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The role of inflammasome activation in Triptolide-induced acute liver toxicity

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ABSTRACT

Triptolide (TP), the major active compound derived from the traditional Chinese medicine *Tripterygium wilfordii* Hook. F, possesses an excellent pharmacological profile of immunomodulatory and anti-tumor activities. However, the application of TP was restricted due to its narrow therapeutic window and side effects, especially its hepatotoxicity. This study was designed to investigate the role of inflammasome in TP-induced acute liver toxicity. After the administration of TP at the dose of 600 μg/kg for 12 h or 24 h, we examined the serum biochemical parameters, liver histopathological changes, the expression of liver inflammatory factors, and the activation of NLRP3 inflammasome. Mice treated with TP displayed liver injury with a time-dependent increase of serum transaminases and activation of NLRP3 inflammasome, accompanied by the elevation of neutrophils infiltration. Further results implied that the activation of TLR4-Myd88-NF-κB pathway and oxidative stress induced by a single dose of TP (600 μg/kg) might participate in the activation of NLRP3 inflammasome. To investigate whether the activation of inflammasome participates in the liver damage induced by TP, a single dose of Ac-Yvad-Cmk (Caspase-1 inhibitor) was injected before TP administration. Ac-Yvad-Cmk pretreatment effectively prevented the increase of Cleaved Caspase-1 and inhibited the maturity of IL-1β. Additional studies revealed that Ac-Yvad-Cmk pretreatment decreased the recruitment of neutrophils and inhibited the production of massive pro-inflammatory factors. Taken together, our results revealed that activation of inflammasome aggravated the acute liver toxicity induced by TP. Inhibition of inflammasome could serve as a novel therapeutic target for the amelioration of TP-induced hepatotoxicity.

1. Introduction

Triptolide (TP), the major active compound derived from a well-known Chinese Traditional Medicine, *Tripterygium wilfordii* Hook. f (TWHF), exhibits valuable bioactivities, including anti-tumor, anti-proliferative, and immunoregulatory activities [1]. Although it possesses numerous pharmacological activities, the side effects and multiple organ toxicities limit TP's wide application. Accumulating evidence suggests that TP could induce severe adverse reactions, including hepatotoxicity, nephrotoxicity, reproductive, and cardiac toxicity, among which the hepatotoxicity has a major concern [2,3].

Previous studies revealed that TP-induced hepatotoxicity was related to mitochondrial impairment, P450s inhibition, lipid peroxidation, glucose metabolism disorder, and inactivation of Sirt1/FXR [4–7]. However, it is unclear whether the immune system is involved in the hepatotoxicity of TP. Our previous study revealed that Th17/Treg imbalance was involved in the acute liver injury induced by TP while anti-IL-17 antibody and adoptive transfer Treg ameliorated TP-induced liver injury, indicating that the adaptive immune response was tightly related to TP-induced acute liver toxicity [8–10]. Nevertheless, how

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innate immune response is involved in TP-induced liver toxicity remained elusive.

As one of the vital organ of the immune system in the body, the liver possesses a special physiological structure that receives the venous blood from the gut through the portal vein and the arterial blood from the hepatic artery. The blood from the portal vein and the hepatic artery is rich in bacterial products, environmental toxins, and food antigens, exposing the liver to sustain stimulation with toxic agents [11–13]. For the removal of potential toxicological agents from the body without initiating a harmful immune response, the liver gradually develops a strong immune system consisting of a large number of innate immune cells [11]. In the initial step of the innate immune response, pattern-recognition receptors (PRRs) play an essential role in the recognition of pathogen-associated molecular patterns (PAMPs) from pathogens and damage-associated molecular patterns (DAMPs) from damaged tissue or necrotic cells [14]. The physiological inflammatory response could help the repairment of the damaged tissue, while excessive inflammatory response might aggravate the tissue damage through the recruitment of circulating leukocytes characterized by the release of uncontrollable inflammatory cytokines and chemokines [15,16].

Among the five PRRs, Toll-like receptors (TLRs), Rig-I-like receptors (RLRs), NOD-like receptors (NLRs), C-type lectin receptors, and AIM2-like receptors (ALRs), NLRs and ALRs promoted the host defense in response to invading pathogens or other harmful signals through the assembly of cytoplasmic signaling complexes, termed inflammasome [17,18]. The inflammasome is the cytosolic multiprotein platform consisted of a sensor protein belongs to NLRs or ALRs, an adaptor protein, ASC, and the cysteine protease, Pro-Caspase-1. The activation of inflammasome always leads to the cleavage of Pro-Caspase-1 into its active form, which further cleaves the inflammatory cytokines, pro-IL-1β and pro-IL-18, into their mature form. Additionally, the activation of inflammasome could also result in the inflammatory form of programmed cell death, called pyroptosis [19]. Besides Caspase-1-dependent canonical inflammasome, Caspase-1-independent, but Caspase-11-dependent (murine) or Caspase-4 and Caspase-5-dependent (human) non-canonical inflammasome had been proposed based on their close similarity to Caspase-1 [20,21]. Murine Caspase-11, and its human homologs Caspase-4 and Caspase-5, are the intracellular receptor of LPS and can be activated by most gram-negative bacteria, such as *Citrobacter rodentium*, *Escherichia coli*, and *Vibrio cholera* [22]. Upon activation, Caspase-11 or Caspase-4 and Caspase-5 could directly induce pyroptosis and releasing the endogenous dangerous molecules in a Caspase-1-independent manner [23]. Also, Caspase-11 could induce mature IL-1β and IL-18 in a Caspase-1-dependent manner [23]. Among the five inflammasomes (NLRP1, NLRP3, NLRC4, Pyrin, and AIM2), the NLRP3 inflammasome has been thoroughly investigated, given its essential role in the development of human diseases [14,24].

The activation of NLRP3 inflammasome is a two-step process. The priming step involves the activation of TLRs, IL-1R, and TNF-R, which results in increasing the transcriptional activity of NF-κB and leading to the up-regulation of the components of NLRP3 inflammasome. The activation step is associated with several cellular events, including potassium flux, lysosomal disruption, calcium signaling, and the production of mitochondrial ROS [19,25]. Upon activation, the NLRP3 inflammasome serves as a platform, prompting the cleavage of Pro-Caspase-1 which in turn cleaves pro-inflammatory cytokines such as pro-IL-1β and pro-IL-18 into their mature form [19]. Recent studies show that several liver diseases were accompanied with the activation of NLRP3 inflammasome, including acetaminophen (APAP)-induced liver injury, liver ischemia-reperfusion injury, viral hepatitis, and liver fibrosis [19,26–28]. The main role of NLRP3 inflammasome in liver diseases has been attributed to the presence of liver immune cells and their ability to trigger inflammation via IL-1β. The autocrine and paracrine release of IL-1β might enhance the transcription of inflammatory cytokines and chemokines, such as tumor necrosis factor alpha (TNF-α) and monocyte chemotactic protein 1 (MCP-1). Also, IL-1β could promote the recruitment of inflammatory cells to the liver and even together with TNF-α trigger the death of hepatocytes [29].

Based on our previous work on TP-induced hepatotoxicity associated with metabolism disorder, as well as the close relationship between inflammasome (especially NLRP3 inflammasome) activation and the progression of the metabolic disease, we supposed the activation of inflammasome might contribute to TP-induced hepatotoxicity [7,30–32]. In this article, we aimed to determine the role of inflammasome (especially NLRP3 inflammasome) in the liver toxicity induced by TP. In addition, we explored the possible mechanisms of NLRP3 inflammasome activation and the participation of inflammasome activation in the liver damage induced by TP.

2. Materials and methods

2.1. Reagents

Triptolide (> 98%, HPLC) was purchased from Sanling Biotech (Guilin, China). Ac-Yvad-Cmk (purity > 95%, HPLC) was purchased from Sigma-Aldrich (MO, USA). Antibodies against NLRP3 (15101), IL-1β (31202) for western blot were purchased from Cell Signaling Technology (MA, USA). Antibodies against ASC (sc-22514-R), Cleaved Caspase-1 (A0914), and GAPDH (AF0006) for western blot were purchased from Santa Cruz Biotechnology (CA, USA), Abclonal Biotechnology (Wuhan, China) and Beyotime Biotechnology (Shanghai, China) respectively. Antibody against MPO (22225-1-AP) for immunohistochemistry (IHC) was purchased from Proteintech (Wuhan, China).

2.2. Animals and experiment treatments

C57BL/6J mice (Female, 18–20 g, 6–8 weeks of age) were purchased from the Vital River Laboratory Animal Technology (Beijing China). All the mice were housed in the pathogen-free conditions (temperature: 22 ± 2 °C, relative humidity: 40 ± 10%) with photoperiod (12 h of light and 12 h of dark) and given free access to food and water. All the procedures for the animal study were performed according to the guidelines for the care and use of animals approved by the Ethical Committee of China Pharmaceutical University and the Laboratory Animal Management Committee of Jiangsu Province (Approval No. 2110748). All the data presented in the manuscript were made from three independent experiments to verify the reproducibility and the most critical independent replicates were presented in Fig.S1 and Fig.S2.

