

Tanshinone IIA pretreatment attenuates hepatic ischemia-reperfusion

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1. ABSTRACT

Tanshinone IIA (Tan IIA), an active component derived from *Salvia miltiorrhiza* root, has been used to treat various ischemic cardiovascular and cerebrovascular diseases. However, its impact on hepatic ischemia/reperfusion (I/R) injury remains unclear. Here, we addressed this issue by using a 90-minute partial liver ischemia model. Mice were administered Tan IIA intragastrically for 3 days before ischemia and were assessed for liver damage 6-h after reperfusion. Tan IIA pretreatment significantly inhibited serum aminotransferases and proinflammatory cytokine levels along with reduced inflammatory infiltration and liver damage. Mechanistic studies revealed that Tan IIA suppressed TLR4 expression in nonparenchymal cells (NPCs) and induced heme oxygenase-1 (HO-1) production in both parenchymal and NPCs. Moreover, the phosphorylation of AKT and ERK1/2 in the liver was enhanced, while the phosphorylation of JNK, p38 and p65 was suppressed. These results suggest Tan IIA can suppress TLR4 signaling which then enhances HO-1 expression along with reduced proinflammatory cytokine expressions in the liver, and Tan IIA could be a useful candidate drug in clinic for prevention and treatment of hepatic I/R injury.

2. INTRODUCTION

Hepatic ischemia/reperfusion (I/R) injury occurs in clinical settings such as shock, elective liver resection, and transplantation. In liver transplantation, I/R injury is associated with early graft dysfunction, higher incidence for acute and chronic rejection, and late complications that carry significant morbidity to the patients. Therefore, I/R injury is one of the leading causes for early organ failure and is a pivotal factor determining post-transplantation outcomes (1, 2). Although many experimental studies show decreased injury and preserved liver function after dampening the inflammatory response, little progress has been made in the development of novel prevention and therapies for I/R injury (3, 4).

The pathophysiology of full-scaled hepatic I/R injury includes direct cellular damage from the ischemic insult and delayed dysfunction resulting from the activation of inflammatory pathways. Hepatic I/R activates Kupffer cells, and to a lesser degree other immune cells, leading to the production of reactive oxygen species and secretion of proinflammatory cytokines/chemokines. These released signaling molecules then recruit monocytes, neutrophils and T cells into the liver to elicit an innate inflammatory response for tissue repair. However, excessive innate

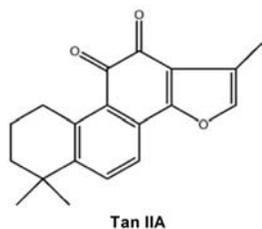


Figure 1. Chemical structure of Tan IIA.

immune response would initiate I/R injury rather than tissue repair. Recent studies suggest that Toll-like receptors (TLRs), especially TLR4, act as modulators of hepatic I/R injury by inducing an overwhelming innate inflammatory response through macrophages, neutrophils, and the complement pathway (5-7). On the other hand, heme oxygenase-1 (HO-1), a rate-limiting stress-responsive enzyme that converts heme into biliverdin, free iron, and carbon monoxide, has been found to be potent in prevention of hepatic I/R injury. Ectopic expression of HO-1 has been shown to significantly decrease local neutrophil accumulation, TNF- α /IL-1 β secretion, IP-10/MCP-1 expression, caspase-3 activity, and apoptosis in the liver during hepatic I/R insult (8-10). Therefore, modulation the axis of TLR4 signaling and HO-1 expression could be a novel approach to prevent hepatic I/R injury in the clinical settings.

Tanshinone IIA (Tan IIA, Figure 1), one of the major active components derived from the traditional Chinese medicinal plant *Salvia miltiorrhiza* root, has been found to be able to eliminate oxygen-derived free radicals, stabilize vascular endothelial function, and ameliorate microcirculation. As a result, it has been widely used in clinic to treat cardiovascular and cerebrovascular diseases (11-14). However, its impact on hepatic I/R injury is yet to be examined. We hypothesized that precondition of mice with Tan IIA would increase their anti-ischemic and anti-inflammatory properties, which in turn would attenuate hepatic I/R injury. Our data revealed that Tan IIA possesses the capability to modulate the axis of TLR4 signaling and HO-1 expression in the liver during I/R insult, and as a result, Tan IIA could be a good candidate drug to prevent hepatic I/R injury in clinic.

3. MATERIALS AND METHODS

3.1. Animals and reagents

Wild-type C57BL/6 male mice (8-10wk old) were purchased from Shanghai Bikai Laboratory Animal Technology Co. LTD (Shanghai, China). All experimental mice were housed under a specific pathogen-free condition in a 12-h day/night rhythm with free access to food and water ad libitum. The animal protocol of this study was approved by the Animal Care and Use committee of the Second Military Medical University, China. Tan IIA used in this study was obtained from Shanghai Bogu Biotech Corporation (Shanghai, China). The purity of Tan IIA was > 95% assessed by high-pressure liquid chromatography (HPLC).

3.2. Experimental groups and mouse hepatic I/R injury model

A nonlethal model of segmental (70%) hepatic warm I/R injury model in mouse was established as described (8). Briefly, an upper midline abdominal incision was made and an atraumatic clip was used to interrupt the left and median lobes (ischemic lobes) of the liver blood supply. After a 90-min of warm ischemia, the clip was removed to initiate hepatic reperfusion. Evidence of ischemia during the clamping period was confirmed by tissue blanching. After removal of the clamp, evidence for reperfusion was confirmed by the immediate color change of the ischemic lobes. Sham-operated mice were underwent the same procedure without clamping.

