International Immunopharmacology 76 (2019) 105870

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Glycyrrhetinic acid pretreatment attenuates liver ischemia/reperfusion injury via inhibiting TLR4 signaling cascade in mice

Xujie Jiang[a,1], Ge Kuang[b,1], Xia Gong[c], Rong Jiang[d], Tianjun Xie[b], Hongtao Tie[e], Shengwang Wu[c], Ting Wang[f], Jingyuan Wan[b,*], Bin Wang[a,*]

[a] Department of Anesthesiology, The First Affiliated Hospital of Chongqing Medical University, Chongqing 400016, China
[b] Chongqing Key Laboratory of Biochemistry and Molecular Pharmacology, Chongqing Medical University, Chongqing 400016, China
[c] Department of Anatomy, Chongqing Medical University, Chongqing 400016, China
[d] Laboratory of Stem Cell and Tissue Engineering, Chongqing Medical University, Chongqing 400016, China
[e] Department of Cardiothoracic Surgery, The First Affiliated Hospital of Chongqing Medical University, Chongqing 400016, China
[f] Department of Orthopaedic Surgery, The Second Affiliated Hospital of Chongqing Medical University, Chongqing 400016, China

ARTICLE INFO

Keywords:
Glycyrrhetinic acid
Liver ischemia/reperfusion injury
Inflammation
Apoptosis
HMGB1
TLR4

ABSTRACT

Glycyrrhetinic acid (GA), the main bioactive substances of glycyrrhiza uralensis Fisch, has been reported to exhibit hepatoprotective and anti-inflammatory properties. However, the effects and underlying mechanisms of GA in liver ischemia/reperfusion (I/R) injury remain elusive. In this study, mice were pretreated with GA (100 mg/kg) three times a day by gavage prior to I/R injury, and then hepatic histopathological damages, biochemical parameters and inflammatory molecules were evaluated. We found that mice performed with liver I/R showed a significantly increase in plasma aminotransferase (ALT), aspartate aminotransferase (AST), liver cell apoptosis and infiltration of neutrophils compared with the control group. GA pretreatment notably improved liver function, histopathology of liver tissues, and lowered liver cell apoptosis and infiltration of neutrophils. Besides, further analysis indicated that GA pretreatment reduced I/R-induced expression of extracellular HMGB1, inhibited activation of TLR4 and following phosphorylation of IRAK1, ERK, P38 and NF-κB, and attenuated TNF-α and IL-1β production. These data suggested that GA protected against liver I/R injury through a HMGB1-TLR4 signaling pathway and it might be a promising drug for future clinical use in liver transplantation.

1. Introduction

Hepatic ischemia/reperfusion (I/R) injury, occurring mainly in liver resection, transplantation and hemorrhagic shock, are a major cause of liver failure and lead to high morbidity and mortality among liver recipients. As for liver transplantation, there are two major types of liver injury included: the “warm” IR injury and the “cold” IR injury, which are characterized by initial hepatocellular damage and hepatic sinusoidal endothelial cell damage, respectively [1,2]. Although I/R injury have been studied extensively for years, the detailed mechanisms of hepatic I/R injury remain to be elucidated. During I/R injury, activation of immunological cascades initiated by predominantly local innate-immune response and following adaptive-immune response is an essential mechanism, thus leading further tissue damage [3].

In our body, the immune response is fundamental to discriminate ‘self’ from ‘nonself’ to impending danger and keep healthy, and the innate immune system has evolved to respond to both invading pathogens and molecular elements released from dying cells [4,5]. Damage-associated molecular patterns (DAMPs), such as HMGB1, histones and heat-shock protein, are normally released by damaged tissues and can activate innate immune system by interacting with pattern recognition receptors [6]. HMGB1, a DNA-binding nuclear protein that mainly organizes DNA and regulates gene transcription in nuclear, is released into the extracellular milieu by numerous immune and non-immune cell upon acute stress. Upon released outside of cells, HMGB1 serves as a prototypic DAMPs that contribute to pathobiology in both infection and sterile inflammation [7,8]. TLR4, a member of pattern recognition receptors located on the cell surface, is the first defined toll-like receptors (TLRs) that triggers inflammatory response through recognizing lipopolysaccharide (LPS) [9]. Upon activation of TLR4-

* Corresponding authors.
E-mail addresses: jywan@cqmu.edu.cn (J. Wan), 13637815096@163.com (B. Wang).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.intimp.2019.105870
Received 3 July 2019; Received in revised form 28 August 2019; Accepted 28 August 2019
Available online 04 September 2019

dependent signaling pathway by endogenous DAMPs, downstream signaling molecules are subsequently activated via MyD88-dependent or MyD88-independent signaling pathways, resulting in activation of nuclear factor-κB (NF-κB) and activating protein-1 (AP-1) [10,11]. Finally, activated transcription factors transfer to nucleus to producing cytokines and chemokines to regulate immune response. Mice with inhibition of TLR4-dependent signaling pathway was protected from I/R injury [12–17].

Glycyrrhetinic acid (GA), an aglycone saponin extracted from glycyrrhizae radix, reportedly exhibits a wide range of pharmacological effects, including anti-inflammation, anti-tumor and hepatoprotection. Recent years, it has been demonstrated that GA is hepatoprotective in many liver damage animal models, like CCl4-induced damage [18], LPS/D-GalN-induced fulminant hepatic failure [19], triptolide-induced hepatotoxicity [20] and free fatty acid-induced liver damage [21]. Although GA has been reported protective in heart I/R injury by reducing both the susceptibility and incidence of fatal ventricular arrhythmia [22], the underlying mechanisms of GA in acute liver injury remains unclear. In this study, we aimed to explore the real role of GA in hepatic I/R injury. We demonstrated that GA protected liver from I/R injury via a HMGB1-TLR4 signaling pathway.

