

Original Article

LPS pretreatment ameliorates D-galactosamine/ lipopolysaccharide-induced acute liver failure in rat

Jin-Zhong Dong^{1*}, Li-Ping Wang^{2*}, Sai-Nan Zhang³, Ke-Qing Shi³, Shao-Long Chen⁴, Nai-Bin Yang³,
Shun-Lan Ni³, Jian-Hua Zhu¹, Ming-Qin Lu³

Departments of ¹Intensive Care Unit, ²Infection Disease, Ningbo First Hospital, Ningbo, China; ³Department of Infection Disease, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China; ⁴Department of Infectious Diseases, Huashan Hospital, Fudan University, Shanghai, China. *Equal contributors.

Received September 17, 2014; Accepted November 1, 2014; Epub October 15, 2014; Published November 1, 2014

Abstract: Acute liver failure (ALF) remains an extremely poor prognosis and high mortality; with no effective treatments. The endotoxin tolerance (ET) phenotype has been reported to exhibit protective activities in several sepsis models. We now investigated the effects and underlying intraperitoneal injection of the same volume of pyrogen-free 0.9% sodium chloride instead of LPS for five consecutive days before D-GalN/LPS injection in rats. The serum levels of TNF- α , IL-6, ALT, AST and TBiL from ET + ALF group and ALF group were measured at different time points. Our results showed that ET + ALF group markedly reduced the serum levels of TNF- α , IL-6, ALT, AST and TBiL and histological features in the ET + ALF group were improved significantly. Furthermore, LPS pre-treatment inhibited D-GalN/LPS-induced NF- κ B activation, Bax activation, signal transducer and activator of transcription-1 (STAT1) and signal transducer and activator of transcription-3 (STAT3) activities. LPS pre-treatment also significantly enhance the expression of suppressors of cytokine signaling 1 (SOCS1) and suppressors of cytokine signaling 3 (SOCS3). Our experimental data indicated that ET might alleviate D-GalN/LPS-induced ALF by inhibiting the inflammatory response, inactivation of STAT1 and STAT3 and up-regulation of SOCS1 and SOCS3.

Keywords: D-galactosamine, lipopolysaccharide, inflammatory cytokines, acute hepatic failure, inflammatory cytokines, JAK/STAT, rat

Introduction

Acute liver failure (ALF) is the appearance of severe complications rapidly after the first signs of liver disease (such as jaundice), and indicates that the liver has sustained severe damage (loss of 80-90% function of liver cells) [1]. As we know, massive hepatocytes necrosis and apoptosis in the liver parenchymal cells will increase systemic inflammatory syndrome which will lead to ALF [2-4]. In clinic, about 60% of all ALF patients fulfill the criteria for systemic inflammatory syndrome irrespective of presence or absence of infection, resulting in multiple organ dysfunction syndrome (MODS) [5].

Endotoxin, as a gram-negative bacterial lipopolysaccharide (LPS), stimulates a wide variety of inflammatory mediators, which are considered to be related to the development of ALF [6-8]. Recent studies showed that reduction of endotoxemia could prevent the death of hepatocytes, which could alleviate the development of

ALF in experimental models [9-11]. Prior exposure of innate immune cells like monocytes/macrophages to minute amounts of endotoxin caused them to become refractory to subsequent endotoxin challenge, a phenomenon called endotoxin tolerance (ET) [12-14]. Therefore, we hypothesized that ET rat might have a lower mortality in D-GalN/LPS-induced ALF. However, the mechanism of ET in ALF still has not been fully clarified.

In this study, we investigated the effect of ET pretreatment on ALF in rat. Moreover, the mechanisms of ET in progression of ALF, including JAK/STAT pathway and cytokines were also explored.

Materials and methods

Animal grouping and treatment

One hundred-six Male SPF Sprague-Dawley rats (200-220 g) were obtained from the Shanghai

Laboratory Animal Center (Shanghai, China). The rats were housed under normal laboratory conditions ($21 \pm 2^\circ\text{C}$, 12 h light-dark cycle) with free access to standard pellet diet and water. All animal procedures were conducted in accordance with the standards set forth in the guidelines for the care and use of experimental animals by the Committee for the Purpose of Control and Supervision of Experiments on Animals and the National Institutes of Health. The study protocol was approved by the Animal Ethics Committee of Wenzhou Medical University.