Mice were randomized into five groups: a) sham operation (negative controls); b) pretreated with phosphate buffered saline (PBS) followed by a 90-min hepatic warm ischemia and then reperfusion; c) pretreated with DMSO before I/R insult as above; d) and e) pretreated with 10 mg/kg/day and 20 mg/kg/day of Tan IIA for three days before the I/R insult, respectively. Blood and liver samples were harvested 6-h after reperfusion to assess liver damage.

3.3. Serum aminotransferases assay and histopathological assessment

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured using the Olympus AU400 Chemistry Analyzer. For histological studies, liver specimens were fixed in 10% buffered formalin and embedded in paraffin. Five-micron sections were stained with hematoxylin-eosin and examined under an Axioplan 2 widefield microscope (Zeiss). The severity of I/R injury was graded using the Suzuki's criteria (15). In this classification, three liver injury indices are graded for a total score of 0 to12: sinusoidal congestion (0~4); hepatocyte necrosis (0~4); and ballooning degeneration (0~4). The sections were scored 0 if necrosis, congestion, or centrilobular ballooning are absent, while the sections were scored 4 in the presence of severe congestion and ballooning degeneration as well as > 60% lobular necrosis.

3.4. Serum proinflammatory cytokine assay

Serum TNF- α (eBioscience), IL-1 β (eBioscience), MCP-1 (Bender) and MIP-2 (PeproTech) levels were assayed using ELISA kits according to the manufacturer's instructions.

3.5. Immunohistochemistic analysis of inflammatory infiltration

Immunohistochemistry for infiltrated macrophages was conducted as described (16). Briefly, paraffin-embedded mouse liver sections were deparaffinized in xylene and rehydrated through graded ethanol series to water. Endogenous peroxidase activity was inhibited with 0.3% H₂O₂. After blocking with 10% normal rabbit serum/phosphate-buffered saline solution, the slides were incubated overnight with primary antibody for macrophages (F4/80) at 4°C in a humidified chamber, followed by incubating with horseradish peroxidase-conjugated rabbit anti-mouse immunoglobulin G for 30-min and diaminobenzidine reagent for 10-min. Control

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serum immunoglobulin G was used as a negative isotype control. The sections were evaluated blindly through the counting of the labeled cells ($\times 200$).

3.6. Preparation of mouse hepatic parenchymal cells and nonparenchymal cells

Hepatic parenchymal cells (hepatocytes) and nonparenchymal cells (NPCs, including Kupffer cells, neutrophils, T cells and endothelial cells) were isolated as described (17). Briefly, the portal vein was cannulated with a 23-gauge plastic cannula, and the liver was perfused with calcium-free Hanks' balanced salt solution (HBSS) at 37°C for 5-min, followed by digestion with a collagenase solution (0.05%) at 37°C for 7-min. After perfusion, the liver was removed into a Petri dish, parenchymal cells and NPCs were liberated from the connective tissue by carefully raking the liver with scissors and shaking the liver gently. The initial cell suspension was filtrated through a nylon mesh, and parenchymal cells were obtained by low-speed centrifugation (two times at 50g for 5-min). The supernatant was centrifuged (at 300g for 7-min) to obtain NPCs. The viability of hepatic cells was assessed by trypan blue staining immediately after isolation and was found to be $> 85\%$. The cells (1×10^6 /well) were then maintained in 1640 medium in 5% CO₂ at 37°C.

3.7. Real-time polymerase chain reaction (RT-PCR)

Total RNA was isolated with TRIzol (Gibco BRL, Carlsbad, CA) according to the manufacturer's directions and reverse-transcribed into cDNA as described (10). The primers were: HO-1 (forward 5'-AAG CCG AGA ATG CTG AGT TCA-3', reverse 5'-GCC GTG TAG ATA TGG TAC AAG GA-3'); TLR4 (forward 5'-ATG GCA TGG CTT ACA CCA CC-3', reverse 5'-GAG GCC AAT TTT GTC TCC ACA-3'); TNF- α (forward 5'-CCC TCA CAC TCA GAT CAT CTT CT-3', reverse 5'-GCT ACG ACG TGG GCT ACA G-3'); IL-1 β (forward 5'-GCA ACT GTT CCT GAA CTC AAC T-3', reverse 5'-ATC TTT TGG GGT CCG TCA ACT-3'); MCP-1 (forward 5'-TTA AAA ACC TGG ATC GGA ACC AA-3', reverse 5'-GCA TTA GCT TCA GAT TTA CGG GT-3'); MIP-2 (forward 5'-CCA ACC ACC AGG CTA CAG G-3', reverse 5'-GCG TCA CAC TCA AGC TCT G-3'); F4/80 (forward 5'-CTT TGG CTA TGG GCT TCC AGT C-3', reverse 5'-GCA AGG AGG ACA GAG TTT ATC GTG-3') (18); Ly-6G (forward 5'-TGG ACT CTC ACA GAA GCA AAG-3', reverse 5'-GCA GAG GTC TTC CTT CCA ACA-3') (19); CD3 (forward 5'-AGA GGG CAA AAC AAG GAG CG-3', reverse 5'-AGA CTG CTC TCT GAT TCA GGC-3'); and GAPDH (forward 5'-ACC ACA GTC CAT GCC ATC AC-3', reverse 5'-TCC ACC ACC CTG TTG CTG TA-3').