2. Materials and methods

2.1. Materials

Glycyrrhetinic acid (GA, purity > 98%) was obtained from Nanjing zelang Biological Technology Corporation (Nanjing, China) and had a purity of > 98% by High Performance Liquid Chromatography (HPLC). The commercial assay kits for the determination of serum ALT and AST levels were acquired from Nanjing Jiancheng Biological Engineering Institute (Nanjing, China). Serum TNF-α and IL-1β levels were measured by a standardized enzymatic assay kits purchased from Becton, Dickinson and Company (New Jersey, USA). The terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay kit was supplied by Roche Applied Science (Basel, Switzerland). Primary antibodies against TLR4, p-IRAK1, p-ERK, p-JNK, p-P38 and p-NF-κB were all purchased from Cell Signaling Technology (Boston, MA, USA). Primary antibody specific for anti-GAPDH was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Primary antibody against HMGB1 and horseradish peroxidise (HRP)-conjugated secondary antibodies were purchased from Abcam (Cambridge, MA, USA). Fluorochrome-labeled antibody against Ly6G was supplied by BD Biosciences (Heidelberg, Germany). Phycoerythrin (PE)-labeled anti-Ly6G and allophycocyanin (APC)-labeled anti-CD45 antibodies were supplied by BD Company (Heidelberg, Germany). The ABC kit and DAPI was purchased from Vectorlab (Burlingame, CA, USA). DAB Peroxidase Substrate was obtained from DAKO North America (Carpinteria, CA, USA).

2.2. Animals

Male C57BL/6 mice (8–12 weeks old) were purchased from the Chongqing Medical University Animal Center. All mice were maintained in a specific pathogen-free condition with a 12-h day–night rhythm, and received food and sterile water. All experimental procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals.

2.3. Liver I/R model and treatment

The mice were stochastically divided into four groups with 10 mice in each group. For the I/R group, a non-lethal model of segmental (70%) hepatic warm I/R was induced as previously reported. In brief, mice were anesthetized with phenobarbital (100 mg/kg), and after a midline laparotomy, the arterial and portal venous blood supplies to the left and middle liver lobes were occluded with an atraumatic clamp. After 90 min reperfusion, the clamp was removed for 6 h reperfusion and the incision was closed. For the GA + I/R group, mice were treated with GA (100 mg/kg) by intraperitoneal injection (i.p.) before the surgery. Mice in control group received an equal volume of olive oil and mice in GA group underwent the same procedure without vascular occlusion. All mice were sacrificed after 6 h of reperfusion, serum samples and liver tissues were collected for further analysis.

2.4. Liver functional assays

Serum ALT and AST levels, which are essential indicators of hepatocellular injury, were all detected by commercial kits with standard curves made from the corresponding manufacturer's instructions.

2.5. Histological analysis

The liver tissues were fixed in 4% polyformaldehyde, embedded in paraffin and sectioned at 5 μm and stained with hematoxylin and eosin (H&E).

2.6. Immunohistochemistry

Paraffin-embedded sections (5 μm) were dewaxed, dehydrated and heat-induced antigen retrieval was performed with citric acid buffer (pH 6.0, 10 mM) for 20 min. Sections were then blocked with 3% H_2O_2 in TBS for 20 min, followed by exposure to 10% goat serum in TBS for 30 min and then incubation with rabbit anti-HMGB1 antibody at 4 °C overnight. After washing three times, the slides were incubated with a biotin-labeled goat anti-rabbit antibody. The signals were amplified with ABC kit and visualized with DAB Peroxidase Substrate.

2.7. Immunofluorescence

For fluorescence stain, the liver tissues were fixed with 4% polyformaldehyde for 6 hfollowed by dehydration with 15%, 30% sucrose in PBS respectively at 4 °C. The tissues were then embedded in O.C.T solution for frozen sections (8 μm). After blocking with 10% goat serum in TBS, the frozen sections were incubated with rat anti-PE-labeled Ly6G antibody. Finally, the slides were counterstained with DAPI and analyzed with fluorescence microscopy.

2.8. TUNEL staining

The apoptosis in liver tissues were examined by the commercial TUNEL kits according to the manufacturer's protocol.

2.9. Detection of caspase 3 activity

The total proteins were extracted with lysis buffer from homogenized liver tissues. The caspase3 activity in hepatic tissues was determined with a commercial colorimetric assay kit according to the manufacturer's instructions.

2.10. Flow cytometry

Liver was perfused with cold saline, and minced into small pieces. Then, it was digested with collagenase (Worthington) for 30 min at 37 °C. Liver fragments were grinded and passed continuously through a 200 μm mesh. Single-cell suspension was centrifuged at 50 × *g* for 5 min and resuspended. The supernatant was further centrifuged at 300 × *g* for 15 min, resuspended and counted. After that, the resuspension was incubated with anti-CD45-APC, anti-Ly6G-PE antibody and analyzed by flow cytometry.

2.11. Western blot analysis

The total protein was extracted from liver tissues using RIPA lysis buffer containing protease inhibitors and protein concentration was determined by Bradford assay. Equal amounts of total protein (40 μg) were then separated on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes. The membranes were blocked for 1 h at room temperature with 5% skimmed milk powder in TBST and then incubated with primary antibodies (anti-TLR4, anti-phospho-IRAK1, anti-phospho-ERK1/2, anti-phospho-JNK and anti-phospho-P38, anti-phospho-NF-κB and anti-GAPDH antibodies) overnight at 4 °C, respectively. After three washes in TBST, the membranes were incubated with horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature. The protein expression levels were visualized by ECL chemiluminescence system (Bio-Rad, USA).

2.12. Statistical analysis

The two-tailed Student's test and ANOVA were used to analyze the comparison between different groups, and $P < 0.05$ was considered statistically significant.

3. Results

3.1. GA ameliorated liver function in hepatic I/R-induced liver injury

Firstly, we determined the change of ALT and AST levels in serum after hepatic I/R. As shown in (Fig. 1B and C), the serum levels of ALT and AST were remarkably higher in I/R group than those in control group, and GA pretreatment significantly inhibited those increases.

3.2. GA improved histopathology of liver tissues

Histopathology of liver tissues was determined to analyze the effect of GA on liver I/R injury. The histopathology of liver tissues was normal in both control group and GA group. In contrast, there were marked sinusoidal congestion, cytoplasmic vacuolization, edema and focal necrosis in I/R group and GA pretreatment evidently reversed those histopathological changes (Fig. 2A and B).

3.3. GA reduced liver cell apoptosis induced by hepatic I/R

Apoptosis, known as programmed cell death, plays an essential role in acute liver injury [23]. In this study, apoptosis was detected by TUNEL assay and results showed that there was no significant difference between control group and GA group and a large number of TUNEL-positive hepatocytes were observed in I/R group, however, few hepatocytes were TUNEL-positive after GA pretreatment (Fig. 3A). As Caspase 3 plays an essential roles in cell apoptosis, we measured the activity of caspase 3. In consistent with the TUNEL staining, pretreatment with GA significantly decreased IR-induced the caspase 3 activity. (Fig. 3B).

