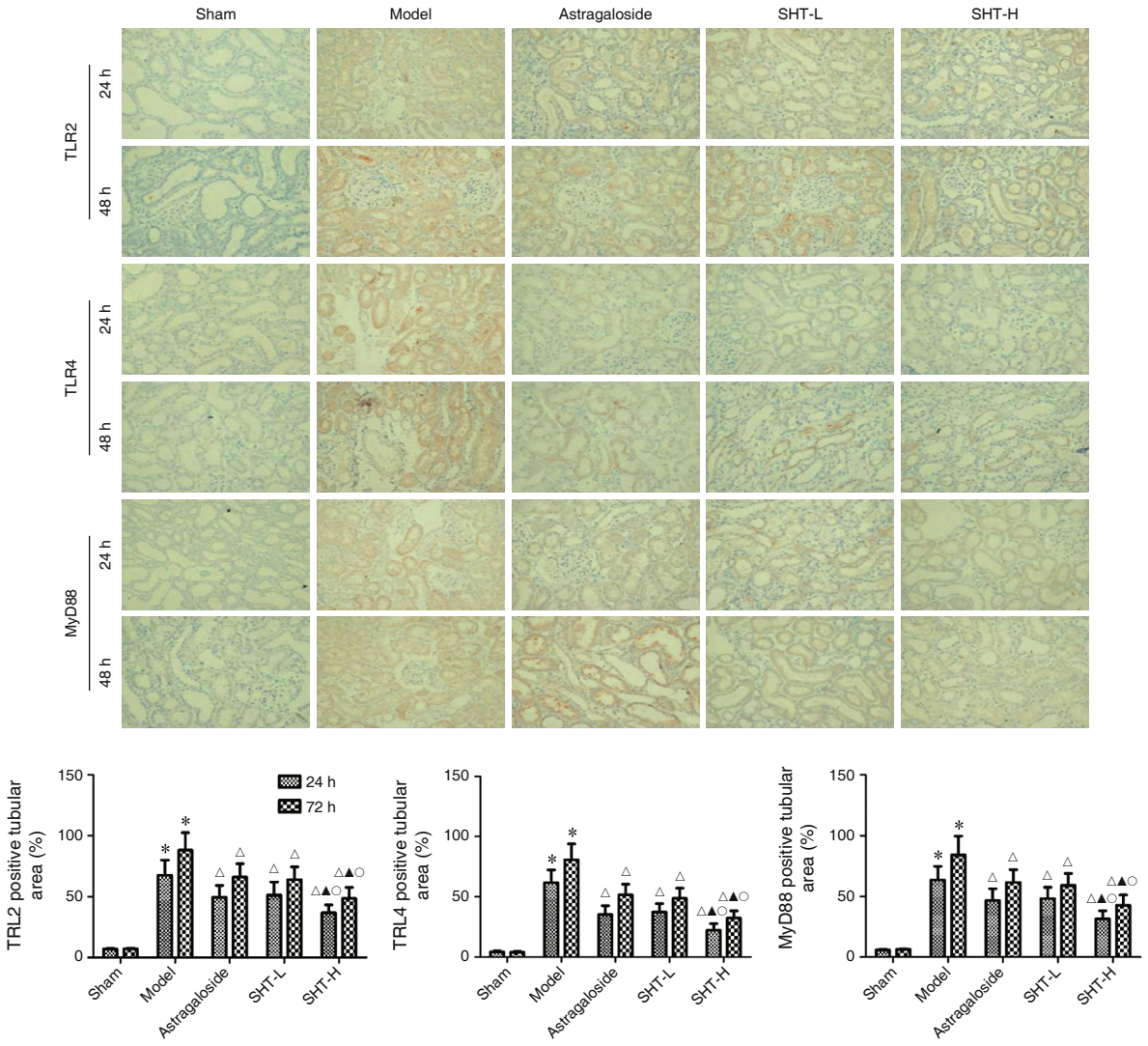

Figure 3. Ratio of Positive Tubular with Immunolocalization Staining in Rats after IRI

SHT Down-Regulates TLRs and MyD88 and Reduces TNF-α and IL-6 of Rats with IRI

Hardly any expression of TLR2, TLR4, and MyD88 was observed in renal tissue of the sham group by immunohistochemical staining. Among other groups, the expression levels of TLR2, TLR4, and MyD88 were the highest in the model group. At 24 and 72 h postoperatively, significantly lower expression levels of TLR2, TLR4, and MyD88 were observed in the astragaloside, SHT-L and SHT-H groups than the model group (P<0.05); in addition, the lowest levels of mentioned factors were observed in the SHT-H group (P<0.05, Figure 3).