3.8. Western blot analysis

Extracted proteins from liver samples (30 μ g/sample) were run on 12% SDS-PAGE gels. The gels were stained with coomassie blue to document equal protein loading. The membranes were blocked with 3% dry milk/0.1% Tween 20 (USB, Cleveland, OH, USA) and incubated with primary antibodies against GAPDH, HO-1, TLR4, and the phosphorylated forms of AKT, ERK1/2, JNK, p38 and p65 (Cell Signaling, Beverly, MA, USA),

respectively. The reactive bands were detected by horseradish peroxidase-conjugated anti-rabbit IgG. The relative amount for each target protein was determined using a densitometric analysis.

3.9. Statistical analysis

All values were expressed as means \pm SD. SPSS13.0 software was used for analysis. Statistical analysis of difference between groups was performed by Student's *t* test or by ANOVA for experiments with more than two subgroups. A *P* value of < 0.05 was considered to be statistically significant.

4. RESULTS

4.1. Tan IIA attenuated hepatic I/R injury

To address whether Tan IIA pretreatment ameliorates hepatic I/R injury, each group of mice were first subjected to analysis of serum ALT and AST levels (IU/L). After I/R, ALT and AST levels in mice from group b and c were significantly increased as compared to those in group a, the sham controls ($P < 0.01$). In sharp contrast, mice received pretreatment of Tan IIA showed significant lower levels of serum transaminase (group d and e, $P < 0.01$), which is in a dose-dependent manner (Figure 2 A).

Histopathological analysis revealed normal morphology of liver cells, no redness or swelling of liver sinusoid and no necrotic cells in mice from group a (Suzuki's score = 2.0 ± 0.71 , Figure 2B a). In contrast, there were a large number of liver cells clustered together in mice derived from group b and c. The nuclei in those cells were narrow, deeply stained, and a portion of the liver cells were necrotic. There was also marked congestion and swelling of liver sinusoids with centrilobular ballooning (Suzuki's score = 9.0 ± 1.58 and 9.0 ± 1.0 , respectively, Figure 2B b and c). However, Tan IIA pretreatment significantly attenuated I/R-mediated liver injury (mice in group d and e) with well preserved structure for hepatic lobules. There was only a slight edema of the liver cells, and a small number of nuclei were deeply stained. The degree of liver sinusoidal congestion in the portal areas was also inhibited (Suzuki's score = 6.0 ± 1.22 and 4.2 ± 0.84 , respectively, Figure 2B d and e).

4.2. Tan IIA inhibited proinflammatory cytokine production

A hallmark of I/R injury is the release of proinflammatory cytokines such as TNF- α (20), IL-1 β (21), MCP-1 (22) and MIP-2 (23, 24) from Kupffer cells, which results in elevated serum transaminase levels and organ damage. To explore whether Tan IIA protects the liver from I/R injury through inhibiting the production of proinflammatory cytokines, TNF- α , IL-1 β , MCP-1 and MIP-2 levels in the liver and peripheral blood were analyzed by RT-PCR and ELISA. As shown in Figure 3, the production of proinflammatory cytokines was significantly increased in mice from group b and c when compared to those in group a ($P < 0.01$). However, Tan IIA dose-dependently suppressed the production of inflammatory cytokines after I/R insult (mice in group d and e, $P < 0.01$).