3.4. GA attenuated infiltration of neutrophils in liver

It is well known that infiltration of neutrophils in liver parenchyma is a vital hallmark of inflammatory response in acute liver injury. We measured the infiltration of neutrophils by immunofluorescence and flow cytometry, the results (Fig. 4A and B) showed that the number of Ly6G+ neutrophils was significantly increased in I/R group compared with control group, as expected, pretreatment with GA evidently inhibited the infiltration of neutrophils.

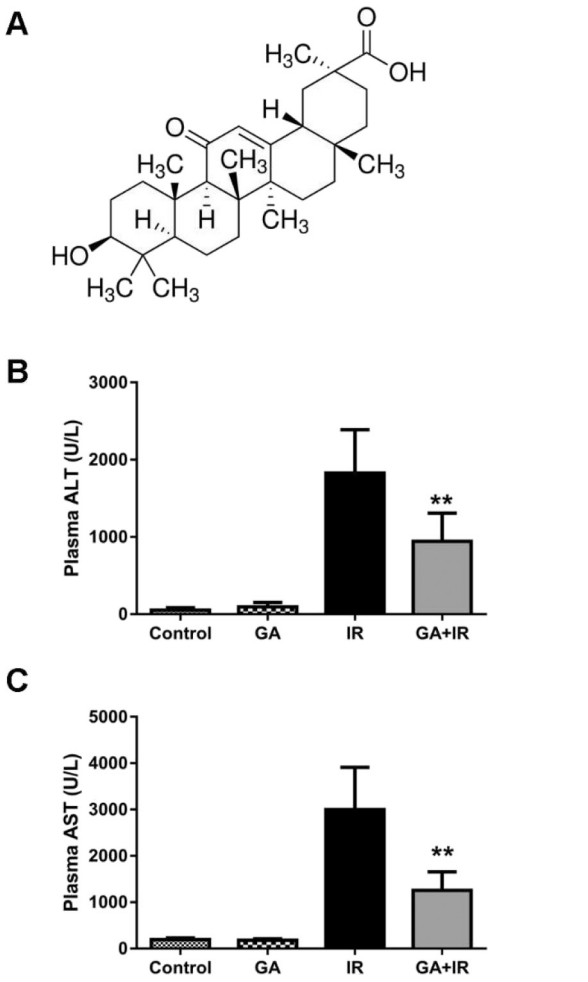

Fig. 1. Effect of GA on serum levels of ALT and AST. Mice ($n = 6$) were given GA at a dose of 100 mg/kg three times (8 h per time) within 24 h before liver I/R. After 6 h reperfusion, the blood were collected and analyzed for serum levels of ALT and AST. (A) Structure of Glycyrrhetinic acid. (B) The level of serum ALT. (C) The level of serum AST. ** $P < 0.01$ compared with I/R model group. GA: glycyrrhetinic acid; I/R: ischemia/reperfusion; ALT: alanine aminotransferase; AST: aspartate aminotransferase.

3.5. GA reduced pro-inflammatory cytokines production induced by hepatic I/R

The expression levels of TNF-α and IL-1β mRNA and protein in liver tissues and serum were detected by RT-PCR and ELISA, respectively. As presented in Fig. 5, I/R injury significantly increased the expression levels of inflammatory mediators TNF-α and IL-1β mRNA and protein in liver tissues and serum, while the GA intervention group reduced these increased expression levels efficiently, with statistically significant differences.

3.6. GA decreased expression of HMGB1 induced by hepatic I/R

As HMGB1, a member of the HMG proteins mainly released from necrotic or apoptotic cells in the early phase of hepatic I/R, was reported to trigger innate immune response in tissues injury, we then explored the level of HMGB1 expression after GA pretreatment. As expected, our data showed that the level of HMGB1 expression was significantly increased after hepatic I/R, while this increase was markedly inhibited by GA pretreatment (Fig. 6A).

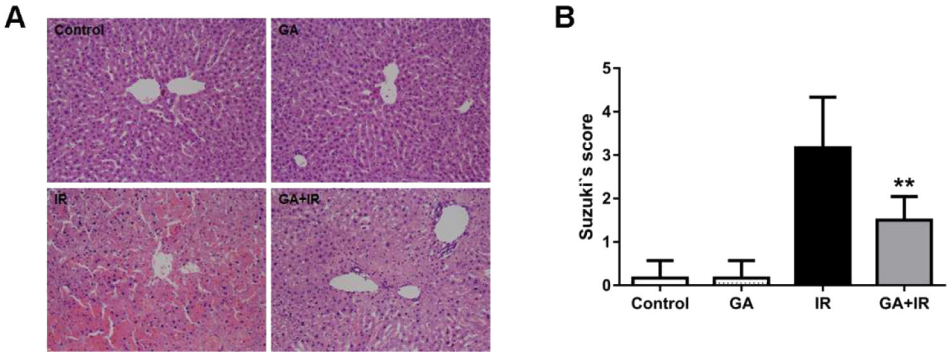

Fig. 2. Effect of GA on histopathological changes of hepatic tissues. GA(100 mg/kg) was administered to mice three times (8 h per time) a day before the surgery, and mice were euthanized after reperfusion for 6 h. The liver tissues were collected and stained with H&E, (A) H&E staining, original magnification ×200. (B) Suzuki's histological grading. Results were mean ± SD (n = 6), ** P < 0.01 versus I/R model group. GA: glycyrrhetinic acid; I/R: ischemia/reperfusion.

3.7. GA inhibited HMGB1-TLR4 signaling pathway after hepatic I/R

Finally, considering the effect of GA pretreatment on HMGB1 expression, we further determined whether this effect of GA pretreatment was associated with TLR4-dependent signal pathway, which was also known to have a major role in sterile inflammation induced by innate immune response. Interestingly, the phosphorylation levels of IRAK1, ERK, P38 and NF-κB were significantly elevated in I/R group, which were markedly inhibited by GA pretreatment. However, there was no significant difference in the levels of TLR4 mRNA and protein, and phosphorylation of JNK between I/R group and GA + I/R group (Fig. 6B and C).