Western blot analyzed TLRs and MyD88 protein expression levels among groups, the extracts from the model group rats showed marked rise in the factors, with almost nonexistent in the sham group. The higher expression levels of these proteins were observed at postoperative 72 h than 24 h in each group. TLR2, TLR4, and MyD88 expression levels were lower in the astragaloside, SHT-L groups than the model group (P<0.05), while significantly lower in the SHT-H group than in others (P<0.05, Figure 4).

Examination of qPCR analyses revealed that the mRNA expression levels of TLR2, TLR4 and MyD88 were the lowest in the sham group and the highest in the model group. All the levels significantly reduced in the astragaloside, SHT-L and SHT-H groups than the model group (P<0.05), and rose slowly during the observation period, particularly lower in the SHT-H group than the astragaloside and SHT-L groups (P<0.05, Figure 5).

Except for the sham group, TNF-α and IL-6

Chin J Integr Med 2019 Jan;25(1):37-44

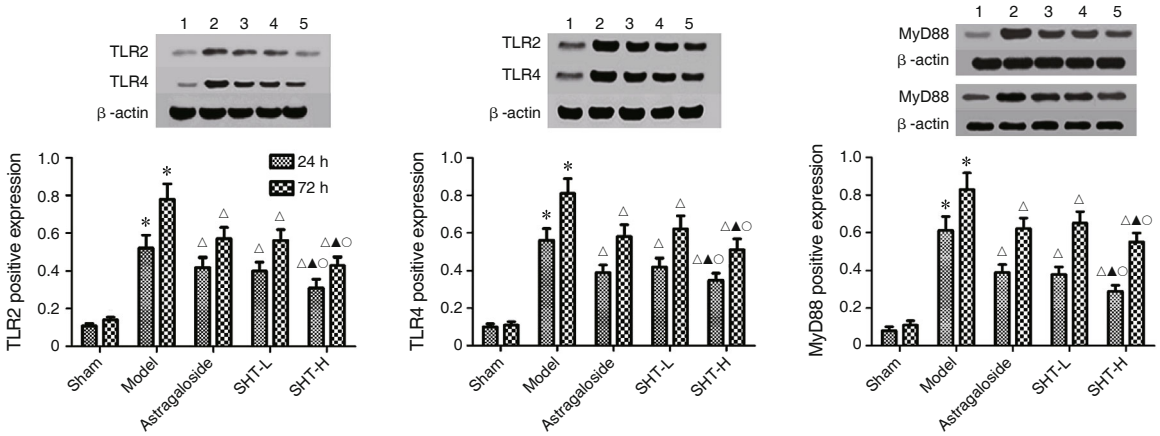

Figure 4. Comparison of TLR2, TLR4 and MyD88 Protein Expressions among Groups after IRI by Western Blot

Notes: 1: Sham; 2: model; 3: astragaloside; 4: SHT-L; 5: SHT-H

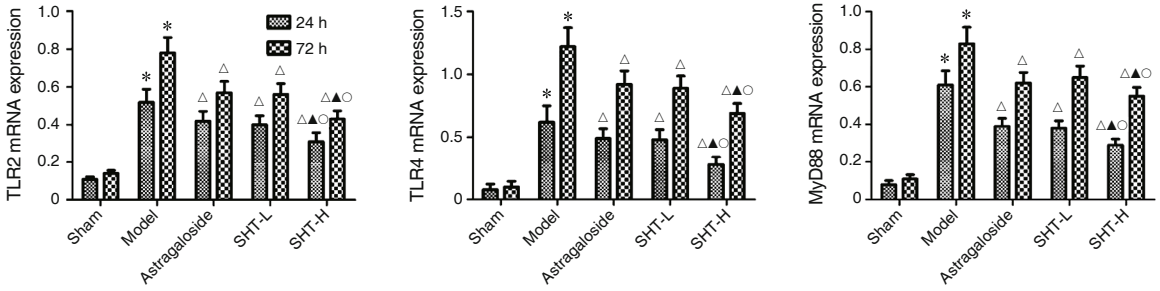

Figure 5. Comparison of TLR2, TLR4, MyD88 mRNA Expressions after IRI among Groups by qPCR Analysis