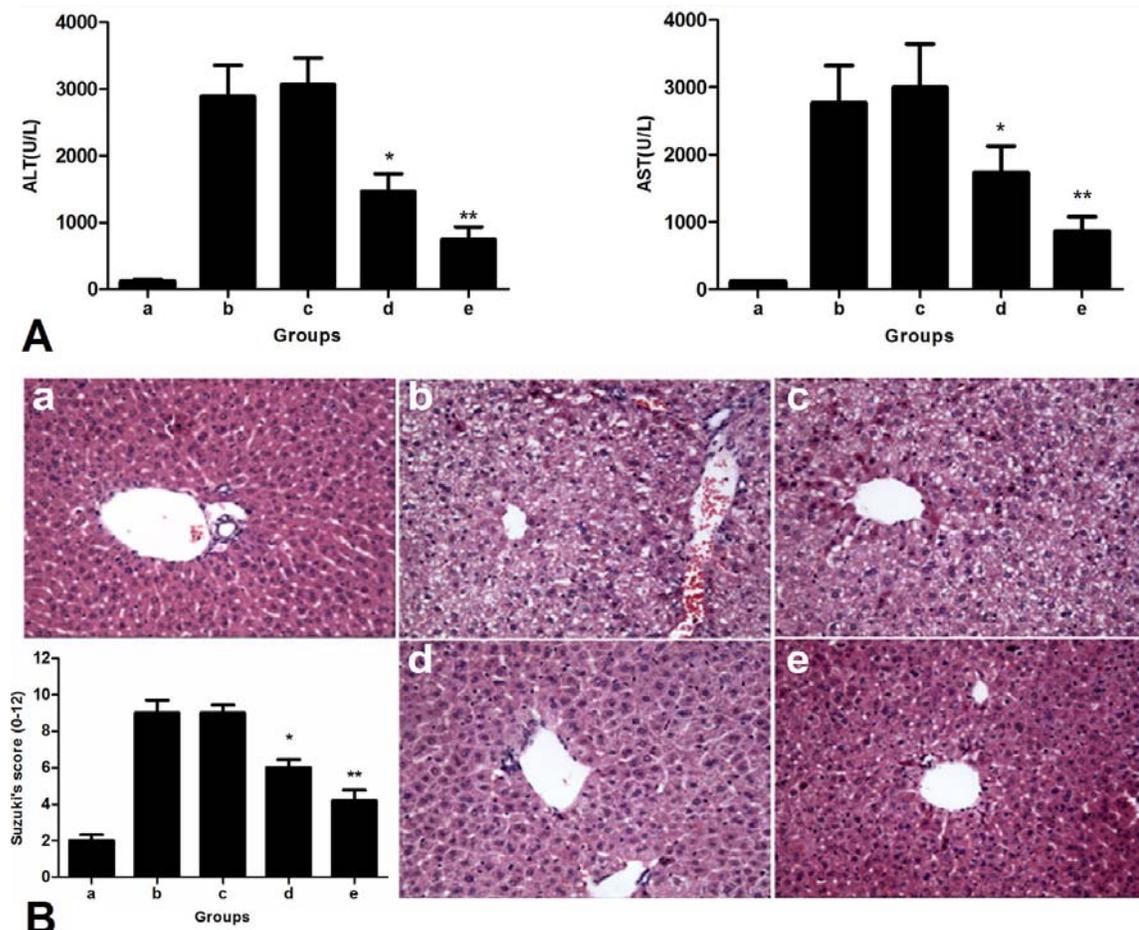


Figure 2. Tan IIA attenuated hepatic I/R injury. Serum ALT and AST (A) levels (IU/L) in mice with different pretreatment (Group a, mice underwent sham operation; Group b, mice pretreated with physiological saline followed by I/R; Group c, mice pretreated with DMSO before I/R; Group d and e, mice pretreated with 10 mg/kg or 20 mg/kg of Tan IIA, respectively, three days before I/R) before 90 minutes of warm liver ischemia followed by 6-h of reperfusion. (B) Hematoxylin eosin staining and Suzuki's score of the livers from mice with different pretreatment. Values were expressed as means \pm SD for 6–8 mice per group. * $P < 0.05$, ** $P < 0.01$ as compared with Group b. (Original magnification $\times 200$).

4.3. Tan IIA inhibited leukocyte infiltration in the liver

As the proinflammatory cytokines are responsible for the recruitment of monocytes, neutrophils and $CD4^+$ T cells into the site of injury (25), which are associated with a secondary wave of inflammatory damage. We further investigated leukocyte infiltration in the liver. As shown in Figure 4A, the F4/80 positive cells in mice from group d and e were significantly decreased than those in group b and c ($P < 0.01$), indicating a inhibited infiltration for macrophages (18). RT-PCR analysis further revealed a significant decrease of mRNA for Ly-6G (neutrophils) and CD3 ($CD4^+$ T cells) (Figure 4B), suggesting that Tan IIA also suppressed neutrophil and $CD4^+$ T cell infiltration.

4.4. Tan IIA regulated the axis for TLR4 signaling and HO-1 expression in the liver

TLRs, especially TLR4, are expressed in monocytes/macrophages and neutrophils (26, 27), and are recognized as a key mediator for I/R injury during the

course of innate inflammatory response in hepatic I/R insult (5–7). To dissect the underlying mechanisms by which Tan IIA prevents hepatic I/R injury, we examined TLR4 expression in the liver by RT-PCR and Western blotting. As shown in Figure 5A, TLR4 expression in mice from group b and c was significantly increased compared to that in group a. In contrast, Tan IIA pretreatment dose-dependently inhibited TLR4 mRNA transcription in mice from group d and e.

Given the factor that other than NPCs hepatic parenchymal cells also express TLR4 (28), a critical question relevant to the above results is whether the reduced TLR4 expression in Tan IIA treated mice was a consequence of inhibited inflammatory infiltration (Figure 4). To address this question, we analyzed Tan IIA targeted cells in the liver. For this purpose, we isolated the hepatic parenchymal cells and NPCs from I/R mice. It was interestingly found that Tan IIA did not cause a significant change for TLR4 expression in hepatic parenchymal cells