4. Discussion

Liver I/R injury are a highly dynamic and complex process that involves initially local ischemic insult and subsequent inflammation-mediated reperfusion injury, resulting in poor early graft function and serious postoperative complication [2,24]. Although many strategies have been explored to alleviate I/R-induced liver injury, the specific strategies to prevent hepatic I/R injury are limited and supportive care is only the available treatment today [25]. In this study, we found that pretreatment with GA significantly reduced infarct size and improved liver function, which were associated with inhibition of apoptosis and infiltration of neutrophils. Besides, pretreatment with GA also decreased release of HMGB1 into the extracellular milieu as well as expression of phosphorylation IRAK1, ERK, P38 and NF-κB. These results suggested GA as a hepatoprotective drug which improved liver I/R injury by inhibition of the HMGB1-TLR4 signaling pathway. Neutrophils, the most abundant circulating leukocyte in humans, has been discovered for centuries and plays a pivotal role in innate immune response [26,27]. Once activated in inflammatory loci, neutrophils can kill invading pathogens by phagocytosis, secretion of preformed granular enzymes and proteins, release of ROS and production of neutrophil extracellular traps, thus protecting the host from infectious pathogens [28]. However, the highly cytotoxic capacity of neutrophil also leads to additional damage of healthy tissues in various infectious and inflammatory diseases [29]. Sterile inflammation (occurs in the absence of microorganisms), such as ischemia-reperfusion injury, trauma and chemically induced injury, is marked by abundant recruitment of neutrophils [4,30,31]. Inhibiting infiltration of neutrophils or activity of neutrophil elastase attenuated ischemia-reperfusion injury in rat liver [32,33]. In the current study, we evaluated the number of neutrophils infiltration in liver parenchyma by immunofluorescence and flow cytometry, and found that GA pretreatment significantly decreased neutrophils infiltration, which was consistent with the previous studies on anti-inflammation effect of GA. GA markedly attenuated acetaminophen-induced hepatic injury and reduced neutrophils recruitment [34]. In helicobacter pylori-infected gastric mucosa model, GA improved the gastritis by attenuation of neutrophils infiltration [35]. Taken together, these data indicated that GA might protect against I/R injury by reducing neutrophils infiltration.

Apoptosis, a form of programmed cell death preceding through either the extrinsic or intrinsic apoptosis pathways, is first formulated in 1972 and has been reported to play a vital role in generation of cell death results from various stress stimuli, such as hypoxia, oxidative stress and DNA damage [23,36]. During liver I/R injury, a number of liver parenchyma cells undergo cell death in the form of apoptosis after ischemic insult, and pharmacological interventions that blocking apoptosis have been demonstrated protective in reperfusion injury [37]. As expected, our results showed that hepatocytes apoptosis were markedly decreased after GA pretreatment compared with I/R group.

HMGB1, the prototypical endogenous DAMPs when released outside of the cell, has been confirmed for decades as an essential cell death marker released by necrotic cells [38]. Indeed, a number of data reported that administration of HMGB1 to mice caused systemic inflammatory responses, while treatment with anti-HMGB1 antibodies or specific antagonists protected mice from various inflammatory and autoimmune diseases, including sepsis, hepatitis, colitis, rheumatoid arthritis, atherosclerosis and trauma [39–44]. Moreover, it was reported that the level of HMGB1 was increased in a time-dependent manner from 1 h to 24 h after liver I/R, and the liver damage promoted

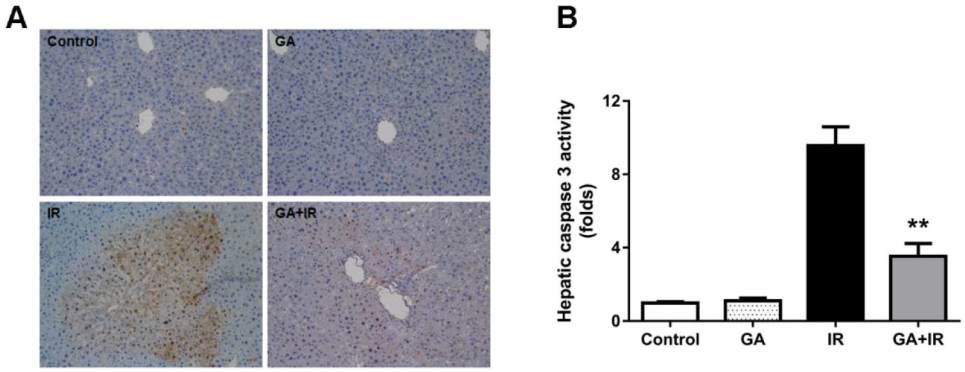

Fig. 3. Effect of GA on hepatic I/R-induced cell apoptosis. Mice were pretreated with GA (100 mg/kg) three times within 24 h before I/R surgery. (A) Representative images of TUNEL (200× magnification). (B) Activity of caspase-3. Results were mean ± SD (n = 6) ** P < 0.01 versus I/R model group. GA: glycyrrhetinic acid; I/R: ischemia/reperfusion.