To analyze the activation of NLRP3 in TP-induced hepatotoxicity, 48 mice were divided randomly into four groups (n = 8/group). TP (600 μg/kg) or the same volume of solvent was administered intragastrically. After the administration of TP for 12 h or 24 h respectively, mice were sacrificed while blood and liver samples were collected at the indicated time intervals.

To explore whether the activation of NLRP3 inflammasome participates in the progression of hepatotoxicity induced by TP, Ac-Yvad-Cmk (12.5 μmol/kg), dissolved in DMSO and PBS (1:9, v/v), was given intraperitoneally 30 min before the gavage of TP (600 μg/kg) and all the groups (n = 8/group) were given the same volume of solvent. After the treatment of TP for 24 h, the blood and liver samples were collected for further analysis.

2.3. Blood chemistry analysis

To examine the serum biochemical index, the blood collected from the mice was kept at room temperature for 1 h. Soon after, the blood was centrifuged at 3500 rpm for 10 min to get the supernatant for further detection. Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) were measured by assay kits from

Whitman Biotech (Nanjing, China). All the kits were operated according to the manufacturer's instructions.

2.4. Histopathological evaluations

The liver sections from mice were fixed in 10% formaldehyde for > 24 h and embedded with paraffin for hematoxylin and eosin (H & E) staining and immunohistochemistry respectively. For histopathology, H &E staining was carried out to investigate the liver morphological changes while MPO staining was used to investigate the neutrophil infiltration between the groups.

2.5. Measurement of MDA and SOD in the liver tissue

After sacrifice, liver tissues were weighed and homogenized with ice-cold physiological saline (1:10; w/v). After centrifugation, the supernatant was collected for further detection. Liver malondialdehyde (MDA, A003-1) and Superoxide dismutase (SOD, A001-3) were measured according to the instruction of the kit from Jiancheng Bioengineering Institute (Nanjing, China) and protein levels were normalized using BCA kit from Beyotime Biotechnology.

2.6. RNA extraction and real-time quantitative PCR

A small liver section was separated from the liver tissue and the entire RNA was isolated with a Trizol agent. After quantification of the RNA concentration with Nanodrop 2000 from Thermo Fisher Scientific (MA, USA), the RNA was reversed to cDNA according to the reverse transcription kit. Real-time quantitative PCR was performed with SYBR Green for targeted genes with the corresponding primers designed by Invitrogen (CA, USA). The primers used in this article are listed in Table 1 and GAPDH was used to normalize the quantity of cDNA. All the reagents used in real-time quantitative PCR were purchased from Vazyme Biotech (Nanjing, China).

2.7. Western blot analysis

The total protein from mice liver was extracted with RIPA buffer from Beyotime Biotechnology and the protein concentration was determined using the BCA kit. The protein was then mixed with 4 × loading buffer from Bio-Rad Laboratories (CA, USA). A total of 50 μg protein was separated by 8% and 12% SDS-PAGE and transferred to polyvinylidene fluoride membranes. After the blocking with 5% BSA at room temperature for 1 h, the membranes were incubated with primary antibody at 4 °C overnight respectively and then incubated with corresponding secondary antibody. The membranes were visualized with the ECL reagent from Millipore (MA, USA). The protein of GAPDH was served as the internal reference.

2.8. Statistical analysis

The data were expressed as the means ± SEM and analyzed using GraphPad Prism 6. One-way ANOVA followed by Bonferroni's Multiple Comparison Test and two-way ANOVA followed by Bonferroni posttests were performed to analyze the difference between groups. *P*-values < 0.05 were considered to be statistically significant.

3. Result

3.1. Time-dependent changes of TP-induced acute hepatotoxicity

In this experiment, C57BL/6J mice were administrated with TP at the dose of 600 μg/kg for 12 h or 24 h respectively according to the previous experiment [9]. To evaluate the hepatotoxicity induced by TP, serum ALT, AST, and liver/body weight were assayed. As shown in Fig. 1A to Fig. 1B and results in Fig.S1A to Fig.S1B from the independent replicates, TP treatment increased the serum ALT and AST in a time-dependent manner. Compared with the control group, although TP-treatment did not increase serum AST significantly at 12 h, it showed the increasing trend (Fig. 1B and Fig.S1B). In addition, TP-treatment slightly increased the liver/body weight ratio, indicating the liver damage induced by TP (Fig. 1C). H & E staining revealed the focal necrosis, reflected as the pyknosis, karyorrhexis, nucleus and cytoplasmic deficiency (Shown as white arrows) and inflammatory infiltration (Shown as black arrows) in the mouse liver, especially at 24 h after TP administration (Fig. 1D and Fig.S1E). These results implied that TP treatment could induce acute liver damage accompanied by inflammatory cell infiltration and hepatocellular necrosis.

3.2. The up-regulation of several pro-inflammatory factors and the recruitment of inflammatory cells were related to TP induced acute liver toxicity

To further prove the inflammatory cells infiltration after the administration of TP, we determined the mRNA levels of several pro-inflammation factors. The DAMPs released from dying hepatocytes and the PAMPs from pathogens bound to the PRRs expressed on the surface of Kupffer cells, which holds a central position in tissue injury, promoting the releasing of pro-inflammatory factors and chemokines, such as IL-1β, TNF-α, CXCL-1, CXCL-2, and CXCL-8. Among the cytokines related to hepatic diseases, TNF-α, IL-1β, and IL-6 are the most important ones. TP treatment enhanced the mRNA levels of TNF-α, IL-1β, and IL-6 at both 12 h and 24 h time intervals (Fig. 2A and Fig. 3D). In addition, TP increased the expression of chemokines, such as CXCL-1,

Table 1
The primer sequences used for real-time quantitative PCR assay in mice.

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)
NLRP3	TCACAACTCGCCCAAGGAGGAA	AAGAGACCACGGCAGAAGCTAG
ASC	CTTGTCAGGGGATGAACTCAAAA	GCCATACGACTCCAGATAGTAGC
Caspase-1	CTTGGAGACATCCTGTCAGGG	AGTCACAAGACCAGGCATATTCT
IL-1β	TGGACCTTCCAGGATGAGGACA	GTTCATCTCGGAGCCTGTAGTG
TNF-α	GGTGCCTATGTCTCAGCCTCTT	GCCATAGAACTGATGAGAGGGAG
IL-6	TACCACTTCACAAGTCGGAGGC	CTGCAAGTGCATCATCGTTGTTC
MCP-1	GCTACAAGAGGATCACCAGCAG	GTCTGGACCCATTCCTTCTTGG
CXCL-1	TCCAGAGCTTGAAGGTGTTGCC	AACCAAGGGAGCTTCAGGGTCA
CXCL-2	CATCCAGAGCTTGAGTGTGACG	GGCTTCAGGGTCAAGGCAAACT
CXCL-5	CCGCTGGCATTTCTGTTGCTGT	CAGGGATCACCTCCAAATTAGCG
MPO	CGTGTCAAGTGGCTGTGCCTAT	AACCAGCGTACAAAGGCACGGT
TLR4	AGCTTCTCCAATTTTTCAGAACTTC	TGAGAGGTGGTGTAAGCCATGC
Myd88	ACCTGTGTCTGGTCCATTGCCA	GCTGAGTGCAAACTTGGTCTGG
IκB-α	TGAAGGACGAGGAGTACGAGC	TTCGTGGATGATTGCCAAGTG
GAPDH	CTTTGGCATTGTGGAAGGGCTC	GCAGGGATGATGTTCTGGGCAG

CXCL-2, and CXCL-5 (Fig. 2A). An increase in both cytokines and chemokines might act as the inducer to attract the recruitment of neutrophils [33–36]. Neutrophils recruitment is not always responsible for hepatic inflammation, however, it can aggravate liver disease by producing additional cytotoxic reactive oxygen and nitrogen species or pro-inflammation cytokines to attract other neutrophils and monocytes [37]. An increase in CXCL-1, CXCL-2, and MCP-1 could also attract monocytes, while the recruitment of neutrophils was further proved by the IHC and mRNA levels of the neutrophil-specific enzyme myeloperoxidase (MPO) (Fig. 2A, Fig. 2B, and Fig. S1E) [15]. Indicators specific for oxidative stress were also detected. TP-treatment decreased the activity of SOD and increased the content of MDA, indicating the oxidative stress induced by TP (Fig. 2C, Fig. 2D, Fig. S1C, and Fig. S1D). Thus, in this part, we verified that TP treatment increased the expression of cytokines and chemokines thereby further recruiting the inflammatory cells, such as the neutrophils and monocytes.