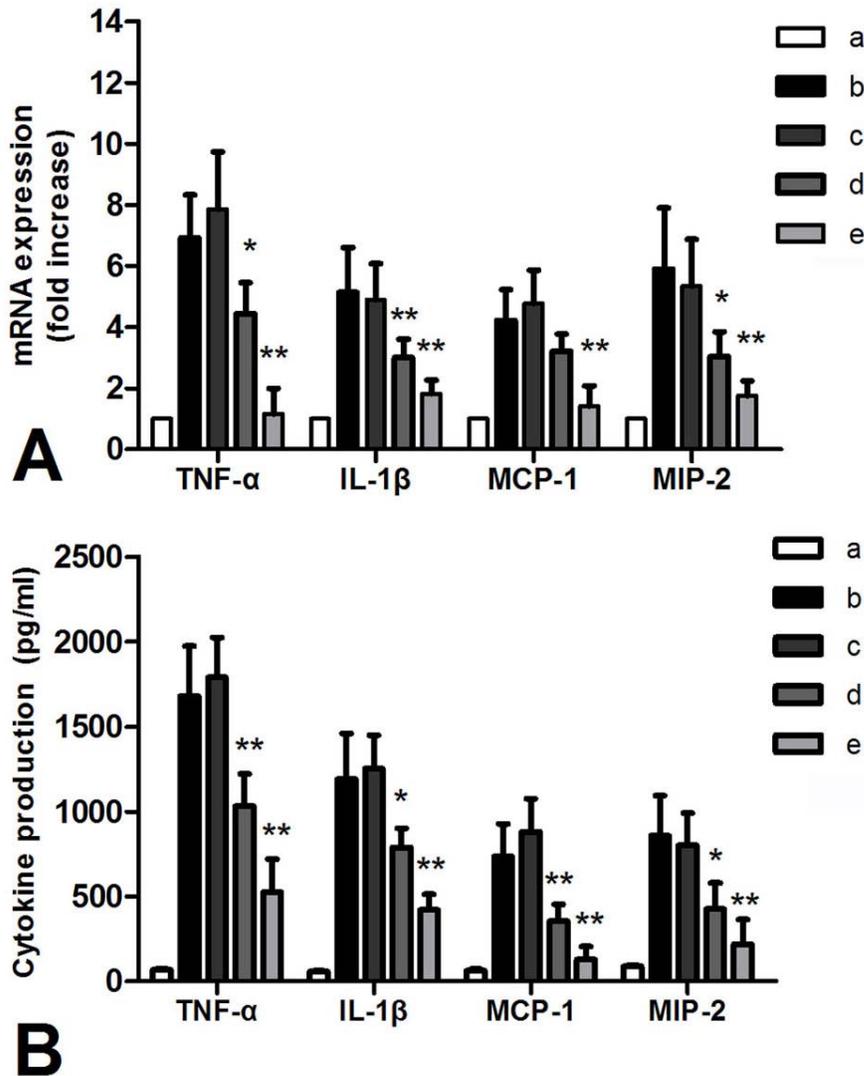


Figure 3. Tan IIA inhibited proinflammatory cytokines production. Proinflammatory cytokines mRNA expression in the liver was measured by RT-PCR (normalized against *GAPDH*), and protein production in the serum was measured by ELISA. Values are represented as means ± SD for 6–8 mice per group. * $P < 0.05$, ** $P < 0.01$ as compared with Group b.

(Figure 5B), instead it selectively suppressed TLR4 expression in NPCs (Figure 5C), which could be caused by the inhibited inflammatory infiltration (Figure 4).

Next, we examined the expression of HO-1, a critical protective molecule in hepatic I/R injury (8, 9, 29, 30). Surprisingly, Tan IIA pretreatment significantly induced HO-1 expression both in the parenchymal cells and NPCs, as compared to that in mice from control groups (Figure 5B and C). These results revealed that Tan IIA regulated the axis for TLR4 signaling and HO-1 expression in the liver.

4.5. Tan IIA triggered the protective signaling pathways in the liver

To further explore the signaling pathways relevant to the protective effect for Tan IIA treatment, we

analyzed the phosphorylation states of cell-signaling proteins. As shown in Figure 6, Tan IIA pretreatment significantly increased the phosphorylation of AKT and extracellular-signal-regulated kinase 1/2 (ERK1/2). On the contrary, Tan IIA selectively inhibited the phosphorylation of JNK, p38 and p65, the three important signal molecules downstream of TLR4 signaling. These results further demonstrate that Tan IIA protects hepatic I/R injury by suppressing TLR4-triggered innate inflammatory response.

5. DISCUSSION

Accumulated evidence demonstrates that Tan IIA regulates hypoxia-induced oxidative stress, and therefore, it has been widely used to treat cardiovascular and cerebrovascular diseases. Wang *et al.* reported that Tan IIA inhibits the expressions of HMGB1, TLR4, RAGE and NF-