Experimental protocols

Animals were randomly divided into the control group ($n = 6$), the ALF group ($n = 70$), ET + ALF group ($n = 30$). In ET + ALF group, rats were treated with intraperitoneal injection of 0.1 mg/kg LPS for five consecutive days for induction of ET. As in the ALF group, rats were injected with the same volume of pyrogen-free 0.9% sodium chloride instead of LPS for five consecutive days. On the six day, all the model group animals were given an intraperitoneal injection of 500 μL saline containing 800 mg/kg D-GalN (Sigma, USA) and 8 μg LPS (Sigma, USA). In a separate experiment, survival rate was monitored for 72 hours after ALF in eighty rats.

Plasma biochemistry and Enzyme-linked immune sorbent assays

In the model groups, animals were anesthetized with urethane (1.0 g/kg, intraperitoneally (i.p.)) at 2, 6, 12, 24, 48 hours, respectively, the blood samples from abdominal aorta were drawn into heparinized injectors, and centrifuged at 1500 rpm at 4°C for 10 min. The serum levels of alanine transaminase (ALT), aspartate transaminase (AST), total bilirubin (TBil) were measured according to the manufacturer's instructions. Plasma concentrations of cytokines, including tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) were assayed using enzyme-linked immune sorbent assay (ELISA) kits according to the manufacturer's instructions.

Liver histological examination

After finishing with the blood collection, the experimental animals were sacrificed, liver samples were dissected and washed with ice-cold saline, then they were immediately stored

at -80°C for further analysis. Partial liver specimens were fixed in 10% neutral formalin and embedded in paraffin. The tissue sections were then cut into 4- μm sections and histological changes were assessed by using H&E staining. Images from different groups were obtained using a video microscope.

RNA isolation and reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from preserved liver tissue from each group using RNAiso Plus reagent, according to the manufacturer's protocol. The RNA samples were then reverse-transcribed into cDNA by reverse transcription reaction according to the following protocol. The above product was then used for amplification of cDNA, and the reaction system totaled 20 μL volume per sample, which consisted of The RNA samples were then reverse-transcribed into cDNA by reverse transcription reaction which contained 1 μL RNA sample, 1 μL Oligo dT-Adaptor primer (50 μM), 1 μL dNTP mixture (10 mM each), 4 μL 5 $\times$ first-strand buffer, 1 μL Power M-MuL Reverse Transcriptase (200U/ μL), 1 μL RNase inhibitor (40 U/ μL), 11 μL RNase-free dH₂O. The above product was then used for amplification of cDNA, and the reaction system was 25 μL volume per sample, which consisted of 12.5 μL 2X Master Mix, 0.5 μL forward primers, 0.5 μL reverse primers, 2.5 μL cDNA sample and 1 μL RNase-free dH₂O. The primers of the different cell cytokines were as follows: SOCS1 (350 bp): forward primers: 5'-CCACTCTGATTA-CCGGCGCATC-3' and reverse primers: 5'-GCT-CCTGCAGCGGCCGACG-3'. SOCS3 (514 bp): forward primers: 5'-ATGGTCACCCACAGCAGTTTC-3' and reverse primers: 5'-CGCCCCAGAA-TAGATGTAGTAAG-3'. P65 (505 bp): forward primers: 5'-TTGAGCAGCCCAAGCAGCGG-3' and reverse primers: 5'-GCAGTGTGGGGGCACGGTT-3'. β -actin (149 bp): forward primers 5'-CAAG-TTCAACGGCACAGTCAA-3' and reverse primers 5'-TGGTGAAGACGCCAGTAGACTC-3'.

The PCR protocol was as follows: the mixture was first denatured at 94°C for 5 min, 35 cycles at 94°C for 30 seconds, 58°C for 30 seconds (P65), 60°C for 30 seconds (SOCS1 and SOCS3), 61°C for 30 seconds (β -actin) followed by 72°C for 20 seconds and a final extension step at 72°C for 5 min. The PCR products were finally analyzed in a 2% agarose gel containing ethidium bromide (EB), and β -actin was used as a loading control.

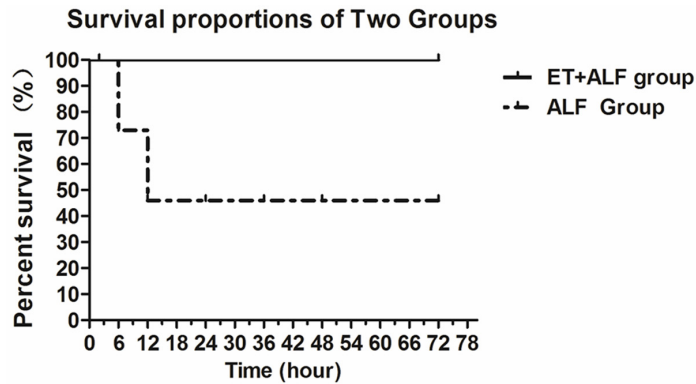


Figure 1. Effect of LPS pretreatment on survival rate after ALF. Rats were pretreated with either five consecutive daily injections of LPS at the dosage of 0.1 mg/kg (ET + ALF, n = 40) or normal saline (ALF, n = 40). ALF was performed on the sixth day and then survival was monitored for 72 hours. LPS pretreatment resulted in a significant improvement in survival compared with ALF group ($P < 0.05$).