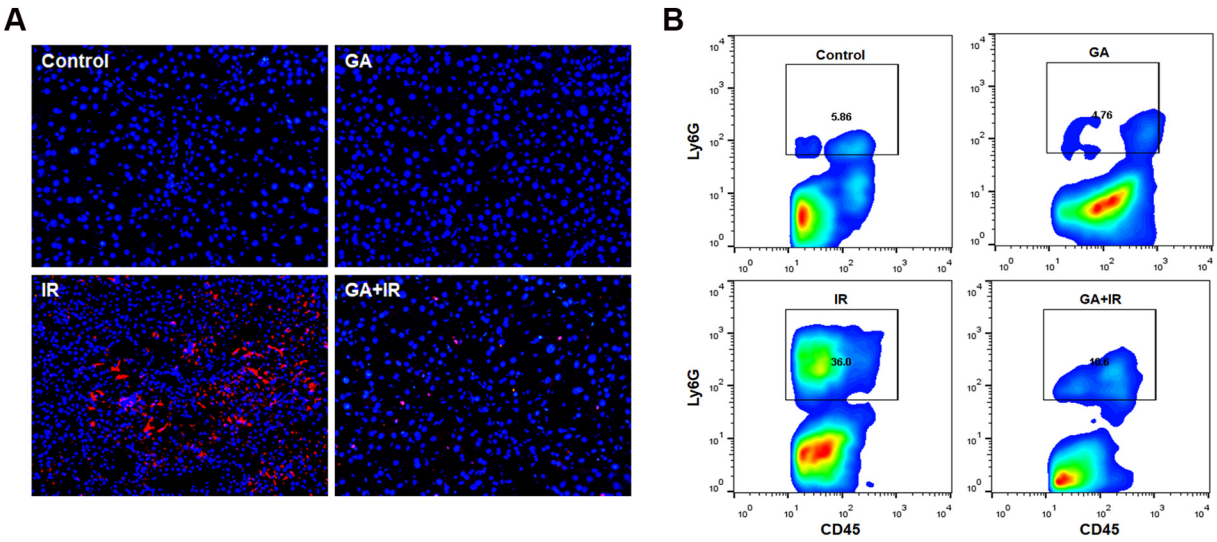

Fig. 4. Effect of GA on infiltration of neutrophils. Number of infiltrated granulocytes in ischemia liver tissues after 6 h reperfusion was analyzed by immunofluorescent assay and flow cytometry. (A) Results of neutrophils immunostaining with anti-Ly6G antibody in four groups (200 × magnification). (B) Number of neutrophils analyzed by flow cytometry. I/R: ischemia/reperfusion;GA: glycyrrhetinic acid.

by TLR4-dependent ROS production and downstream CaMK-mediated signaling was significantly attenuated after inhibition of HMGB1 with neutralizing antibody, [45,46]. In this study, GA pretreatment significantly reduced the level of HMGB1 that was increased after I/R injury. These data suggested that the hepatoprotective effects conferred by GA might be partially associated with inhibition of HMGB1 released extracellularly.

Since recent studies reported that extracellular HMGB1 activated cell signaling pathways of innate immune response by interacting with pattern recognition receptors, such as TLR4, integrins, receptor for advanced glycation end products (RAGE), and the triggering receptor expressed on myeloid cells-1 (TREM-1), among which the HMGB1-TLR4 axis is the most vital in driving immunopathology in a wide range of experimental models, including sterile and microbes-induced inflammation [39,47,48]. Indeed, the HMGB1-TLR4 receptor interaction is firstly confirmed in vivo by a model of liver I/R injury in 2005, during which it identified that the ischemia insult is a potent trigger for the passive release of HMGB1, and mice with TLR4 defection or mice subjected to anti-HMGB1 antibody treatment all exhibited less damage after hepatic I/R respectively, however, treatment with anti-HMGB1 antibody failed to provide protection in TLR4-defective mice, implicating that TLR4 was essential to HMGB1-induced liver I/R injury [46]. Considering that the gene transcription and translation are two relatively independent processes, and the regulation has temporal and spatial effects, thus the differences in mRNA and protein expression levels of TLR4 could be explained [49]. To determine the detail mechanisms of TLR4 signal pathway after GA pretreatment in liver I/R, we explored TLR4 expression and its downstream signaling molecules activation. It is known to all that TLR4 signaling pathway is initiated by interacting of DAMPs and pattern recognition receptors, and caused phosphorylation of a series of protein kinases including IRAK1, ERK, JNK, and p38 MAPK, leading to generation of NF-κB and AP-1 in innate immune response [10]. IRAK1, a member of IRAK family mediating TLRs/IL-1Rs signaling pathways, plays a prominent role in initiating a cascade of signaling events and leading to production of inflammatory cytokines, and down-regulation of IRAK1 exhibited less tissue damage

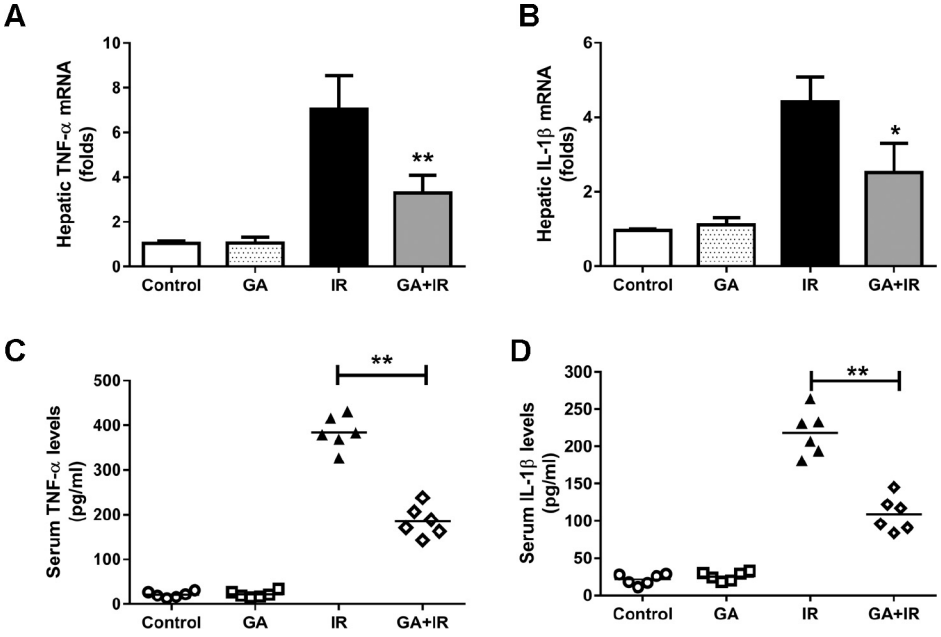

Fig. 5. Effect of GA on pro-inflammatory-cytokines release. Serum and liver tissues were collected after 6 h reperfusion. Hepatic TNF-α (A) and IL-1β mRNA were quantified by RT-PCR, and serum TNF-α and IL-1β protein were determined by ELISA. Data are expressed as mean ± SD. * $P < 0.05$, ** $P < 0.01$ versus I/R model group. GA: glycyrrhetinic acid; I/R: ischemia/reperfusion.