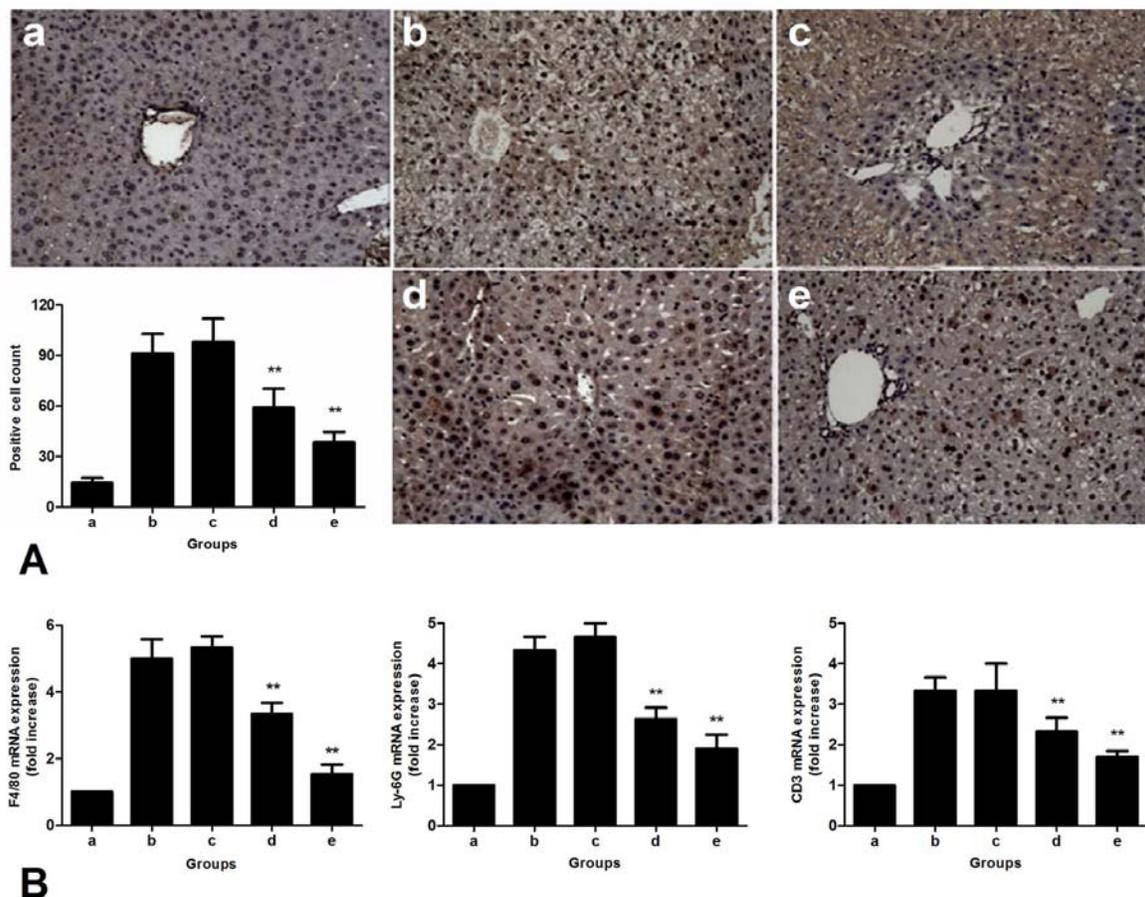


Figure 4. Tan IIA inhibited leukocytes infiltration into the liver. (A) Immunostaining of liver tissue from mice 6 hours after reperfusion (F4/80 immunostained). Original magnification, $\times 200$. Positively stained cells were counted blindly by two observers per slide. Data are shown as means \pm SD. (B) mRNA transcription of molecular markers for monocytes/macrophages (F4/80), neutrophils (Ly-6G) and T cells (CD3) in the liver was measured by RT-PCR (normalized against *GAPDH*). Values are represented as means \pm SD for 6–8 mice per group. * $P < 0.05$, ** $P < 0.01$ as compared with Group b.

κ B in ischemic brain tissue caused by permanent middle cerebral artery occlusion (31). Studies also found that addition of Tan IIA to UW (University of Washington) solution could decrease skeletal muscle I/R injury (32). Fu *et al.* further revealed that Tan IIA pretreatment protects rat cardiac myocytes against oxidative stress-induced apoptosis, which is associated with increased scavenging of oxygen free radicals, reduced lipid peroxidation and upregulated Bcl-2/Bax ratio (12). However, the role of Tan IIA in hepatic I/R injury largely remains enigmatic.

This is the first report that shows the capability of Tan IIA to modulate the axis of TLR4 signaling and HO-1 expression during the course of I/R-induced innate immune response. Our results demonstrate the feasibility for Tan IIA in prevention and treatment of I/R induced liver injury. Mice pretreated with Tan IIA show attenuated hepatic I/R injury both biologically and histopathologically, and the protective effect was associated with decreased serum TNF- α , IL-1 β , MCP-1 and MIP-2 levels, inhibited leukocyte infiltration, and suppressed JNK, p38 and p65 activation in the liver. Tan IIA selectively inhibits TLR4

expression in NPCs, but enhances HO-1 expression during hepatic I/R insult, indicating that Tan IIA attenuates hepatic I/R injury by modulating the axis of TLR4 signaling and HO-1 expression in the liver.

We first demonstrated convincing evidence that Tan IIA attenuates hepatic I/R injury both biochemically and histopathologically. These effects were at least partially mediated by promoting the expression of HO-1 which shows antioxidative cytoprotective effect (9, 30). Recently, Ke *et al.* reported that adoptive transfer of Ad-HO-1-transduced macrophages prevented I/R-induced hepatocellular damage, as evidenced by depressed serum AST levels and preserved liver histology (Suzuki's scores). However, concomitant treatment with HO-1 siRNA completely reversed this beneficial effect (29). Transfer of Ad-HO-1-transduced macrophages significantly decreased local neutrophil accumulation, TNF- α /IL-1 β , IFN- γ /E-selectin, and IP-10/MCP-1 expression, caspase-3 activity, and the frequency of apoptotic cells. Similar as these results, we found in our model that upregulated HO-1 expression is correlated with decreased secretion of