Protein isolation and Western blotting

Total protein was isolated using lysis buffer supplemented with a protease inhibitor. Following heat denaturation at 95°C for 5 min, the samples (15 µg protein each) were subjected to polyacrylamide gel electrophoresis (SDS-PAGE) and subsequently transferred onto a nitrocellulose membrane. The membrane was then blocked by skimmed milk for 90 min at room temperature. The primary antibodies against STAT1 (cst), STAT3 (cst), SOCS1 (cst), SOCS3 (cst), P65 (Santa Cruz), Bax (anbo) and β-actin (Santa Cruz) were used for incubation with the membrane overnight at 4°C, respectively. After being washed with three times of TBST, the membrane was incubated with the secondary antibody at room temperature for 1 hour. Then the film exposure was performed.

Statistical analysis

All data were expressed as the means ± standard deviation (SD). Statistical significances were determined using one-way analysis of variance (ANOVA) or the least significant difference (LSD) test. SPSS19.0 software (IBM, USA) was used for statistical analyses; $P < 0.05$ was considered to have statistical significance.

Results

Effect of LPS pretreatment on the survival rate of rats after ALF

In a separate experiment, eighty rats were randomly divided into two groups: ALF group (40)

and ET + ALF (40), and survival rate was monitored for 72 hours after ALF. In the ALF group, twenty rats died in the first 12 hours. Among them, four rats died during 12-48 hours. The mortality rate reached 60% at 72 hours. However, in the ET + ALF group, all rat alive. The mortality rate was 0% at 72 hours (**Figure 1**).

Liver histopathology after D-GaIN/LPS injection

Histopathological study of the liver was performed to investigate events after D-GaIN/LPS injection. In ALF group, hepatocytes become to swell, a large number of inflammatory cells infiltrated at 6 hours, and significant-

ly degeneration and necrosis were observed at the later time (**Figure 2E**). However, in ET + ALF group, the histopathological injuries caused by D-GaIN/LPS were significantly alleviated (**Figure 2E**).

Plasma indicators for liver function (ALT, AST, TBIL) after D-GaIN/LPS injection

Following the administration of D-GaIN/LPS, serum ALT, AST levels of rats in both the ALF and ET + ALF group became to increase at 2 hours and were significantly elevated at 12 hours. Meanwhile, serum TBIL levels continuously increased after 2 hours. However, the expressions of all above factors in ALF group were higher compared to the ET + ALF group at each time point ($P < 0.01$, **Figure 2B, 2D**).

Cytokines production after D-GaIN/LPS injection

After the administration of D-GaIN/LPS, serum TNF-α and IL-6 levels became to increase at 2 hours and were significantly elevated at 24 hours both in ALF group and ET + ALF group compare to control group ($p < 0.05$, **Figure 1**). In addition, the expressions of all above data in ALF group remained higher than in ET + ALF group at each time point ($P < 0.05$, **Figure 3A, 3B**).

Expression of P65, SOCS1 and SOCS3 genes after D-GaIN/LPS injection

To clarify the effect of D-GaIN/LPS on the expression of P65, SOCS1 and SOCS3, liver tis-