3.3. The priming and activation of NLRP3 inflammasome in TP-induced acute liver toxicity

Given the fact that NLRP3 inflammasome and IL-1β are closely related to the progress of the liver diseases and play a role in amplifying the inflammatory reaction through the recruitment of inflammatory cells, we detected the mRNA and protein levels of NLRP3 inflammasome [27]. The activation of NLRP3 inflammasome requires two steps. The priming step results from the up-regulation of the transcription of NLRP3 components and the second step triggers by a variety of activators, ultimately leading to the cleavage of Pro-Caspase-1 and mature IL-1β. As described in Fig. 3A to Fig. 3E as well as the results of independent replicates presented in Fig.S1F, TP-treatment increased the mRNA levels of NLRP3, ASC, Caspase-1 together with IL-1β and up-regulated the protein levels of NLRP3, ASC, suggesting that TP-treatment engaged in the priming step of NLRP3 inflammasome. In addition, TP-treatment increased the protein levels of Cleaved Caspase-1 (P20) and mature IL-1β (P17), indicating the activation of NLRP3 inflammasome after TP-treatment (Fig. 3E, H, I, and Fig.S1F). As one of the two ways accounting for the production of IL-1β and the crucial role of IL-1β in the hepatic inflammatory response, we supposed that the activation of NLRP3 inflammasome could in some part cause the increase of hepatic inflammatory reactions induced by TP [36].

3.4. TLR4-Myd88-NF-κB pathway and oxidative stress induced by TP might be related to the activation of NLRP3 inflammasome

NLRP3 inflammasome formation requires a priming step that occurs through the activation of TLRs, IL-1R, and TNFR [25]. The activation of these pathways could result in the degradation of NF-κB inhibitor alpha (IκB-α) and activation of nuclear factor-κB (NF-κB), simultaneously leading to the increased transcription level of IκB-α [38]. The activation of NF-κB also enhanced the transcription of NLRP3 components as well as IL-1β. To further investigate the related pathway involved in the priming step of NLRP3 inflammasome, we detected the mRNA levels of TLR4, myeloid differentiation primary response 88 (Myd88) and IκB-α. As presented in Fig. 4A to Fig. 4C, TP-treatment enhanced the mRNA levels of TLR4, Myd88, and IκB-α in a time-dependent manner, suggesting that TLR4-Myd88-NF-κB pathway might be responsible for the priming step of NLRP3 inflammasome formation. Previous studies revealed that TP could impair the mitochondrial respiratory chain and enhance the oxidative stress, while the oxidative stress state and the mitochondrial-derived reactive oxygen species (ROS) severed as the activator for NLRP3 inflammasome [39,40]. The data presented in Fig. 2C, Fig. 2D as well as Fig.S1C and Fig.S1D revealed that the activity of hepatic SOD was significantly inhibited by TP, while TP treatment increased the hepatic MDA levels, suggesting that TP could disrupt the antioxidant defense system in the liver. Taken together, the up-regulation of the TLR4-Myd88-NF-κB pathway and the oxidative stress induced by TP might be the reasons accounting for the priming and activation steps of the NLRP3 inflammasome.

3.5. Inhibition of the activation of inflammasome alleviated TP induced liver injury

To explore whether the activation of inflammasome participate in the progress of liver toxicity induced by TP, we pretreated the mice with a Caspase-1 inhibitor (Ac-Yvad-Cmk) 30 min before the administration of TP to inhibit the cleavage of Pro-Caspase-1 to Cleaved Caspase-1. The results from Fig. 5A to Fig. 5E and the results of independent replicates presented in Fig.S2D revealed that Ac-Yvad-Cmk could dampen the activation of NLRP3 inflammasome induced by TP. Also, the hepatoprotective effect of Ac-Yvad-Cmk was reflected by the alleviation of serum ALT and AST (Fig. 6A, Fig. 6B, Fig.S2A, and Fig.S2B). Compared with the TP-treated group, although Ac-Yvad-Cmk pretreatment did not significantly decrease the liver/body weight ratio induced by TP, it only showed the decreasing trend (Fig. 6C). Inhibition of the activation of inflammasome also decreased the production of cytokines and weakened the recruitment of inflammatory cells after TP-treatment showed by IHC of MPO staining (Table.2, Fig. 6D, and Fig.S2C, shown as black arrows). The protective effect of Ac-Yvad-Cmk against TP was also confirmed by H&E staining (Fig. 6D and Fig.S2C), as inhibiting the activity of inflammasome reduced the inflammatory cells infiltration (Shown as black arrows) and necrotic area (Shown as white arrows) compared with the TP-treated group. Thus, we concluded that the activation of NLRP3 inflammasome resulted in the maturity of IL-1β, which further augmented the release of cytokines and recruited the inflammatory cells, such as the neutrophils and monocytes. The accumulation of inflammatory cells in the liver may also augment the liver damage induced by TP. Our speculation for the role of the inflammasome in the acute hepatotoxicity induced by TP was illustrated by Fig. 7.

4. Discussion

Drug-induced liver injury is the key factor responsible for impeding the development and application of drugs worldwide. Despite exhibiting the immunomodulatory and antitumor activities, the narrow therapeutic window and severe hepatotoxicity limit the TP's application. Considerable studies related to TP-induced hepatotoxicity have been published and several groups are working on synthesizing the analogs of TP, such as (5*R*)-5-hydroxytriptolide and minnelide, in order to avoid the occurrence of toxic reactions while retaining its bioactivity [3,41–45]. Nevertheless, a few studies focused on the immunotoxicity induced by TP. Accordingly, a more comprehensive understanding of TP's immunotoxicity is needed. This study proved the involvement of the innate immune system in the process of liver damage induced by TP. The fundamental role of the liver innate immune system relies on its PRRs expressed on the innate immune cells to sense the invading pathogens from the gut and the endogenous harmful signals from dying cells [46,47]. However, patients with chronic liver disease are always accompanied by the abnormal activation of PRRs through exposing the liver to excessive gut-derived endotoxins resulting from the decreased mucosal integrity and intestinal mucosal barrier, which could further contribute to liver inflammation and fibrogenesis [48]. Meanwhile, over-production of heat shock proteins, high mobility group box 1, and hyaluronan, the contents released from dying cells or damaged tissues, can also be recognized by PPRs expressed on liver cells and accelerate the development of liver disease via inducing the production of pro-inflammatory cytokines [14]. Given the essential role of PRRs in the deterioration of several liver diseases, we supposed that hyper-activation of PRRs might be related to TP-induced hepatotoxicity.

Published data pointed out that there is a connection between NLRP3 inflammasome and the adaptive immune response [30,49–51].