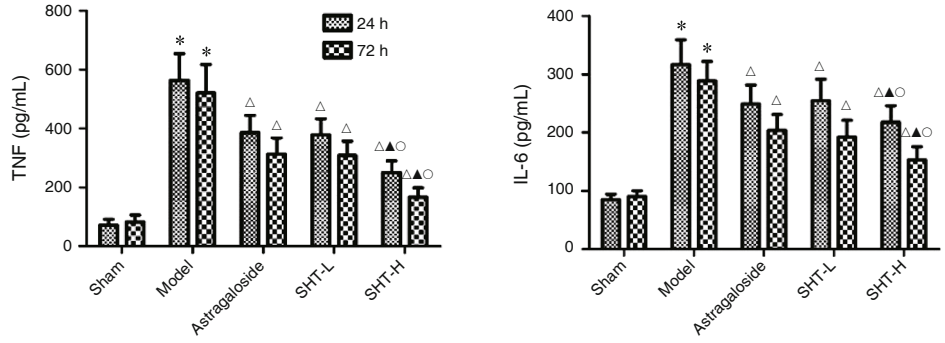

Figure 6. Comparison of TNF-α and IL-6 Expressions after IRI among Groups by ELISA

mRNA expression increased to the highest level in each group of 24 h after IRI and gradually declined at 72 h. The inflammation factors in the three treatment groups significantly manifested lower expression than that in the model group after IRI and presented different decreasing speed (P<0.05). The mRNA expressions of TLR2, TLR4 and MyD88 in the SHT-H group was the most obvious (P<0.05, Figure 6).

DISCUSSION

AKI, often arised from insults to ischemia, toxic substance and septicemia, is a frequently seen disease with a high morbidity and mortality, in particular, renal IRI is one of the primary causes of AKI.[16] The one of core mechanisms of IRI is the rapid activation to innate immune responses. The pathogenesis includes multiple mediators, such as inflammation reaction, reactive oxygen species, adhesion molecules/chemokines, and the activation of leukocytes and endothelial cells, above reasons lead to tubular damage, endothelial disordering, and inflammation responses.[17] Increasing evidence has suggested that ischemia-induced immune-inflammatory responses are primarily stimulated through the activation of TLRs. TLRs are type Ⅰ transmembrane receptors that serve as key sensors of tissue damages.[18] TLR2 and TLR4 mRNA are the primary TLRs expressed in tubular epithelial cells, and the expression levels of both of these receptors increase after IRI.[9] The activation of TLRs is

known to produce macrophage accumulation in mice during acute tubular necrosis induced by renal IRI.[19] TLRs act through two intracellular signal transduction pathways: the MyD88-dependent pathway and the MyD88-independent pathway. The MyD88-dependent pathway is the main pathway activated in renal injury.[7] It leads to subsequent downstream activation of complicated signal transduction pathways,[20] promote the transcript of cytokines and chemokines. A study considered this to be the dominant pathway mediating TLR-4-associated with kidney IRI since MyD88-/mice was protected against kidney dysfunction and therefore targeting the MyD88-dependent pathway would serve as an effective strategy against renal IRI.[8] Another experiment observed that MyD88 knockout mice exhibit less tubular damage. So, the TLRs can be an important spot for AKI.[21]

Recently, research about the efficacy of Chinese medicine has attracted increasing worldwide attention and stand a good chance to provide an innovative direction of the treatment.[22] One significant difference between Chinese medicine and Western medicine is that the active ingredients of Chinese medications produce multi-targeted therapeutic effects. SHT is composed of *Astragalus membranaceus* (Fisch.) Bge, *Ligustrum lucidum*, *Atractylodes macrocephala* koidz, *Rhizoma Curcumae*, and *Lonicera japonica*. Studies have reported that astragaloside can reduce damage to IRI in rat lungs.[23] Atractylodes polysaccharides have been shown to reduce IRI damage after liver autotransplantation in rats.[24] Our previous studies have demonstrated that proteinuria of some IgA nephropathy patients were clinically reduced, with renal function protected and glomerulosclerosis alleviated by SHT.[9,10] Furthermore, SHT can alleviate renal IRI though increasing reduced levels of Na^+-K^+-ATPase.[11]