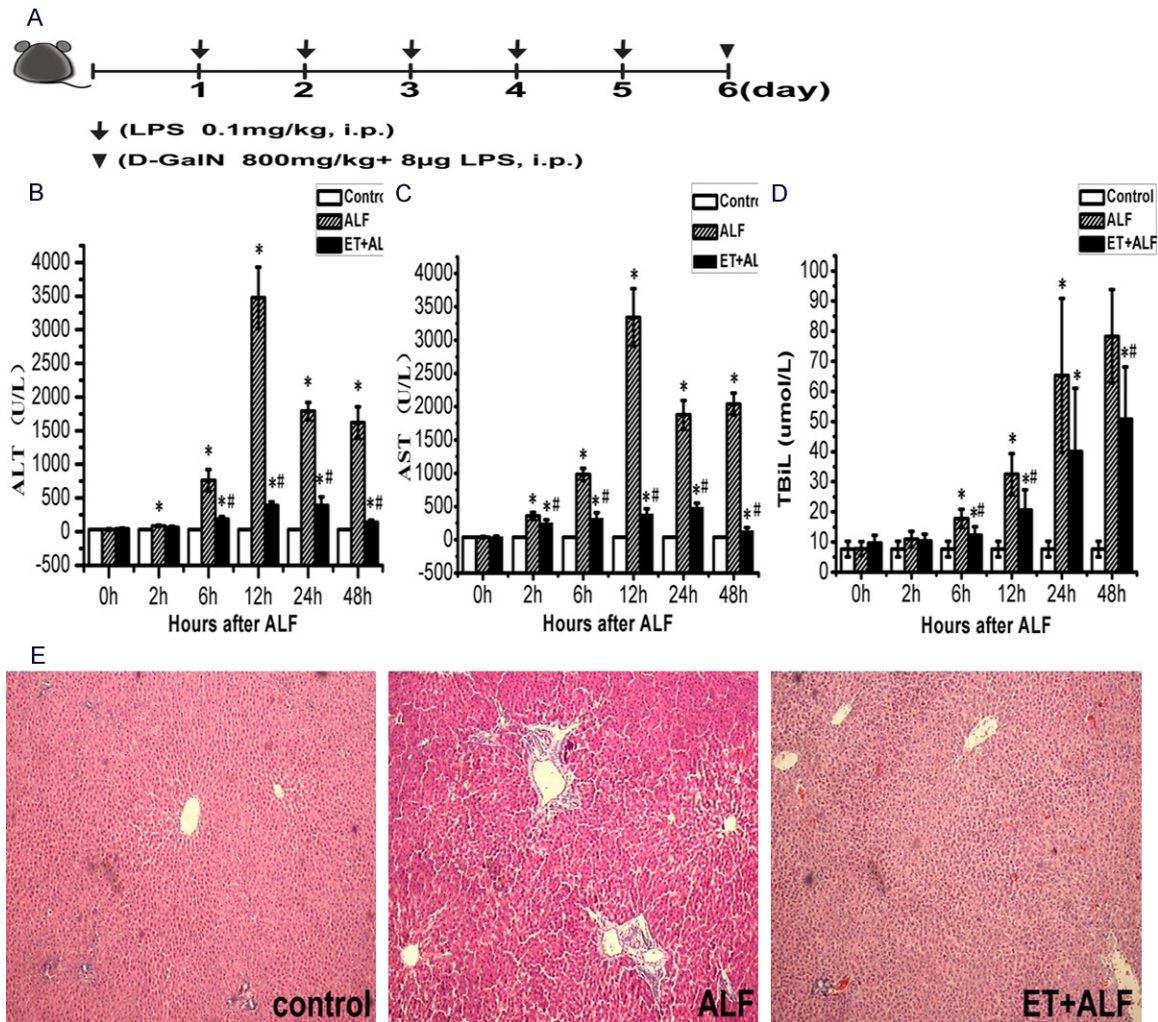


Figure 2. LPS pretreatment attenuates liver damage and inhibited the release of cytokines induced by D-GalN/LPS. (A) Rats were treated with pyrogen-free 0.9% sodium chloride or LPS (0.1 mg/kg). Five days later, D-GalN/LPS (800 mg/kg D-GalN + 8 μg LPS) was injected. Serum samples were collected at indicated time points after D-GalN/LPS injection. ALT (B), AST (C) and TBIL (D) in serum were assayed by using an automated blood chemistry analyzer. (*significant compared to control group, $P < 0.05$; #significant compared to each subgroup, $P < 0.05$). Liver samples were collected 12 h after D-GalN/LPS injection for HE staining (original magnification: $\times 100$) (E).

sues of ALF group and ET + ALF group were assayed by RT-PCR. In the ALF group, the mRNA levels of P65 began to rise at 2 hours after D-GalN/LPS injection and significantly elevated at 12 hours, and the mRNA levels of SOCS1 and SOCS3 increased gradually at 2 hours after D-GalN/LPS injection and significantly elevated at 12 hours, and 6 hours, respectively, then were down-regulated (Figure 4B, 4D). Additionally, the above expression pattern of P65, SOCS1 and SOCS3 were also observed in the ET + ALF group. The mRNA levels of P65 was lower than in ALF group, while the mRNA levels of SOCS1 and SOCS3 were both higher than in ALF group (Figure 4B, 4D).