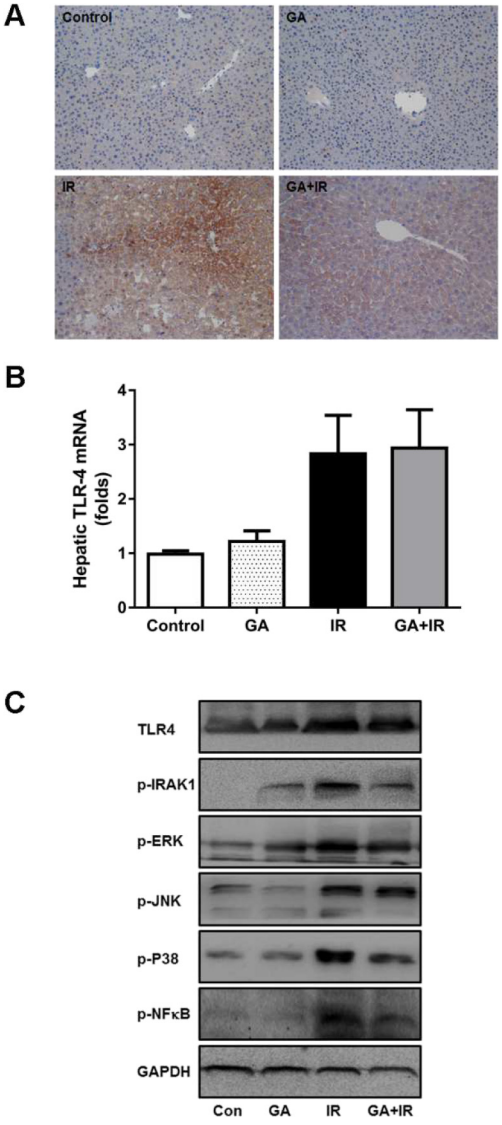

Fig. 6. Effect of GA on TLR4 signaling pathway and HMGB1 expression. After 6 h reperfusion, the liver tissues were collected for analysis of HMGB1 and TLR4 signaling pathway by immunohistochemistry, western blot, and RT-PCR, respectively. (A) The expression of HMGB1 expression in liver tissues. (B) The level of TLR4 mRNA in liver tissues. (c) The levels of proteins in TLR4 signaling pathway. Data are expressed are expressed as mean ± SD GA: glycyrrhetinic acid; I/R: ischemia/reperfusion.

in acute stress [50–53]. Inhibition of IRAK1 and down-regulation of MAPK cascade exhibited less tissue damage in I/R injury by decreasing oxidative stress, apoptosis and inflammation [54–56]. Herein, our present study indicated that GA pretreatment inhibited the level of phosphorylated IRAK1 as well as the phosphorylation of ERK, p38 MAPK and NF-κB, indicating that GA could effectively inhibit TLR4 signal activation, although there was no significant difference in TLR4 expression and phosphorylation of JNK between I/R group and GA + I/R group. In fact, the sustained JNK activation after GA pretreatment might be associated with inhibition of NF-kB activation, during which NF-kB activation played a negative role in modulating TNF-α-mediated JNK activation [57,58]. In summary, all these results suggested that GA might protect against hepatic I/R injury by inhibition of the HMGB1-TLR4 signaling pathway.

In conclusion, GA attenuated liver I/R injury by minimizing inflammation and reducing apoptosis, which might be associated with inhibiting the HMGB1-TLR4 signaling pathway.

Declaration of competing interest

The authors declare no competing financial interests.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (NO. 81200540).

References

[1] L. Lu, H. Zhou, M. Ni, X. Wang, R. Busuttil, J. Kupiec-Weglinski, Y. Zhai, Innate immune regulations and liver ischemia-reperfusion injury, Transplantation 100 (2016) 2601–2610.
[2] Y. Zhai, H. Petrowsky, J.C. Hong, R.W. Busuttil, J.W. Kupiec-Weglinski, Ischaemia-reperfusion injury in liver transplantation–from bench to bedside, Nat Rev Gastroenterol Hepatol 10 (2013) 79–89.
[3] H.K. Eltzschig, T. Eckle, Ischemia and reperfusion–from mechanism to translation, Nat. Med. 17 (2011) 1391–1401.
[4] G.Y. Chen, G. Nunez, Sterile inflammation: sensing and reacting to damage, Nat. Rev. Immunol. 10 (2010) 826–837.
[5] D.R. Green, T. Ferguson, L. Zitvogel, G. Kroemer, Immunogenic and tolerogenic cell death, Nat. Rev. Immunol. 9 (2009) 353–363.
[6] T.O. Crisan, M.G. Netea, L.A. Joosten, Innate immune memory: implications for host responses to damage-associated molecular patterns, Eur. J. Immunol. 46 (2016) 817–828.
[7] G.P. Sims, D.C. Rowe, S.T. Rietdijk, R. Herbst, A.J. Coyle, HMGB1 and RAGE in inflammation and cancer, Annu. Rev. Immunol. 28 (2010) 367–388.
[8] H. Yang, H. Wang, S.S. Chavan, U. Andersson, High mobility group box protein 1 (HMGB1): the prototypical endogenous danger molecule, Mol. Med. 21 (Suppl. 1) (2015) S6–s12.
[9] A. Poltorak, X. He, I. Smirnova, M.Y. Liu, C. Van Huffel, X. Du, D. Birdwell, E. Alejos, M. Silva, C. Galanos, M. Freudenberg, P. Ricciardi-Castagnoli, B. Layton, B. Beutler, Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene, Science 282 (5396) (1998) 2085–2088.
[10] T. Kawai, S. Akira, TLR signaling, Cell Death Differ. 13 (2006) 816–825.