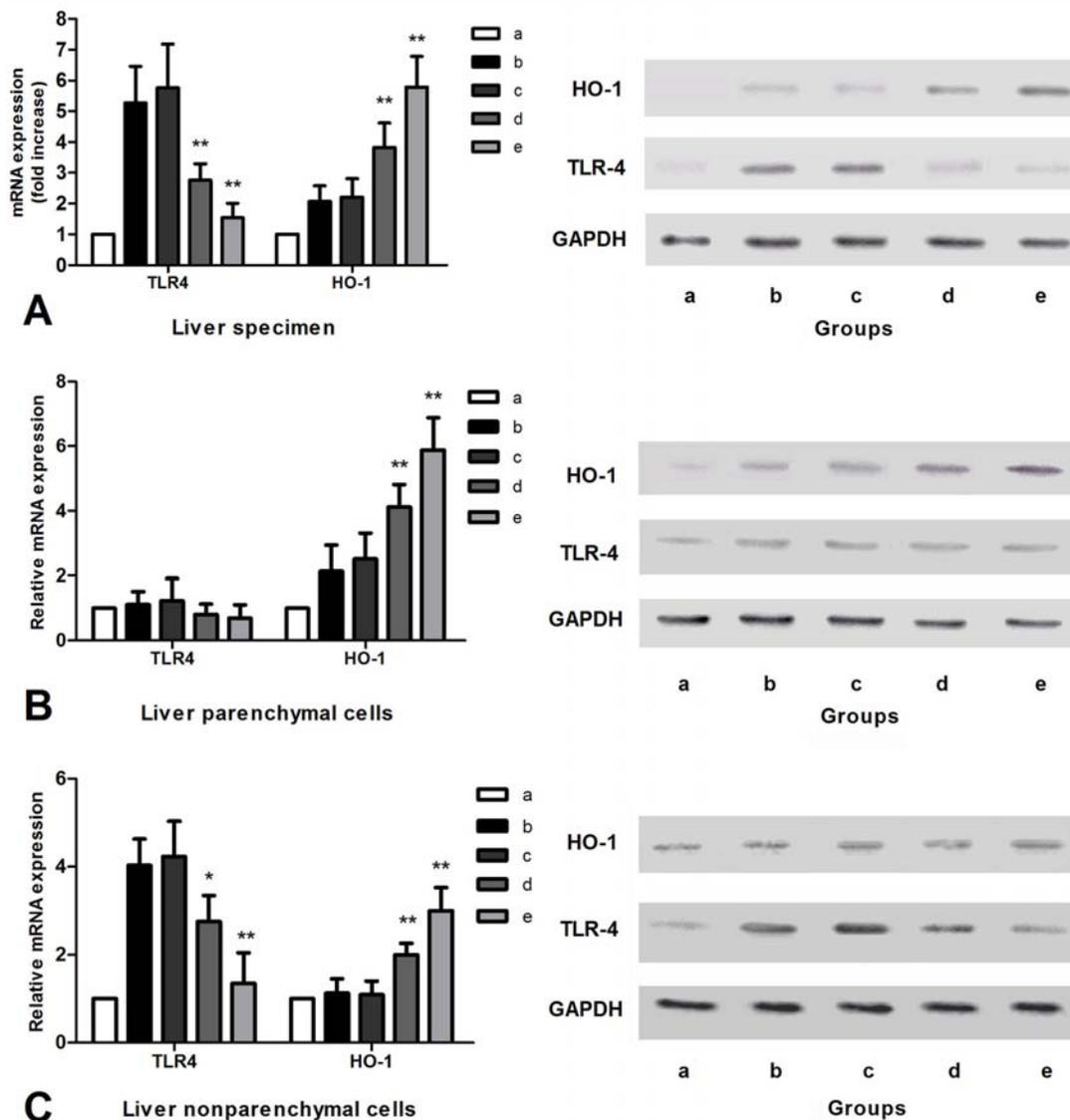


Figure 5. Tan IIA regulated the axis for TLR4 signaling and HO-1 expression in the liver. (A) TLR4 and HO-1 mRNA transcription and protein production in the liver specimens from mice with different pretreatment (Group a, mice underwent sham operation; Group b, mice pretreated with physiological saline followed by I/R; Group c, mice pretreated with DMSO before I/R; Group d and e, mice pretreated with 10 mg/kg/day or 20 mg/kg/day of Tan IIA, respectively, three days before I/R). Mice liver parenchymal (B) and nonparenchymal cells (C) were isolated to test the mRNA and protein levels of TLR4 and HO-1. Data are normalized against *GAPDH* and expressed as means \pm SD for 6~8 mice per group. * $P < 0.05$; ** $P < 0.01$ as compared with Group b.

inflammatory mediators such as TNF- α , IL-1 β , MCP-1 and MIP-2 both in the liver and peripheral blood. As these proinflammatory cytokines are responsible for the recruitment of monocytes, neutrophils and T cells into the liver, (24, 25, 33), inflammatory infiltration and liver damage were significantly inhibited as determined by both immunohistological and RT-PCR analyses.

Of interestingly note, unlike its effect on HO-1, Tan IIA selectively inhibits TLR4 expression in the liver, particularly in NPCs. TLR belongs to the IL-1R family and is conventionally considered to recognize bacterial/viral-

specific pathogen-associated molecular patterns (34, 35). Nevertheless, recent studies consistently demonstrated that TLRs such as TLR4 also recognizes damage-associated molecular patterns such as HMGB1 (36) and heat shock proteins (37) that are passively released innate alarmins during the course of tissue or organ injury. TLR-triggered innate inflammatory response then activates systemic responses to recruit leukocytes to the sites of inflammation. Ellett *et al.* (38). reported that loss of TLR4 in steatotic grafts led to an improved animal survival and liver injury. Using a 90-min lobar warm ischemia model, Shen *et al.* (8) further highlighted the importance of cross talk between