In this study, firstly, we established a rat model of renal IRI by performing a unilateral nephrectomy combined with the clamping of the contralateral renal pedicle. Compared with the stable levels of the sham group, Scr in the model group was significantly elevated to postoperative 24 h, indicating the success of model establishment. Compared to 24 h, the parameters of renal function at 72 h showed a downward trend. Light microscopy indicated that the highest level of renal tissue pathological injury occurred at the same time with the renal function failure. This injury has slowly recovered in 72 h after the surgery. This result suggests that, after renal IRI, recovery from pathological tissue injury requires a longer duration than the renal function. Therefore, histological rehabilitation should be carefully concerned during therapeutic interventions in renal IRI. Meanwhile, the results of our study demonstrated that after IRI, the protein and mRNA expression levels of TLR2, TLR4, and MyD88 exhibited synchronized uptrend from postoperative 24 to 72 h. These results are consistent with the findings of extant literature,[4-8] however, not synchronized with the pathological and functional recovery. In the meantime, our study not only showed that TNF-α and IL-6 expressions were elevated at 24 h postoperatively but also observed the down-regulated secretion at 72 h after the establishment of renal IRI. The finding prompts that, besides the regulation to the inflammatory cytokines, TLRs may be associated with the mediation in pathological changes in renal IRI.

A series of findings by this suggests that astragaloside and SHT can reduce Scr and renal tissue pathological injury, meanwhile high doses of SHT present more effective protection for renal IRI than either low doses of SHT or a single dose of astragaloside. This result indicates that a Chinese medicinal compound with multiple components produced superior effects to a single Chinese medicinal agent, and that the observed effects exhibited a strong dose-response.

Expressions of the above TLRs and relevant parameters were the highest in the model group, significantly lower in the SHT-L and astragaloside groups especially in the SHT-H group. This result further confirmed the involvement of TLR2 and TLR4 in renal IRI through the MyD88-dependent pathway. Thus, it was observed that the SHT could significantly inhibit increases in TLRs expression and decrease the release of inflammatory factors through the MyD88-dependent pathway, thereby suppressing the immune-inflammatory response. SHT therefore plays a protective role in preventing AKI.

In conclusion, our study examined that SHT can protect renal function of ischemic AKI. The underlying mechanism for this protection may relate to the regulation of renal TLR2 and TLR4 expression in the MyD88-dependent signal transduction pathway. SHT could inhibit the production and release of the cytokines; this responsiveness would mitigate the

immune-inflammatory response produced by renal IRI, so diminishing IRI-related pathological damage of renal tissue and improving renal function failure. Thus, SHT plays a protective role in rats that had experienced IRI. For the clinical prevention and treatment of ischemic AKI, this study introduces a novel treatment approach and provides experimental evidence for the use of the approach.

Conflict of Interest

All authors declare that they have no competing interests. The funders are not involved in the experimental design, data collection and analysis, and manuscript preparation.

Author Contributions

All authors participated in the review of the manuscript. LQP, ZXY conducted the experiment and analyzed data, LQP wrote the manuscript. STY and YX acquired data. YZ guided the experiment. WRB guided the writing and revision of the paper. CXM provided advice for the experimental design.