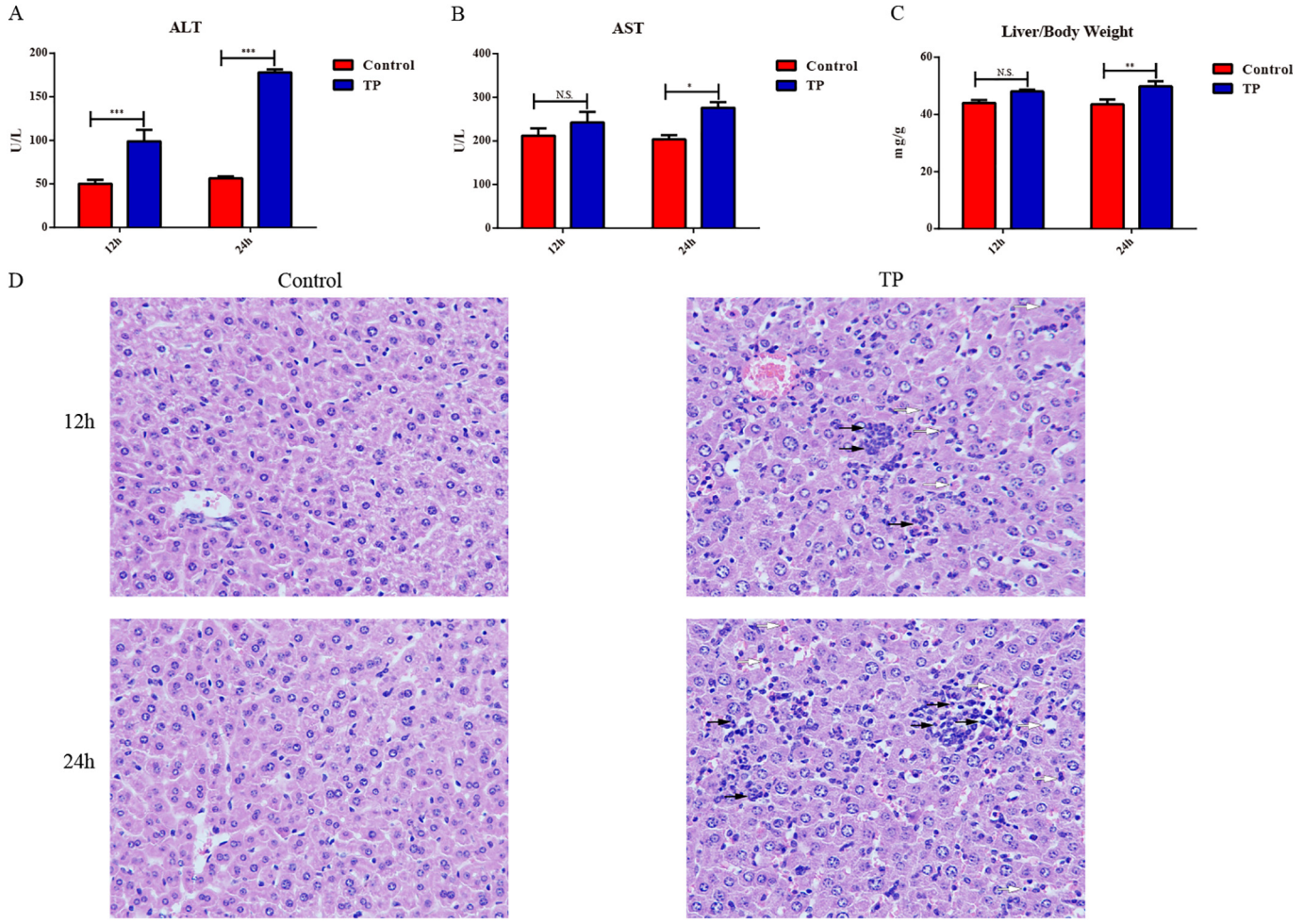

Fig. 1. Time-dependent hepatotoxicity of TP in C57BL/6J mice. (A–C) After the administration of TP for 12 h or 24 h respectively, blood samples and the whole liver tissues were collected to assess the serum ALT, AST, and liver index ($n = 8$). (D) The liver sections were stained with H&E (200×) to examine the histopathological change (white arrows and black arrows represents the necrosis and inflammatory cells respectively). Results were expressed as means ± SEM. Statistical analysis was performed using two-way ANOVA followed by Bonferroni post-test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control group.

Meanwhile, our previous studies revealed the involvement of the adaptive immune response in TP-induced liver damage [8–10]. Those results prompted us to investigate whether innate immune-mediated inflammasome activation played a role in TP-induced liver injury. We administered TP at a dose of 600 μg/kg for 12 h and 24 h respectively according to our previous study [9]. A single dose of TP increased the serum ALT and AST contents in the time-dependent manner (Fig. 1A and Fig. 1B). H&E staining presented in Fig. 1D showed necrosis and inflammatory cell infiltration in liver tissue treated with TP. TP was reported to serve as the immunosuppressive agent for the treatment of autoimmune diseases by inhibiting NF-κB and down-regulating the pro-inflammatory factors at the low dose [2]. However, we found that the pro-inflammatory action of TP occurred only at the high dose in mice (Fig. 2A and Fig. 3D). Combined with the necrotic area shown in H&E staining and increased neutrophil infiltration in IHC staining of MPO, we believed that the increase of pro-inflammatory factors and the recruitment of neutrophils might be accounting for the direct cytotoxicity induced by TP (Fig. 1D and Fig. 2B).

The NLRP3 inflammasome can be activated in response to a variety of stimuli, including infection, metabolic disorder, and tissue damage. The activation of NLRP3 inflammasome triggers the proteolytic cleavage of dormant Pro-Caspase-1 into the active form, which additionally converts the cytokines precursor pro-IL-1β into IL-1β. We found that TP increased both the mRNA levels of NLRP3 inflammasome and promoted the cleavage of Pro-Caspase-1 as well as mature IL-1β, revealing TP-treatment could affect both the priming step and the activation step of NLRP3 inflammasome (Fig. 3A to Fig. 3I). Cell priming with the NF-κB dependent transcription step was the most common reason leading to the induction of NLRP3, IL-1β and the formulation of NLRP3 inflammasome, while the second signal includes a variety of molecular and cellular events, including K^+ efflux, $Ca2^+$ signaling, lysosomal rupture, mitochondrial dysfunction, and the production of reactive oxygen species (ROS) [19]. IκB-α, a protein that inhibits the activation of NF-κB through masking the nuclear localization signals, can be activated by TLR4-Myd88 pathway. The activation and degradation of IκB-α, in turn, lead to the activation of NF-κB dependent transcription, accompanied by the increase in the mRNA level of IκB-α. The results shown in Fig. 4A to Fig. 4C suggested that TP might activate the priming step of NLRP3 inflammasome through TLR4-Myd88-NF-κB pathway. The impairment of mitochondria respiratory chain and the excessive ROS production by NAPDH oxidase were initially thought to be the common signals for the activation of NLRP3 inflammasome [40]. Excessive production of ROS leads to the elimination of anti-oxidative SOD level and an increase in MDA content, one of the major parameter of lipid peroxidation [52]. According to the published data, TP could disrupt the mitochondrial respiratory chain in vivo and lead to the excessive production of ROS in vitro [4,39]. Thus, we detected the contents of MDA and SOD to establish the possible relationship between NLRP3 activation and the occurrence of oxidative stress induced by TP (Fig. 2C to Fig. 2D).

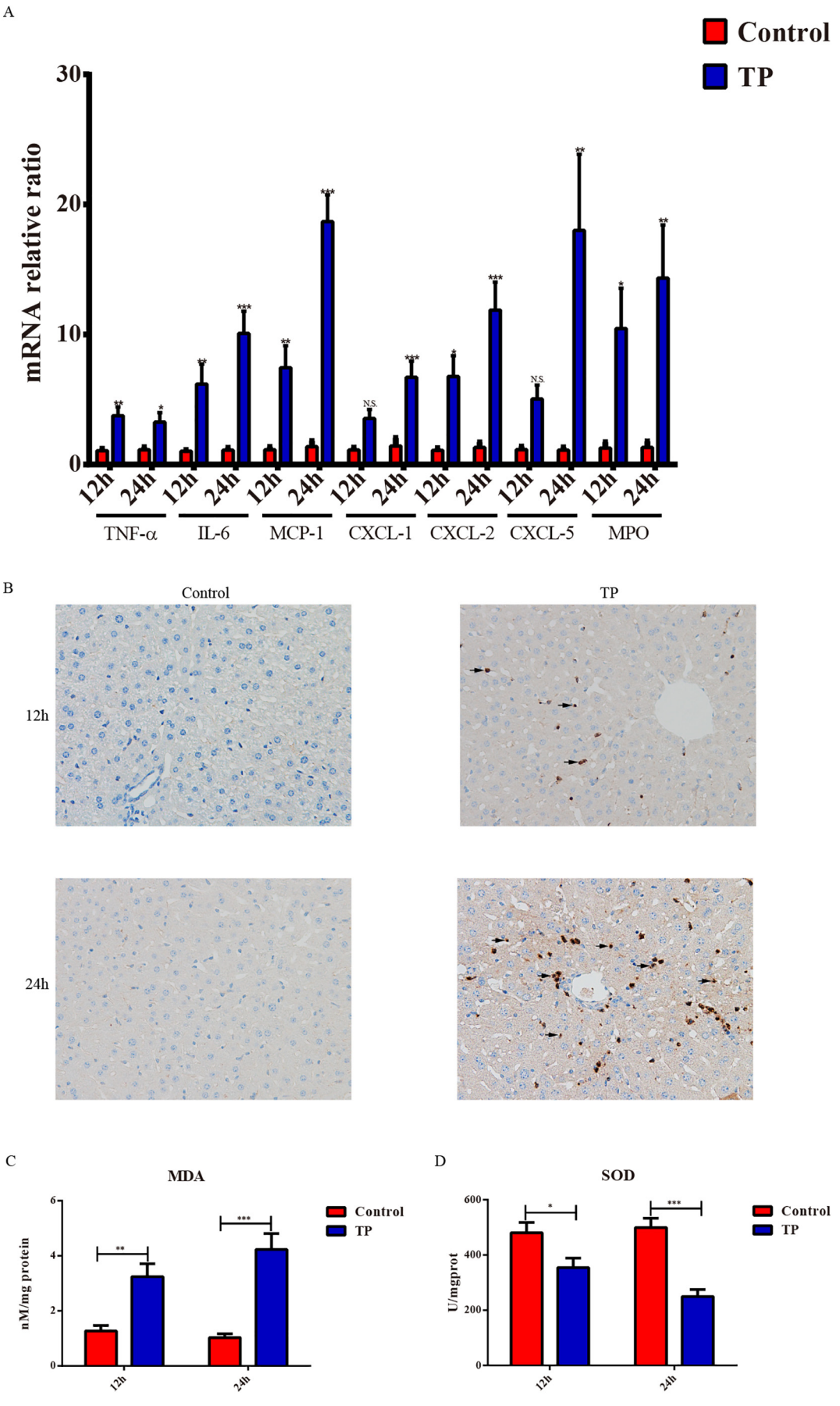

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Fig. 2. Administration of TP up-regulated the expression of pro-inflammatory factors and recruited the inflammatory cells to the liver. (A) Relative mRNA levels of TNF-α, IL-6, MCP-1, CXCL-1, CXCL-2, CXCL-5, and MPO after TP treatment (n = 8). (B) Immunohistochemical staining of MPO (200×) indicated the aggregation of neutrophils followed by TP-treatment (Black arrows represent the neutrophils). Results were expressed as means ± SEM. (C–D) Levels of MDA and SOD were detected using their kits respectively to verify the involvement of oxidative stress in TP-induced liver toxicity (n = 8). Statistical analysis was performed using two-way ANOVA followed by Bonferroni post-test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control group; n.s. no statistical difference vs. control group.