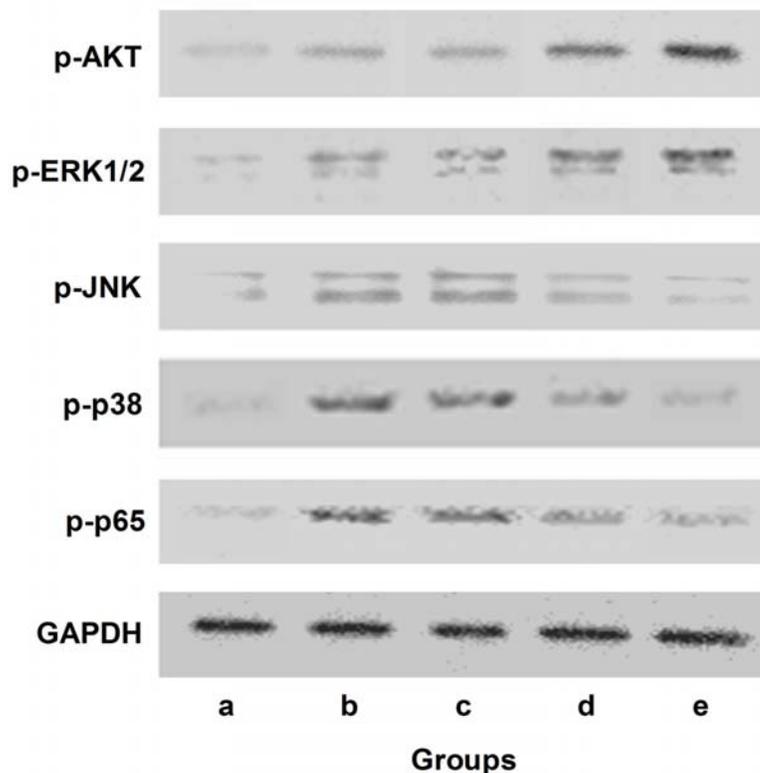


Figure 6. Tan IIA triggered the protective signaling pathways in the liver. Whole-tissue lysates from mouse liver were analyzed for the phosphorylation of AKT, ERK1/2, JNK, p38 and p65 by western blotting. Immunoblots are representative of three independent experiments.

HO-1 and the TLR system in the underlying mechanism of hepatic I/R injury. This represents a case for innate immunity where HO-1 modulates proinflammatory responses that are triggered via TLR4 signaling. They found that defective TLR4 but not TLR2 signaling increased HO-1 mRNA and protein expression. In contrast, tin protoporphyrin-mediated HO-1 inhibition restored hepatic damage in otherwise I/R injury-resistant TLR4 mutant/KO mice. Likewise, cobalt protoporphyrin-induced HO-1 overexpression ameliorated hepatic damage in I/R injury-susceptible TLR2 KO mice. Together, these studies suggest that TLR4 signaling may act as an HO-1 repressor. Therefore, the enhanced HO-1 expression in the liver in our model could be caused by the decreased TLR4 expression.

We further investigated the signal pathways relevant to the protective effect by Tan IIA pretreatment. It was found that the phosphorylation of AKT and ERK1/2 was increased. This result is consistent with previous studies in which activation of AKT and ERK1/2 is essential for the induction of HO-1 expression (39, 40). Jang and colleagues (41) reported that Tan IIA may inhibit LPS-induced κ B α degradation and NF- κ B activation via suppression of the NIK-IKK pathway as well as the MAPKs (p38, ERK1/2, and JNK) pathway in RAW 264.7 cells. In line with their results, we also detected reduced phosphorylation for JNK, p38 and p65 in the liver of mice treated with Tan IIA. As JNK and p38 are important downstream signal molecules in TLR4 signaling and NF-

κ B plays an essential role in the transcription of proinflammatory cytokines and adhesion molecules in inflammatory cells (42, 43), these results support the conclusion that Tan IIA exerts its anti-inflammatory cytoprotective function via suppressing TLR4 signaling.

In summary, this study provides evidence for an anti-inflammatory cytoprotective function of Tan IIA in hepatic I/R injury, which is probably mediated by modulating the axis of TLR4 signaling and HO-1 expression during I/R insult in the liver. This protective effect is due, at least in part, to the capability of Tan IIA to enhance AKT and ERK1/2 activation, and to suppress JNK, p38 and p65 activation. These findings provide a new insight into the understanding of the complex mechanisms underlying Tan IIA regulation of innate immune response during I/R insult and provided feasible evidence that Tan IIA could be a good candidate drug to prevent hepatic I/R injury in clinic.

6. ACKNOWLEDGEMENTS

Yan-yan Qi and Liang Xiao contributed equally to this study. This work was supported by grants from the National Key Basic Research Program of China (2009CB522402), the National Nature Science Foundation of China (81072956, 30772056, U0832009) and the Science and Technology Foundation of Shanghai, China (07JC14067).

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Abbreviations: Tan IIA:Tanshinone IIA; I/R: ischemia/reperfusion; TLR4: toll-like receptor 4; HO-1: heme oxygenase-1; AST: aspartate aminotransferase; ALT: alanine aminotransferase; MCP-1: monocyte

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chemoattractant protein-1; MIP-2: macrophage inflammatory protein-2.

Key Words: Tanshinone IIA, Hepatic ischemia/reperfusion injury, Heme oxygenase-1, Toll-like receptor, Mouse

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