Protein expression of STAT1, STAT3, SOCS1, SOCS3 P65 and Bax after D-GalN/LPS injection

To further investigate the underlying mechanisms, the protein expressions of STAT1, STAT3 (Figure 5B, 5C), SOCS1, SOCS3 (Figure 6B, 6C), P65 and Bax (Figure 7B, 7C) both in the ALF group and ET + ALF group are detected by Western Blotting, then all the above data was normalized against those of β -actin. In the ALF group, the protein levels of STAT1 and STAT3 increased at 2 hours after D-GalN/LPS injection. And the protein levels of P65 and Bax also increased at 2 hour after D-GalN/LPS injection.

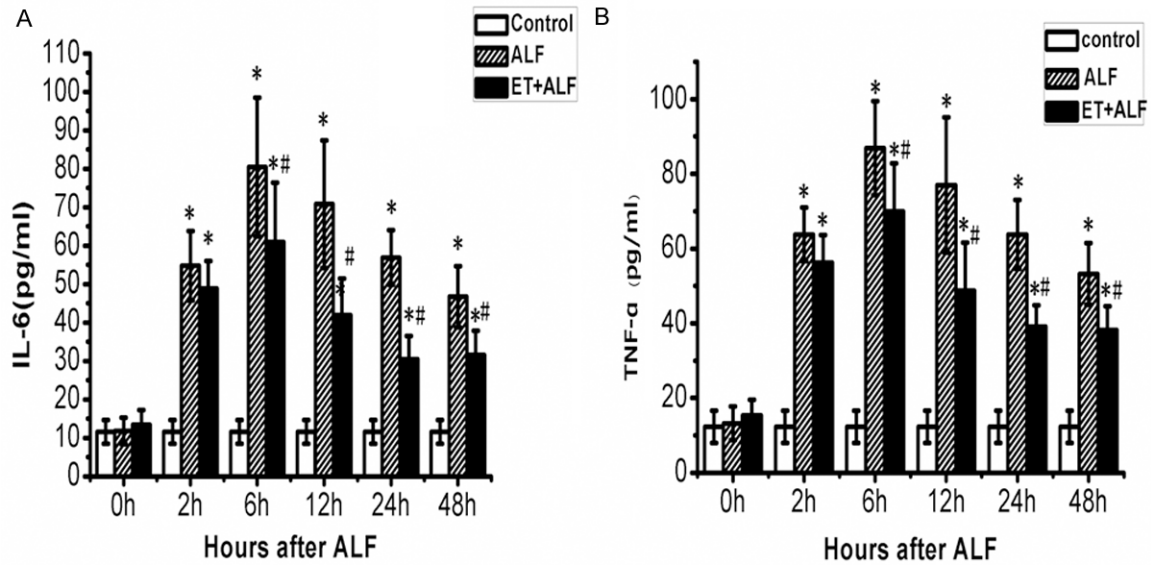


Figure 3. LPS pretreatment inhibited the release of cytokines induced by D-GalN/LPS. Serum samples were collected at indicated time points after D-GalN/LPS injection. IL-6 (A), and TNF-α (B) in serum were assayed by ELISA. *significant compared to control group, $P < 0.05$; #significant compared to each subgroup, $P < 0.05$.