The acute liver toxicity induced by exogenous substance is always deteriorated by the recruitment of inflammatory cells via inflammatory mediators. For example, an increasing presence of the neutrophils could both release cytotoxic signals and recruit additional neutrophils and monocytes to aggravate liver damage [53–55]. TP treatment increased the mRNA levels of CXCL-1, CXCL-2, CXCL-5, MPO, and MCP-1 in the liver, suggesting the aggregation of the neutrophils and monocytes after TP treatment (Fig. 2). In view of the key role of the IL-1 family in the recruitment of inflammatory cells, we speculated that there might be a relationship between the inflammasome activation and the damage induced by massive inflammatory cells [56]. This was confirmed by Ac-Yvad-Cmk pretreatment. Compared with TP alone, TP plus Ac-Yvad-Cmk co-treatment diminished the damage induced by TP presented with the reduction of inflammatory cells in the liver (Fig. 6). As the

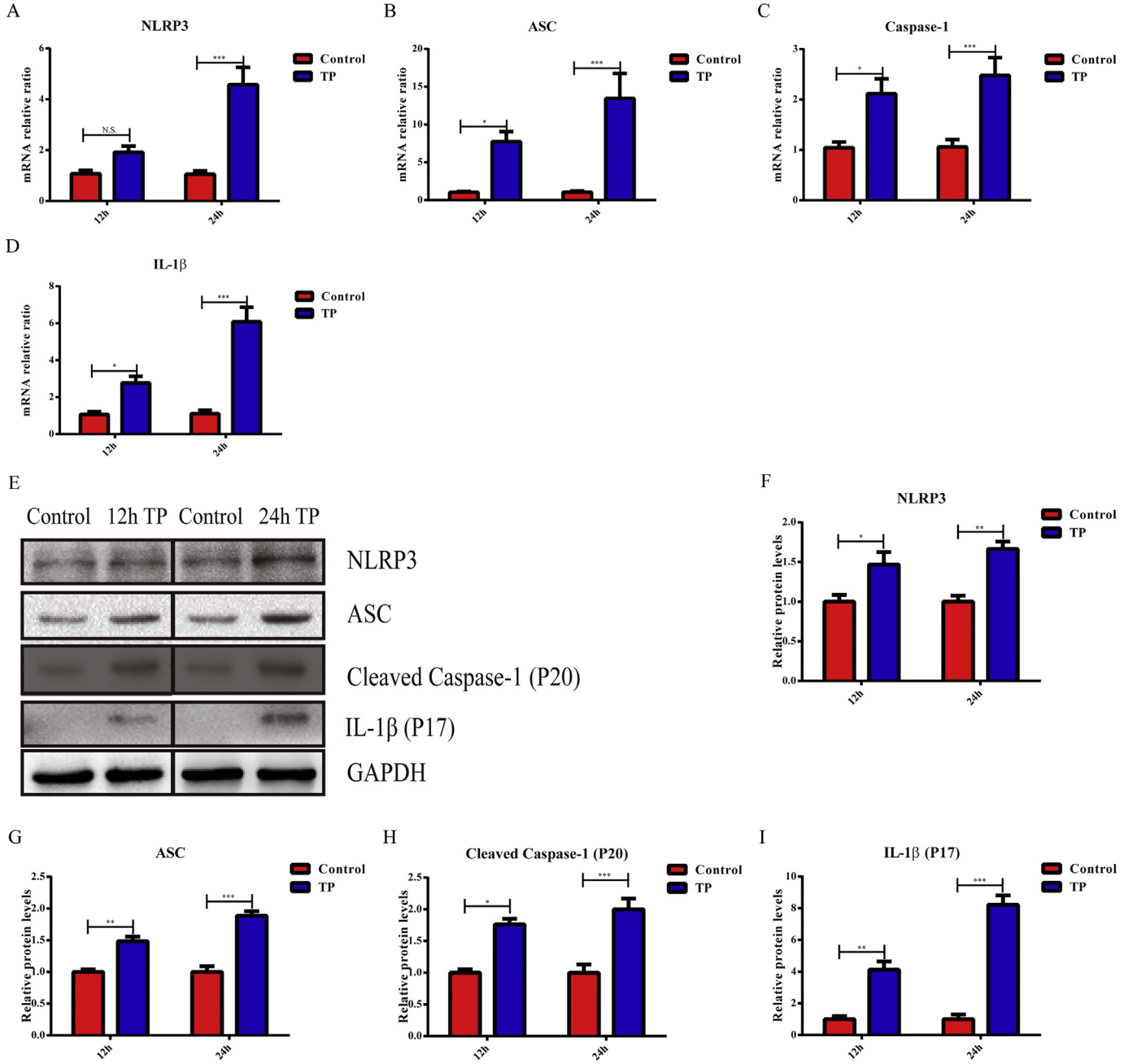

Fig. 3. TP-induced acute hepatotoxicity was accompanied by the activation of NLRP3 inflammasome. (A–D) The mRNA levels of the components of NLRP3 inflammasome and IL-1β were detected to verify whether TP could affect the priming step of NLRP3 inflammasome (n = 8). (E–G) The protein levels of NLRP3, ASC, Cleaved Caspase-1 (P20), and mature IL-1β were detected with GAPDH as the loading control (n = 4). Results were expressed as means ± SEM. Statistical analysis was performed using two-way ANOVA followed by Bonferroni post-test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control group.

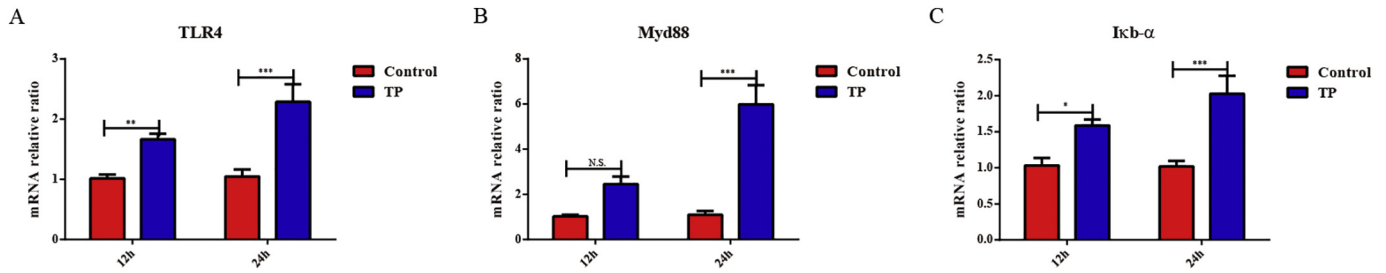

Fig. 4. Effects of TP on TLR4-Myd88-NF-κB pathway. (A-C) The mRNA levels of TLR4, Myd88, IκB-α were detected using RT-PCR to verify the involvement of TLR4-Myd88-NF-κB pathway in the regulation of the priming step of NLRP3 inflammasome after TP administration (n = 8). Results were expressed as means ± SEM. Statistical analysis was performed using two-way ANOVA followed by Bonferroni post-test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control group.

activation of all inflammasomes, NLRP1, NLRP3, NLRC4, AIM2, and Pyrin, is involved in the cleavage of Pro-Caspase-1 and Ac-Yvad-Cmk pretreatment was not only specific for NLRP3 inflammasome, we cannot rule out the possibility of NLRP1, NLRC4, AIM2, and Pyrin activation in TP-induced acute hepatotoxicity [57]. The precise role of NLRP3 inflammasome in TP-induced liver toxicity needs additional experiments such as the application of NLRP3-deficient mice or the specific inhibitors of NLRP3 inflammasome. Our speculations about the role NLRP3 inflammasome in TP induced liver damage are presented in Fig. 7.

5. Conclusion

In this study, we proved that the hyper-activation of NLRP3 inflammasome is involved in TP induced acute hepatotoxicity. The abnormal activation of NLRP3 inflammasome would result in the maturity of IL-1β, subsequently, recruitment of the inflammatory cells to the damaged tissue and aggravate the liver damage. Pretreatment with the pharmacological inhibitor of inflammasome activation partly alleviated TP-induced hepatotoxicity. Our results revealed that targeting PRRs, especially inflammasome, might serve as a potential target for the treatment of TP-induced liver damage.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2019.105754.

Declaration of Competing Interest

We declare that there is no conflict of interest.