[11] M.S. Hayden, S. Ghosh, Shared principles in NF-kappaB signaling, Cell 132 (2008) 344–362.
[12] A. Shimamoto, A.J. Chong, M. Yada, S. Shomura, H. Takayama, A.J. Fleisig, M.L. Agnew, C.R. Hampton, C.L. Rothnie, D.J. Spring, T.H. Pohlman, H. Shimpo, E.D. Verrier, Inhibition of toll-like receptor 4 with eritoran attenuates myocardial ischemia-reperfusion injury, Circulation 114 (1 Suppl) (2006) I270–I274.
[13] Y. Qi, M. Zhao, Y. Bai, L. Huang, W. Yu, Z. Bian, M. Zhao, X. Li, Retinal ischemia/reperfusion injury is mediated by toll-like receptor 4 activation of NLRP3 inflammasomes, Invest. Ophthalmol. Vis. Sci. 55 (2014) 5466–5475.
[14] H. Wu, G. Chen, K.R. Wyburn, J. Yin, P. Bertolino, J.M. Eris, S.I. Alexander, A.F. Sharland, S.J. Chadban, TLR4 activation mediates kidney ischemia/reperfusion injury, J. Clin. Invest. 117 (2007) 2847–2859.
[15] J. Kanellis, F.Y. Ma, R. Kandane-Rathnayake, J.P. Dowling, K.R. Polkinghorne, B.L. Bennett, G.C. Friedman, D.J. Nikolic-Paterson, JNK signalling in human and experimental renal ischaemia/reperfusion injury, Nephrol. Dial. Transplant. 25 (2010) 2898–2908.
[16] J.R. Caso, J.M. Pradillo, O. Hurtado, J.C. Leza, M.A. Moro, I. Lizasoain, Toll-like receptor 4 is involved in subacute stress-induced neuroinflammation and in the worsening of experimental stroke, Stroke 39 (2008) 1314–1320.
[17] H. Meimei, M. Dejin, C. Erzhen, S. Minmin, J. Songyao, L. Jianfang, C. Hao, Inhibiting the toll-like receptor 4 toll/interleukin-1 receptor domain protects against hepatic warm ischemia and reperfusion injury in mice, Crit. Care Med. 42 (2014) e123–e131.
[18] L. Cao, W. Ding, R. Jia, J. Du, T. Wang, C. Zhang, Z. Gu, G. Yin, Anti-inflammatory and hepatoprotective effects of glycyrrhetinic acid on CCl4-induced damage in precision-cut liver slices from Jian carp (Cyprinus carpio var. jian) through inhibition of the nf-kB pathway, Fish Shellfish Immunol 64 (2017) 234–242.
[19] X. Yin, X. Gong, L. Zhang, R. Jiang, G. Kuang, B. Wang, X. Chen, J. Wan, Glycyrrhetinic acid attenuates lipopolysaccharide-induced fulminant hepatic failure in d-galactosamine-sensitized mice by up-regulating expression of interleukin-1 receptor-associated kinase-M, Toxicol. Appl. Pharmacol. 320 (2017) 8–16.
[20] G. Yang, L. Wang, Protective effect of 18beta-glycyrrhetinic acid against Triptolide-induced hepatotoxicity in rats, 2017 (2017) 3470320.
[21] X. Wu, L. Zhang, E. Gurley, E. Studer, J. Shang, T. Wang, C. Wang, M. Yan, Z. Jiang, P.B. Hylemon, A.J. Sanyal, W.M. Pandak Jr., H. Zhou, Prevention of free fatty acid-induced hepatic lipotoxicity by 18beta-glycyrrhetinic acid through lysosomal and mitochondrial pathways, Hepatology 47 (2008) 1905–1915.
[22] H.J. Wu, J.Y. Yang, M. Jin, S.Q. Wang, D.L. Wu, Y.N. Liu, X. Yan, C. Yang, G. Zhang, J. He, Glycyrrhetinic acid protects the heart from ischemia/reperfusion injury by attenuating the susceptibility and incidence of fatal ventricular arrhythmia during the reperfusion period in the rat hearts, Cell. Physiol. Biochem. 36 (2015) 741–752.
[23] L. Campisi, R.J. Cummings, J.M. Blander, Death-defining immune responses after apoptosis, Am. J. Transplant. 14 (2014) 1488–1498.
[24] C. Nastos, K. Kalimeris, N. Papoutsidakis, M.K. Tasoulis, P.M. Lykoudis, K. Theodoraki, D. Nastou, V. Smyrniotis, N. Arkadopoulos, Global consequences of liver ischemia/reperfusion injury, Oxidative Med. Cell. Longev. 2014 (2014)