REFERENCES

1. Li YW, Zhang Y, Zhang L, Li X, Yu JB, Zhang HT, et al. Protective effect of tea polyphenols on renal ischemia/reperfusion injury via suppressing the activation of TLR4/NF-κB p65 signal pathway. Gene 2014;542:46-51.
2. Moreth K, Frey H, Hubo M, Zengbrouwers J, Nastase MV, Hsieh TH, et al. Biglycan-triggered TLR-2- and TLR-4-signaling exacerbates the pathophysiology of ischemic acute kidney injury. Matrix Biol J Int Soc Matrix Biol 2014;35:143-151.
3. Kusch A, Hoff U, Bubalo G, Zhu Y, Fechner M, Schmidtullrich R, et al. Novel signalling mechanisms and targets in renal ischaemia and reperfusion injury. Acta Physiol 2013;208:25-40.
4. Wu H, Ma J, Wang P, Corpuz TM, Panchapakesan U, Wyburn KR, et al. Hmgb1 contributes to kidney ischemia reperfusion injury. J Am Society Nephrol Jasn 2010;21:1878-1890.
5. Friedewald JJ, Rabb H. Inflammatory cells in ischemic acute renal failure. Kidney Int 2004;66:486-491.
6. Kim BS, Lim SW, Li C, Kim JS, Sun BK, Ahn KO, et al. Ischemia-reperfusion injury activates innate immunity in rat kidneys. Transplantation 2005;79:1370-1377.
7. Wolfs TG, Buurman WA, Van SA, De VB, Daemen MA, Hiemstra PS, et al. *In vivo* expression of Toll-like receptor 2 and 4 by renal epithelial cells: IFN-gamma and TNF-alpha mediated up-regulation during inflammation. J Immunol 2002;168:1286-1293.
8. Wu H, Chen G, Wyburn KR, Yin J, Bertolino P, Eris JM, et al. TLR4 activation mediates kidney ischemia/reperfusion injury. J Clin Invest 2007;117:2847-2859.
9. Chen XM, Chen YP, Chen J. Multicentered, randomized, controlled clinical trial on patients with IgA nephropathy of qi-yin deficiency syndrome type. Chin J Integr Tradit West Med (Chin) 2007;27:101-105.
10. Li JJ, Chen XM, Gu Y, Wei RB, Du J, Shi SZ, et al. Effect of compound Shenhua Tablet on macrophage migration inhibition factor in renal tissue of 5/6 nephrectomized rats. Chin J Integr Tradit West Med (Chin) 2005;25:150-153.
11. Yang Y, Wei RB, Zheng XY, Qiu Q, Cui SY, Yin Z, et al. Effects of compound Shenhua Tablet, on renal tubular Na^+-K^+-ATPase in rats with acute ischemic reperfusion injury. Chin J Integr Med 2014;20:200-208.
12. Daemen MA, van de Ven MW, Heineman E, Buurman WA. Involvement of endogenous interleukin-10 and tumor necrosis factor-alpha in renal ischemia-reperfusion injury. Transplantation 1999;67:792-800.
13. Daemen MA, van Veer C, Wolfs TG, Buurman WA. Ischemia/reperfusion-induced IFN-gamma up-regulation: involvement of IL-12 and IL-18. J Immunol 1999;162:5506-5510.
14. Yamada K, Miwa T, Liu J, Nangaku M, Song WC. Critical protection from renal ischemia reperfusion injury by CD55 and CD59. J Immunol 2004;172:3869-3875.
15. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\triangle\triangle Ct}$ method. Methods 2001;25:402-408.
16. Devarajan P. Update on mechanisms of ischemic acute kidney injury. J Am Soc Nephrol Jasn 2006;17:1503-1520.
17. Yano T, Nozaki Y, Kinoshita K, Hino S, Hirooka Y, Niki K, et al. The pathological role of IL-18Rα in renal ischemia/reperfusion injury. Lab Invest 2015;95:78-91.
18. Shigeoka AA, Holscher TD, King AJ, Hall FW, Kiosses WB, Tobias PS, et al. TLR2 is constitutively expressed within the kidney and participates in ischemic renal injury through both MyD88-dependent and -independent pathways. J Immunol 2007;178:6252-6258.
19. Tsuboi N, Yoshikai Y, Matsuo S, Kikuchi T, Iwami K, Nagai Y, et al. Roles of toll-like receptors in c-c chemokine production by renal tubular epithelial cells. J Immunol 2002;169:2026-2033.
20. Wong ET, Tergaonkar V. Roles of NF-kappaB in health and disease: mechanisms and therapeutic potential. Clin Sci 2009;116:451-465.
21. Li M, Khan AM, Maderdrut JL, Simon EE, Batuman V. The effect of Pacap38 on MyD88-mediated signal transduction in ischemia-/hypoxia-induced acute kidney injury. Am J Nephrol 2010;32:522-532.
22. Lam W, Bussom S, Guan F, Jiang Z, Zhang W, Gullen EA, et al. The four-herb Chinese medicine PHY906 reduces chemotherapy-induced gastrointestinal toxicity. Sci Translat Med 2010;245:2841-2857.
23. Gong SG. Protection of amrinone against lung injury induced by ischemia/reperfusion in rats. Chin J Contemp Pediatr (Chin) 2007;9:233-236.
24. Zhang PJ, Jin C, Lang J. Effect of atractylodes macrocephalaon polysaccharides for reducing liver ischemia/reperfusion injury in rats after orthotopic liver autotransplantation. Chin J Integr Tradit West Med (Chin) 2010;30:1193-1196.

(Accepted December 17, 2016; First Online May 3, 2017)
Edited by YUAN Lin