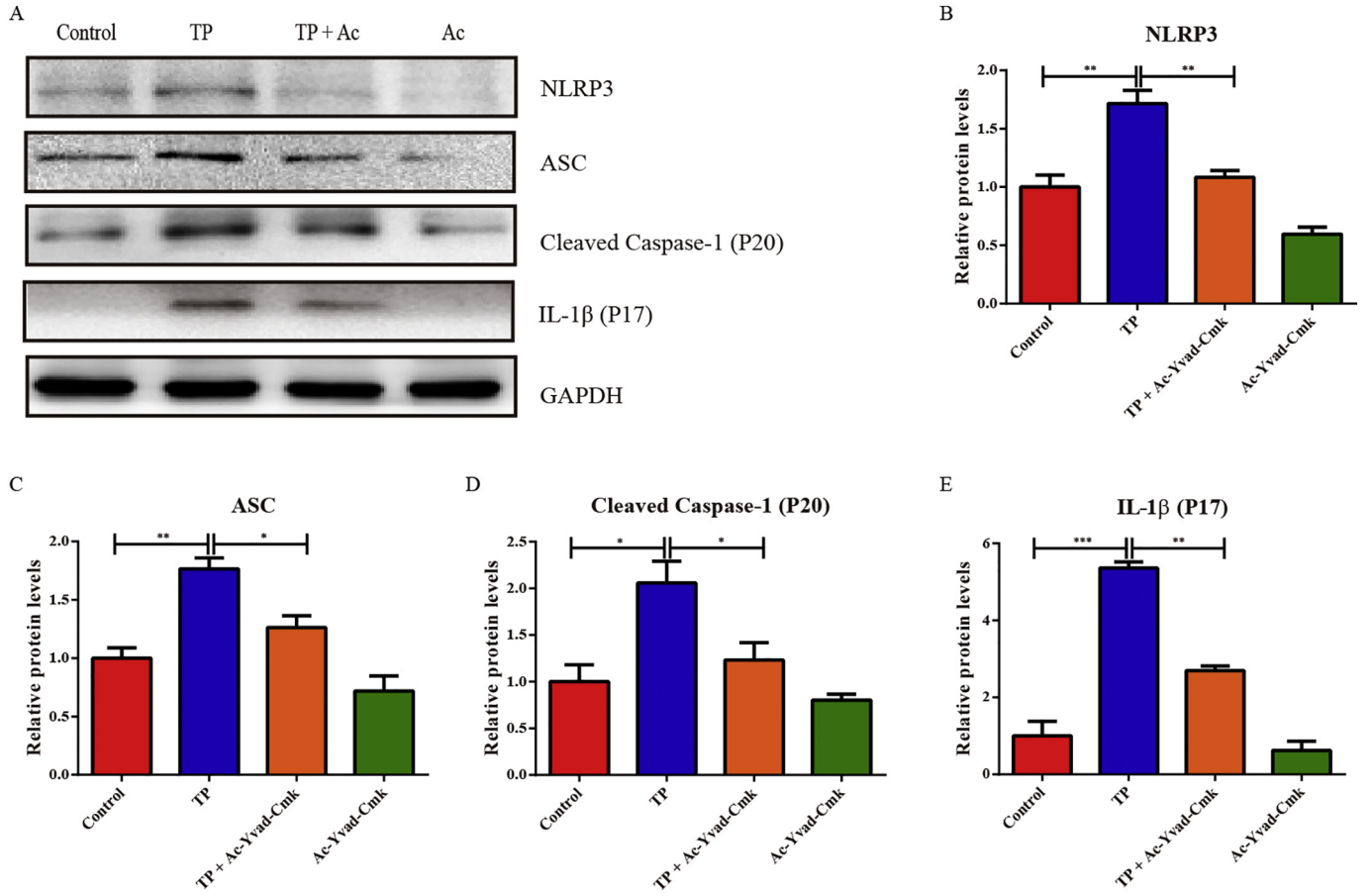

Fig. 5. Ac-Yvad-Cmk pre-treatment alleviated the activation of NLRP3 inflammasome and the maturity of IL-1β induced by TP. (A–E) The protein levels of NLRP3, ASC, Cleaved Caspase-1 (P20), and IL-1β (P17) were detected using western blot with GAPDH as the loading control (n = 4). Results were expressed as means ± SEM. Statistical analysis was performed using one-way ANOVA followed by Bonferroni's Multiple Comparison Test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

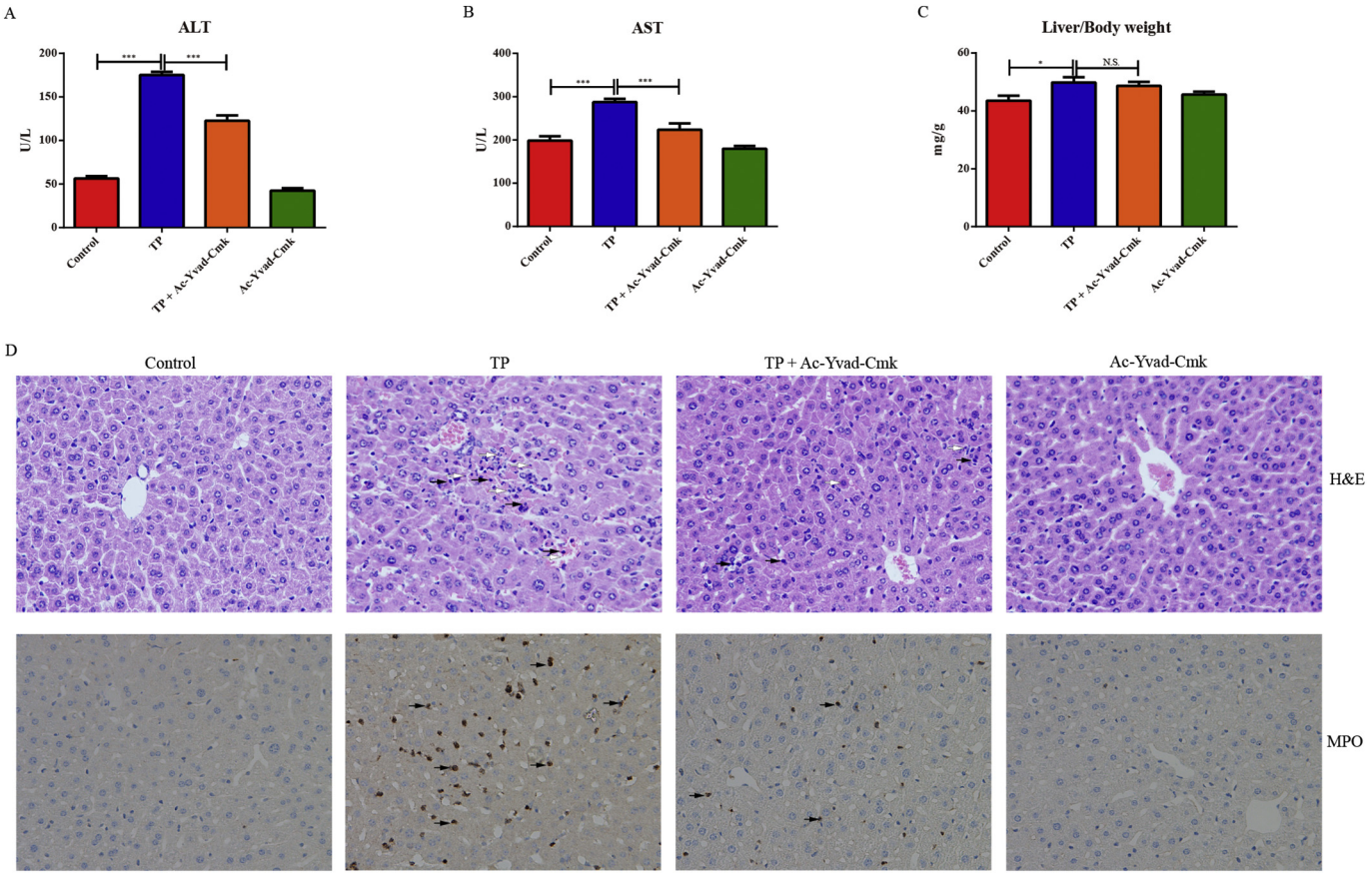

Fig. 6. Ac-Yvad-Cmk pre-treatment alleviated the liver toxicity induced by TP. (A-C) Ac-Yvad-Cmk pre-treatment partly diminished the up-regulation of serum ALT, AST and liver index after the treatment of TP for 24 h (n = 8). (D) Ac-Yvad-Cmk pre-treatment weakened the morphological changes and the recruitment of neutrophils induced by TP as reflected by H&E (200×) staining and immunohistochemical staining of MPO (200×) (White arrows and black arrows represent necrosis and the inflammatory cells in H < E staining; Black arrows represents neutrophils in MPO staining). Results were expressed as means ± SEM. Statistical analysis was performed using one-way ANOVA followed by Bonferroni's Multiple Comparison Test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, n.s. no statistical difference.