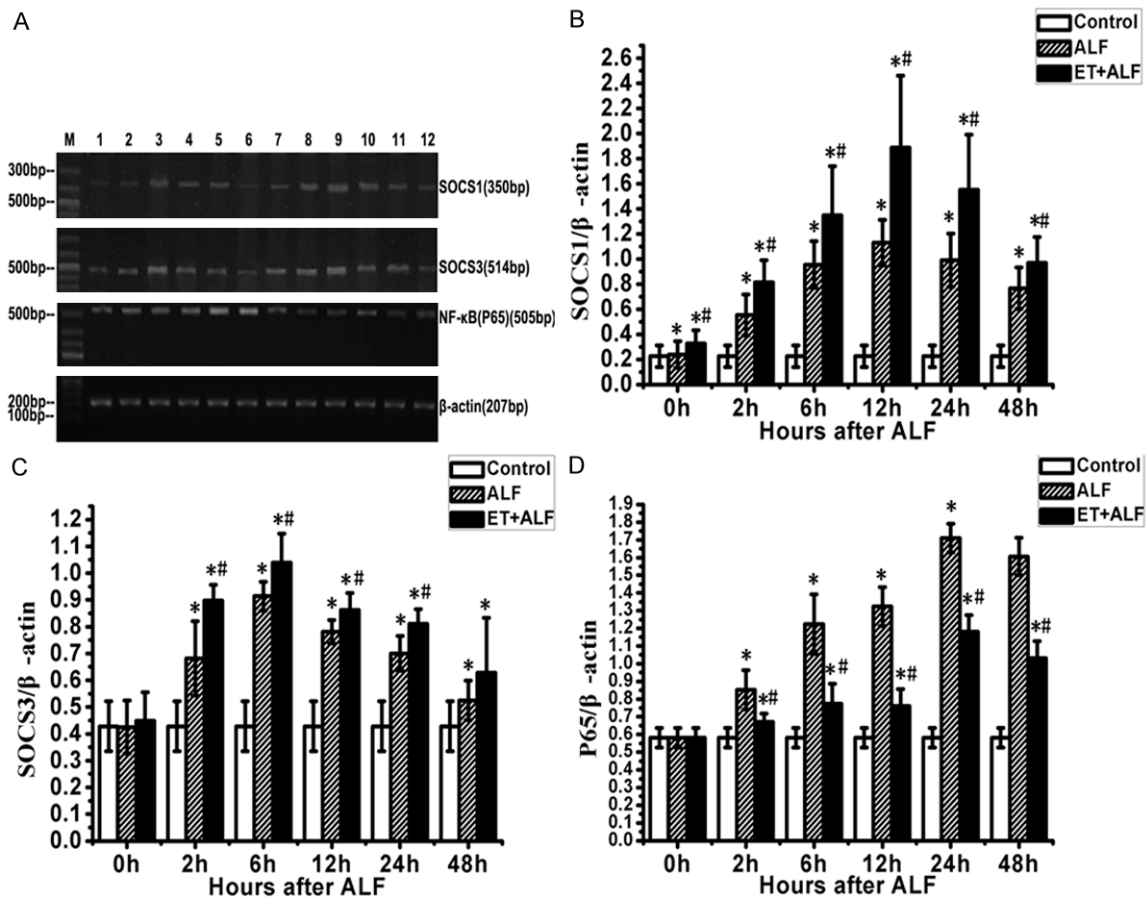


Figure 4. Effect of LPS pretreatment on the changes of SOCS1 (B), SOCS3 (C) and P65 (D) mRNA expression in liver of rat. (A) M: marker; 1: The control group; 2: ALF 2 h group; 3: ALF 6 h group; 4: ALF 12 h group; 5: ALF 24 h group; 6: ALF 48 h group; 7: ETT 2 h group; 8: ETT 6 h group; 9: ETT 12 h group; 10: ETT 24 h group; 11: ETT 48 h group

Liver samples were collected at indicated time points after D-GalN/LPS (800 mg/kg D-GalN + 8 µg LPS) injection to determine the mRNA level of P65, SOCS1 and SOCS3 mRNA by RT-PCR. Data represent means $\pm$ SD. * P < 0.05 (n = 6). *significant compared to control group, #significant compared to each subgroup, P < 0.05.