906965.
[25] L.A. Orci, C. Toso, G. Mentha, P. Morel, P.E. Majno, Systematic review and meta-analysis of the effect of perioperative steroids on ischaemia-reperfusion injury and surgical stress response in patients undergoing liver resection, Br. J. Surg. 100 (2013) 600–609.
[26] C.D. Sadik, N.D. Kim, A.D. Luster, Neutrophils cascading their way to inflammation, Trends Immunol. 32 (2011) 452–460.
[27] C. Nathan, Neutrophils and immunity: challenges and opportunities, Nat. Rev. Immunol. 6 (2006) 173–182.
[28] J. Wang, H. Arase, Regulation of immune responses by neutrophils, Ann. N. Y. Acad. Sci. 1319 (2014) 66–81.
[29] C. Summers, S.M. Rankin, A.M. Condliffe, N. Singh, A.M. Peters, E.R. Chilvers, Neutrophil kinetics in health and disease, Trends Immunol. 31 (2010) 318–324.
[30] B. McDonald, K. Pittman, G.B. Menezes, S.A. Hirota, I. Slaba, C.C. Waterhouse, P.L. Beck, D.A. Muruve, P. Kubes, Intravascular danger signals guide neutrophils to sites of sterile inflammation, Science 330 (2010) 362–366.
[31] H. Jaeschke, T. Hasegawa, Role of neutrophils in acute inflammatory liver injury, Liver Int. 26 (2006) 912–919.
[32] Y. Yamaguchi, E. Akizuki, O. Ichiguchi, F. Matsumura, M. Goto, N. Miyanari, K. Mori, S. Yamada, M. Ogawa, Neutrophil elastase inhibitor reduces neutrophil chemoattractant production after ischemia-reperfusion in rat liver, Gastroenterology 112 (1997) 551–560.
[33] S. Suzuki, L.H. Toledo-Pereyra, F.J. Rodriguez, D. Cejalvo, Neutrophil infiltration as an important factor in liver ischemia and reperfusion injury. Modulating effects of FK506 and cyclosporine, Transplantation 55 (1993) 1265–1272.
[34] G. Yang, L. Zhang, L. Ma, R. Jiang, G. Kuang, K. Li, H. Tie, B. Wang, X. Chen, T. Xie, X. Gong, J. Wan, Glycyrrhetinic acid prevents acetaminophen-induced acute liver injury via the inhibition of CYP2E1 expression and HMGB1-TLR4 signal activation in mice, Int. Immunopharmacol. 50 (2017) 186–193.
[35] D. Cao, J. Jiang, L. You, Z. Jia, T. Tsukamoto, H. Cai, S. Wang, Z. Hou, Y.E. Suo, X. Cao, The protective effects of 18beta-glycyrrhetinic acid on helicobacter pylori-infected gastric mucosa in Mongolian gerbils, Biomed. Res. Int. 2016 (2016) 4943793.
[36] J.F. Kerr, A.H. Wyllie, A.R. Currie, Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics, Br. J. Cancer 26 (1972) 239–257.
[37] F. Eefting, B. Rensing, J. Wigman, W.J. Pannekoek, W.M. Liu, M.J. Cramer, D.J. Lips, P.A. Doevendans, Role of apoptosis in reperfusion injury, Cardiovasc. Res. 61 (2004) 414–426.
[38] D.V. Krysko, P. Agostinis, O. Krysko, A.D. Garg, C. Bachert, B.N. Lambrecht, P. Vandenabeele, Emerging role of damage-associated molecular patterns derived from mitochondria in inflammation, Trends Immunol. 32 (2011) 157–164.
[39] H. Yang, H. Wang, C.J. Czura, K.J. Tracey, The cytokine activity of HMGB1, J. Leukoc. Biol. 78 (2005) 1–8.
[40] C. Wang, H. Nie, K. Li, Y.X. Zhang, F. Yang, C.B. Li, C.F. Wang, Q. Gong, Curcumin inhibits HMGB1 releasing and attenuates concanavalin A-induced hepatitis in mice, Eur. J. Pharmacol. 697 (2012) 152–157.
[41] R. Vitali, F. Palone, S. Cucchiara, A. Negroni, L. Cavone, M. Costanzo, M. Aloi, A. Dilillo, L. Stronati, Dipotassium glycyrrhizate inhibits HMGB1-dependent inflammation and ameliorates colitis in mice, PLoS One 8 (2013) e66527.
[42] Y.B. Li, P. Xu, K. Xu, Y.S. Cai, M.Y. Sun, L. Yang, J. Sun, S.M. Lu, Methotrexate affects HMGB1 expression in rheumatoid arthritis, and the downregulation of HMGB1 prevents rheumatoid arthritis progression, Mol. Cell. Biochem. 420 (2016) 161–170.
[43] Y. Hirata, H. Kurobe, M. Higashida, D. Fukuda, M. Shimabukuro, K. Tanaka, Y. Higashikuni, T. Kitagawa, M. Sata, HMGB1 plays a critical role in vascular inflammation and lesion formation via toll-like receptor 9, Atherosclerosis 231 (2013) 227–233.
[44] X. Ruan, S.S. Darwiche, C. Cai, M.J. Scott, H.C. Pape, T.R. Billiar, Anti-HMGB1 monoclonal antibody ameliorates immunosuppression after peripheral tissue trauma: attenuated T-lymphocyte response and increased splenic CD11b (+) Gr-1 (+) myeloid-derived suppressor cells require HMGB1, Mediat. Inflamm. 2015 (2015) 458626.
[45] A. Tsung, J.R. Klune, X. Zhang, G. Jeyabalan, Z. Cao, X. Peng, D.B. Stolz, D.A. Geller, M.R. Rosengart, T.R. Billiar, HMGB1 release induced by liver ischemia involves Toll-like receptor 4 dependent reactive oxygen species production and calcium-mediated signaling, J. Exp. Med. 204 (2007) 2913–2923.
[46] A. Tsung, R. Sahai, H. Tanaka, A. Nakao, M.P. Fink, M.T. Lotze, H. Yang, J. Li, K.J. Tracey, D.A. Geller, T.R. Billiar, The nuclear factor HMGB1 mediates hepatic injury after murine liver ischemia-reperfusion, J. Exp. Med. 201 (2005) 1135–1143.
[47] R. Kang, R. Chen, Q. Zhang, W. Hou, S. Wu, L. Cao, J. Huang, Y. Yu, X.G. Fan, Z. Yan, X. Sun, H. Wang, Q. Wang, A. Tsung, T.R. Billiar, H.J. Zeh 3rd, M.T. Lotze, D. Tang, HMGB1 in health and disease, Mol. Asp. Med. 40 (2014) 1–116.
[48] A. Tsung, S. Tohme, T.R. Billiar, High-mobility group box-1 in sterile inflammation, J. Intern. Med. 276 (2014) 425–443.
[49] J. Rivera-Torres, Analysis of gene and Protein expression in atherosclerotic mouse aorta by Western blot and quantitative real-time PCR, J. Methods Mol Biol. 1399 (2015) 309–322.
[50] S. Gottipati, N.L. Rao, W.P. Fung-Leung, IRAK1: a critical signaling mediator of innate immunity, Cell. Signal. 20 (2008) 269–276.
[51] C. Chassin, C. Hempel, S. Stockinger, A. Dupont, J.F. Kubler, J. Wedemeyer, A. Vandewalle, M.W. Hornef, MicroRNA-146a-mediated downregulation of IRAK1 protects mouse and human small intestine against ischemia/reperfusion injury, EMBO Mol. Med. 4 (2012) 1308–1319.
[52] E.H. Joh, J.J. Jeong, D.H. Kim, Kalopanaxsaponin B inhibits LPS-induced inflammation by inhibiting IRAK1 kinase, Cell. Immunol. 279 (2012) 103–108.
[53] N. Singh, L. Li, Reduced oxidative tissue damage during endotoxemia in IRAK-1 deficient mice, Mol. Immunol. 50 (2012) 244–252.
[54] D. Yu, M. Li, Y. Tian, J. Liu, J. Shang, Luteolin inhibits ROS-activated MAPK pathway in myocardial ischemia/reperfusion injury, Life Sci. 122 (2015) 15–25.
[55] J.H. Chen, H.C. Kuo, K.F. Lee, T.H. Tsai, Magnolol protects neurons against ischemia injury via the downregulation of p38/MAPK, CHOP and nitrotyrosine, Toxicol. Appl. Pharmacol. 279 (2014) 294–302.
[56] J. Chen, W. Wang, Q. Zhang, F. Li, T. Lei, D. Luo, H. Zhou, B. Yang, Low molecular weight fucoidan against renal ischemia-reperfusion injury via inhibition of the MAPK signaling pathway, PLoS One 8 (2013) e56224.
[57] G. Tang, Y. Minemoto, B. Dibling, N.H. Purcell, Z. Li, M. Karin, A. Lin, Inhibition of JNK activation through NF-kappaB target genes, Nature 414 (2001) 313–317.
[58] D. Javelaud, F. Besancon, NF-kappa B activation results in rapid inactivation of JNK in TNF alpha-treated Ewing sarcoma cells: a mechanism for the anti-apoptotic effect of NF-kappa B, Oncogene 20 (2001) 4365–4372.