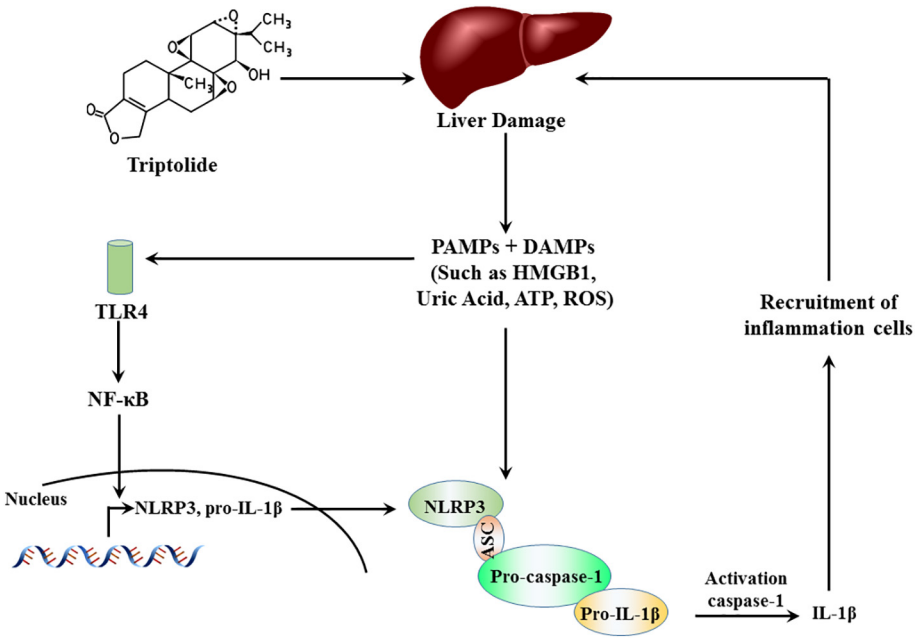

Fig. 7. The graph represents the possible mechanism of NLRP3 inflammasome activation and its associated pathway in the acute liver toxicity induced by TP.

Table 2
The effect of Ac-Yvad-Cmk on the mRNA levels of pro-inflammatory factors induced by TP-treatment at 24 h.

	Group					
Gene	Control	TP	TP + Ac-Yvad-Cmk	Ac-Yvad-Cmk	P value (Control VS TP)	P value (TP VS TP + Ac-Yvad-Cmk)
IL-1β	1.074 ± 0.1844	6.757 ± 0.7468***	1.607 ± 0.3727###	0.1591 ± 0.03375	P < 0.001	P < 0.001
TNF-α	1.053 ± 0.1460	3.589 ± 0.7642**	1.753 ± 0.3260#	0.3411 ± 0.06816	P < 0.01	P < 0.05
IL-6	1.082 ± 0.1801	11.76 ± 1.798***	4.560 ± 0.7621###	0.5044 ± 0.1075	P < 0.001	P < 0.001
CXCL-1	1.135 ± 0.3127	9.295 ± 1.840***	5.403 ± 1.233	0.5524 ± 0.07553	P < 0.01	*P* = 0.092
CXCL-2	1.247 ± 0.3710	10.42 ± 1.797***	3.556 ± 1.176###	0.2932 ± 0.06098	P < 0.001	P < 0.001
CXCL-5	1.070 ± 0.1845	26.09 ± 7.417***	6.094 ± 2.640##	0.1505 ± 0.02661	P < 0.01	P < 0.01
MCP-1	1.152 ± 0.2775	20.73 ± 2.272***	9.117 ± 0.6981###	0.1132 ± 0.03427	P < 0.001	P < 0.001
MPO	1.054 ± 0.1442	20.24 ± 5.394***	5.983 ± 1.448##	0.9830 ± 0.4082	P < 0.01	P < 0.01

Results were expressed as means ± SEM (n = 6-8). Statistical analysis was performed using one-way ANOVA followed by Bonferroni's Multiple comparison Test.*, #P < 0.05, **, ##P < 0.01, ***, ###P < 0.001; *P, **P, ***P, compared between TP-treated and control group; #P, ##P, ###P, compared between TP-treated and TP plus Ac-Yvad-Cmk co-treatment group.

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References

[1] S. Ziaei, R. Halaby, Immunosuppressive, anti-inflammatory and anti-cancer properties of triptolide: a mini review, Avicenna journal of phytomedicine 6 (2) (2016) 149–164.
[2] Q. Liu, Triptolide and its expanding multiple pharmacological functions, Int. Immunopharmacol. 11 (3) (2011) 377–383.
[3] X.J. Li, Z.Z. Jiang, L.Y. Zhang, Triptolide: progress on research in pharmacodynamics and toxicology, J. Ethnopharmacol. 155 (1) (2014) 67–79.
[4] J. Yao, Z. Jiang, W. Duan, J. Huang, L. Zhang, L. Hu, L. He, F. Li, Y. Xiao, B. Shu, C. Liu, Involvement of mitochondrial pathway in triptolide-induced cytotoxicity in human normal liver L-02 cells, Biol. Pharm. Bull. 31 (4) (2008) 592–597.
[5] Y. Lu, T. Xie, Y. Zhang, F. Zhou, J. Ruan, W. Zhu, H. Zhu, Z. Feng, X. Zhou, Triptolide induces hepatotoxicity via inhibition of CYP450s in rat liver microsomes, BMC Complement. Altern. Med. 17 (1) (2017) 15.
[6] Z. Jiang, X. Huang, S. Huang, H. Guo, L. Wang, X. Li, X. Huang, T. Wang, L. Zhang, L. Sun, Sex-related differences of lipid metabolism induced by Triptolide: the possible role of the LXRα/SREBP-1 signaling pathway, Front. Pharmacol. 7 (2016).
[7] J. Yang, L. Sun, L. Wang, H.M. Hassan, X. Wang, P.B. Hylemon, T. Wang, H. Zhou, L. Zhang, Z. Jiang, Activation of Sirt1/FXR signaling pathway attenuates Triptolide-induced hepatotoxicity in rats, Front. Pharmacol. 8 (2017) 260.
[8] X. Wang, Z. Jiang, W. Cao, Z. Yuan, L. Sun, L. Zhang, Th17/Treg imbalance in triptolide-induced liver injury, Fitoterapia 93 (2014) 245–251.
[9] X. Wang, Z. Jiang, M. Xing, J. Fu, Y. Su, L. Sun, L. Zhang, Interleukin-17 mediates triptolide-induced liver injury in mice, food and chemical toxicology: an international journal published for the British industrial, Biological Research Association 71 (2014) 33–41.
[10] X. Wang, L. Sun, L. Zhang, Z. Jiang, Effect of adoptive transfer or depletion of regulatory T cells on triptolide-induced liver injury, Front. Pharmacol. 7 (2016) 99.
[11] B. Gao, W.I. Jeong, Z. Tian, Liver: an organ with predominant innate immunity, Hepatology 47 (2) (2008) 729–736.
[12] V. Racanelli, B. Rehermann, The liver as an immunological organ, Hepatology 43 (2 Suppl 1) (2006) S54–S62.
[13] A.W. Thomson, P.A. Knolle, Antigen-presenting cell function in the tolerogenic liver environment, Nat. Rev. Immunol. 10 (11) (2010) 753–766.
[14] O. Takeuchi, S. Akira, Pattern recognition receptors and inflammation, Cell 140 (6) (2010) 805–820.
[15] F. Marra, F. Tacke, Roles for chemokines in liver disease, Gastroenterology 147(3) (2014) 577–594 e1.
[16] R. Medzhitov, Origin and physiological roles of inflammation, Nature 454 (7203) (2008) 428–435.
[17] K.M. Storek, D.M. Monack, Bacterial recognition pathways that lead to inflammasome activation, Immunol. Rev. 265 (1) (2015) 112–129.
[18] F. Martinon, K. Burns, J. Tschopp, The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta, Mol. Cell 10 (2) (2002) 417–426.
[19] Y. He, H. Hara, G. Nunez, Mechanism and regulation of NLRP3 Inflammasome activation, Trends Biochem. Sci. 41 (12) (2016) 1012–1021.
[20] S. Wang, M. Miura, Y. Jung, H. Zhu, V. Gagliardini, L. Shi, A.H. Greenberg, J. Yuan, Identification and characterization of Ich-3, a member of the interleukin-1beta converting enzyme (ICE)/Ced-3 family and an upstream regulator of ICE, J. Biol. Chem. 271 (34) (1996) 20580–20587.
[21] J. Shi, Y. Zhao, Y. Wang, W. Gao, J. Ding, P. Li, L. Hu, F. Shao, Inflammatory caspases are innate immune receptors for intracellular LPS, Nature 514 (7521) (2014) 187–192.
[22] N. Kayagaki, S. Warming, M. Lamkanfi, L. Vande Walle, S. Louie, J. Dong, K. Newton, Y. Qu, J. Liu, S. Heldens, J. Zhang, W.P. Lee, M. Roose-Girma, V.M. Dixit, Non-canonical inflammasome activation targets caspase-11, Nature 479 (7371) (2011) 117–121.
[23] I. Stowe, B. Lee, N. Kayagaki, Caspase-11: arming the guards against bacterial infection, Immunol. Rev. 265 (1) (2015) 75–84.
[24] D. De Nardo, E. Latz, NLRP3 inflammasomes link inflammation and metabolic disease, Trends Immunol. 32 (8) (2011) 373–379.
[25] E.I. Elliott, F.S. Sutterwala, Initiation and perpetuation of NLRP3 inflammasome activation and assembly, Immunol. Rev. 265 (1) (2015) 35–52.
[26] A.B. Imaeda, A. Watanabe, M.A. Sohail, S. Mahmood, M. Mohamadnejad, F.S. Sutterwala, R.A. Flavell, W.Z. Mehal, Acetaminophen-induced hepatotoxicity in mice is dependent on Tlr9 and the Nalp3 inflammasome, J. Clin. Invest. 119 (2) (2009) 305–314.
[27] Y. Inoue, K. Shirasuna, H. Kimura, F. Usui, A. Kawashima, T. Karasawa, K. Tago, K. Dezaki, S. Nishimura, J. Sagara, T. Noda, Y. Iwakura, H. Tsutsui, S. Taniguchi, K. Yanagisawa, T. Yada, Y. Yasuda, M. Takahashi, NLRP3 regulates neutrophil functions and contributes to hepatic ischemia-reperfusion injury independently of inflammasomes, J. Immunol. 192 (9) (2014) 4342–4351.
[28] S. Guo, C. Yang, B. Diao, X. Huang, M. Jin, L. Chen, W. Yan, Q. Ning, L. Zheng, Y. Wu, Y. Chen, The NLRP3 Inflammasome and IL-1beta accelerate immunologically mediated pathology in experimental viral fulminant hepatitis, PLoS Pathog. 11 (9) (2015) e1005155.
[29] G. Szabo, J. Petrasek, Inflammasome activation and function in liver disease, Nat. Rev. Gastroenterol. Hepatol. 12 (7) (2015) 387–400.
[30] M. Haneklaus, L.A. O'Neill, NLRP3 at the interface of metabolism and inflammation, Immunol. Rev. 265 (1) (2015) 53–62.
[31] J. Wang, Z. Jiang, J. Ji, X. Wang, T. Wang, Y. Zhang, T. Tai, M. Chen, L. Sun, X. Li, L. Zhang, Gene expression profiling and pathway analysis of hepatotoxicity induced by triptolide in Wistar rats, Food and chemical toxicology: an international journal published for the British Industrial Biological Research Association 58 (2013) 495–505.
[32] H. Hao, L. Cao, C. Jiang, Y. Che, S. Zhang, S. Takahashi, G. Wang, F.J. Gonzalez, Farnesoid X receptor regulation of the NLRP3 Inflammasome underlies cholestasis-associated sepsis, Cell Metab. 25 (4) (2017) 856–867 e5.
[33] H.J. Schlayer, H. Laaff, T. Peters, M. Woort-Menker, H.C. Estler, U. Karck, H.E. Schaefer, K. Decker, Involvement of tumor necrosis factor in endotoxin-triggered neutrophil adherence to sinusoidal endothelial cells of mouse liver and its modulation in acute phase, J. Hepatol. 7 (2) (1988) 239–249.
[34] M. Rincon, Interleukin-6: from an inflammatory marker to a target for inflammatory diseases, Trends Immunol. 33 (11) (2012) 571–577.
[35] M.L. Bajt, A. Farhood, H. Jaeschke, Effects of CXC chemokines on neutrophil activation and sequestration in hepatic vasculature, Am. J. Physiol. Gastrointest. Liver Physiol. 281 (5) (2001) G1188–G1195.
[36] G. Schett, J.M. Dayer, B. Manger, Interleukin-1 function and role in rheumatic disease, Nat. Rev. Rheumatol. 12 (1) (2016) 14–24.
[37] F. Heymann, F. Tacke, Immunology in the liver–from homeostasis to disease, Nat. Rev. Gastroenterol. Hepatol. 13 (2) (2016) 88–110.
[38] M.S.J. Mangan, E.J. Olhava, W.R. Roush, H.M. Seidel, G.D. Glick, E. Latz, Targeting the NLRP3 inflammasome in inflammatory diseases, Nat. Rev. Drug Discov. 17 (2018) 588–606.
[39] Q. Fu, X. Huang, B. Shu, M. Xue, P. Zhang, T. Wang, L. Liu, Z. Jiang, L. Zhang, Inhibition of mitochondrial respiratory chain is involved in triptolide-induced liver injury, Fitoterapia 82 (8) (2011) 1241–1248.