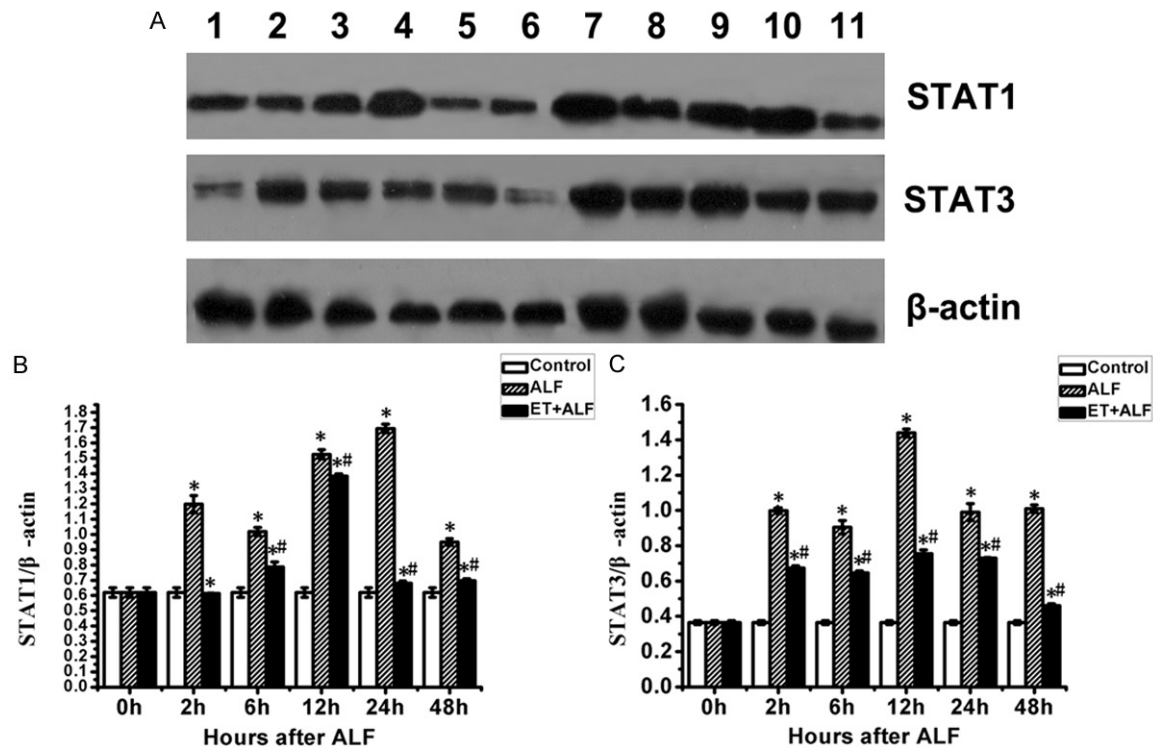


Figure 5. Effect of LPS pretreatment on the changes of STAT1 (B) and STAT3 (C) protein expression in liver of rat. (A) 1: The control group; 2: ET 2 h group; 3: ET 6 h group; 4: ET 12 h group; 5: ET 24 h group; 6: ET 48 h group; 7: ALF 2 h group; 8: ALF 6 h group; 9: ALF 12 h group; 10: ALF 24 h group; 11: ALF 48 h group. Liver samples were collected at indicated time points after D-GalN/LPS (800 mg/kg D-GalN + 8 µg LPS) injection to determine the protein level of STAT1 and STAT3 by Western blot. Data represent means $\pm$ SD. * P < 0.05 (n = 6). #significant compared to each subgroup, P < 0.05.

A similar result was found in the ET + ALF group. Our data showed that the protein levels of STAT1, STAT3, P65 and Bax in the ALF group were higher than the ET + ALF group. SOCS1 and SOCS3 in the ET + ALF group elevated gradually at 2 hours after D-GalN/LPS injection and significantly elevated at 12 hours, those levels were higher than ALF group.

Discussion

Endotoxin is a gram-negative bacterial lipopolysaccharide (LPS) and releases a wide variety of inflammatory mediators, which are considered to be related to the development of acute liver failure as well as to multiple organ failure. D-GalN is a specific hepatotoxic agent that induces liver injury by selective depletion of uridine nucleotides in hepatocytes. Taken together, can dramatically increase their susceptibility

to the lethal effects of LPS and may leads to fulminant hepatic. Hence, combined administration of D-GalN/LPS together has been widely used as to mimic the sequences of events in human hepatitis [8, 15, 16]. In our study, we found that pretreated of rats with low dose LPS for five days could significantly ameliorate D-GalN/LPS-induced liver injury. ET significantly increased the survival rate in rats from 40% to 100% after D-GalN/LPS injection. Both in ALF group and ET + ALF group, the serum ALT, AST levels increased at 2 hour and were significantly elevated at 12 hour, and serum TBIL levels increased persistently from 2 hour. Additionally, we examined the expression of Bax proteins in liver. However, serum ALT, AST, and TBIL levels in the ET + ALF group were significant lower than HF group at each time point. Hence, our results demonstrated that pre-exposure of a sub-lethal dose of LPS in rats not