[40] R. Zhou, A.S. Yazdi, P. Menu, J. Tschopp, A role for mitochondria in NLRP3 inflammasome activation, Nature 469 (7329) (2011) 221–225.
[41] S.R. Chen, Y. Dai, J. Zhao, L. Lin, Y. Wang, Y. Wang, A mechanistic overview of Triptolide and Celastrol, natural products from Tripterygium wilfordii hook F, Front. Pharmacol. 9 (2018) 104.
[42] R. Chugh, V. Sangwan, S.P. Patil, V. Dudeja, R.K. Dawra, S. Banerjee, R.J. Schumacher, B.R. Blazar, G.I. Georg, S.M. Vickers, A.K. Saluja, A preclinical evaluation of Minnelide as a therapeutic agent against pancreatic cancer, Sci. Transl. Med. 4 (156) (2012) 156ra139.
[43] S.R. Hingorani, J.D. Potter, Pancreas cancer meets the thunder god, Sci. Transl. Med. 4 (156) (2012) 156ps21.
[44] W. Tang, Y. Yang, F. Zhang, Y.C. Li, R. Zhou, J.X. Wang, Y.N. Zhu, X.Y. Li, Y.F. Yang, J.P. Zuo, Prevention of graft-versus-host disease by a novel immunosuppressant, (5R)-5-hydroxytriptolide (LLDT-8), through expansion of regulatory T cells, Int. Immunopharmacol. 5 (13–14) (2005) 1904–1913.
[45] R. Zhou, F. Zhang, P.L. He, W.L. Zhou, Q.L. Wu, J.Y. Xu, Y. Zhou, W. Tang, X.Y. Li, Y.F. Yang, Y.C. Li, J.P. Zuo, (5R)-5-hydroxytriptolide (LLDT-8), a novel triptolide analog mediates immunosuppressive effects in vitro and in vivo, Int. Immunopharmacol. 5 (13–14) (2005) 1895–1903.
[46] G. Szabo, A. Dolganiuc, P. Mandrekar, Pattern recognition receptors: a contemporary view on liver diseases, Hepatology 44 (2) (2006) 287–298.
[47] V. Bieghs, C. Trautwein, The innate immune response during liver inflammation and metabolic disease, Trends Immunol. 34 (9) (2013) 446–452.
[48] E. Seki, B. Schnabl, Role of innate immunity and the microbiota in liver fibrosis: crosstalk between the liver and gut, J. Physiol. 590 (3) (2012) 447–458.
[49] C. Feriotti, E.F. de Araujo, F.V. Loures, T.A. da Costa, N.A.L. Galdino, D.S. Zamboni, V.L.G. Calich, NOD-like receptor P3 Inflammasome controls protective Th1/Th17 immunity against pulmonary paracoccidioidomycosis, Front. Immunol. 8 (2017) 786.
[50] F. Ghiringhelli, L. Apetoh, A. Tesniere, L. Aymeric, Y. Ma, C. Ortiz, K. Vermaelen, T. Panaretakis, G. Mignot, E. Ullrich, J.L. Perfettini, F. Schlemmer, E. Tasdemir, M. Uhl, P. Genin, A. Civas, B. Ryffel, J. Kanellopoulos, J. Tschopp, F. Andre, R. Lidereau, N.M. McLaughlin, N.M. Haynes, M.J. Smyth, G. Kroemer, L. Zitvogel, Activation of the NLRP3 inflammasome in dendritic cells induces IL-1beta-dependent adaptive immunity against tumors, Nat. Med. 15 (10) (2009) 1170–1178.
[51] G. Meng, F. Zhang, I. Fuss, A. Kitani, W. Strober, A mutation in the Nlrp3 gene causing Inflammasome Hyperactivation potentiates Th17 cell-dominant immune responses, Immunity 30 (6) (2009) 860–874.
[52] N. Chondrogianni, I. Petropoulos, S. Grimm, K. Georgila, B. Catalgol, B. Friguet, T. Grune, E.S. Gonos, Protein damage, repair and proteolysis, Mol. Asp. Med. 35 (2014) 1–71.
[53] H. Liu, Q. Wu, T. Chu, Y. Mo, S. Cai, M. Chen, G. Zhu, High-dose acute exposure of paraquat induces injuries of swim bladder, gastrointestinal tract and liver via neutrophil-mediated ROS in zebrafish and their relevance for human health risk assessment, Chemosphere 205 (2018) 662–673.
[54] C.K. Ayata, S.C. Ganal, B. Hockenjos, K. Willim, R.P. Vieira, M. Grimm, B. Robaye, J.M. Boeynaems, F. Di Virgilio, P. Pellegatti, A. Diefenbach, M. Idzko, P. Hasselblatt, Purinergic P2Y(2) receptors promote neutrophil infiltration and hepatocyte death in mice with acute liver injury, Gastroenterology 143 (6) (2012) 1620–1629 (e4).
[55] H. Horiguchi, E. Oguma, Acute exposure to cadmium induces prolonged neutrophilia along with delayed induction of granulocyte colony-stimulating factor in the livers of mice, Arch. Toxicol. 90 (12) (2016) 3005–3015.
[56] J.E. Sims, D.E. Smith, The IL-1 family: regulators of immunity, Nat. Rev. Immunol. 10 (2) (2010) 89–102.
[57] G. Szabo, T. Csak, Inflammasomes in liver diseases, J. Hepatol. 57 (3) (2012) 642–654.