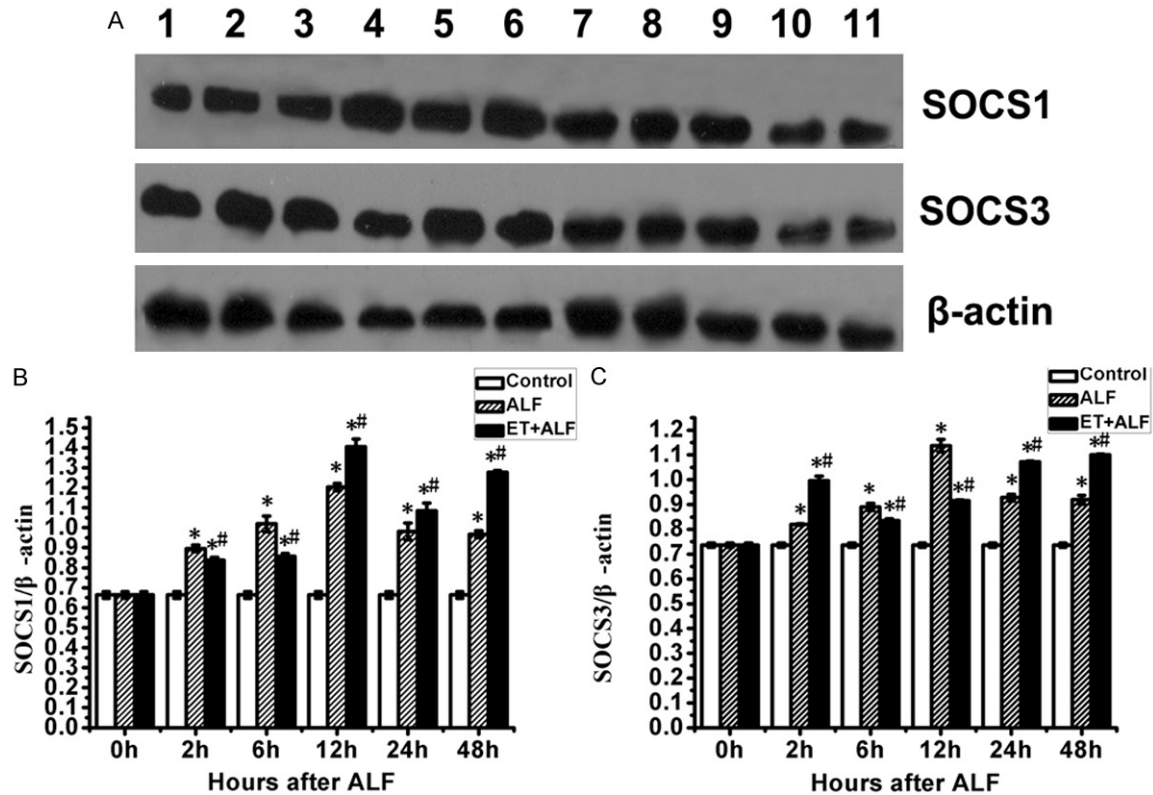


Figure 6. Effect of LPS pretreatment on the changes of SOCS1 (B) and SOCS3 (C), protein expression in liver of rat. (A) 1: The control group; 2: ET 2 h group; 3: ET 6 h group; 4: ET 12 h group; 5: ET 24 h group; 6: ET 48 h group; 7: ALF 2 h group; 8: ALF 6 h group; 9: ALF 12 h group; 10: ALF 24 h group; 11: ALF 48 h group. Liver samples were collected at indicated time points after D-GalN/LPS (800 mg/kg D-GalN + 8 µg LPS) injection to determine the protein level of SOCS1 and SOCS3 by Western blot. Data represent means ± SD. * $P < 0.05$ ($n = 6$). #significant compared to each subgroup, $P < 0.05$.

only attenuated histopathological injuries but also increased their survive rate caused by D-GalN/LPS.

As we know, various cytokines and inflammatory mediators are involved in the pathogenesis of ALF. TNF- α , one of the most pivotal mediators in the pathogenesis of LPS and Gram-negative bacterial infection, has been widely investigated [8, 17, 18]. In addition, NF-kappa B plays a key role in the regulation of TNF- α transcription stimulated by LPS [19, 20]. After exposure to LPS, NF-kappa B is translocated to the nucleus and binds to NF-kappa B promoter sites on DNA, activating gene transcription of cytokines such as TNF- α , and accelerating liver injury [21, 22]. In our study, we exhibited that the plasma levels of TNF- α increased at 2 hours and were significantly elevated at 24 hours in ALF group. In ET + ALF group, TNF- α level was lower than in ALF group. Additionally, Bax protein encoded by Bax gene belongs to the BCL2 protein family, which leads to the loss in mem-

brane potential and the release of cytochrome c, leading to cell death [23]. Our data showed that Bax protein levels in ALF group continuously increased after 2 hour. However, the expressions of Bax in ET + ALF group were lower compared to the ALF group at each time point, except at 12 hours. Those results indicated that mitochondrial in the ALF may be are serious losses and massive hepatic cell death. Therefore, pre-exposure of a sub-lethal dose of LPS significantly increased the survival rate via depressing the levels of pro-inflammatory mediators.

Following liver injury, liver cells, including Kupffer cells [24], hepatocytes [25, 26], natural killer cells [27], and dendritic cells [28], could produce pro-inflammatory cytokines and anti-inflammatory cytokines. IL-6 can activated the STAT3, which playing key roles in inducing the acute-phase response in the liver, protecting against hepatocellular damage, and promoting liver regeneration [29]. Furthermore, studies

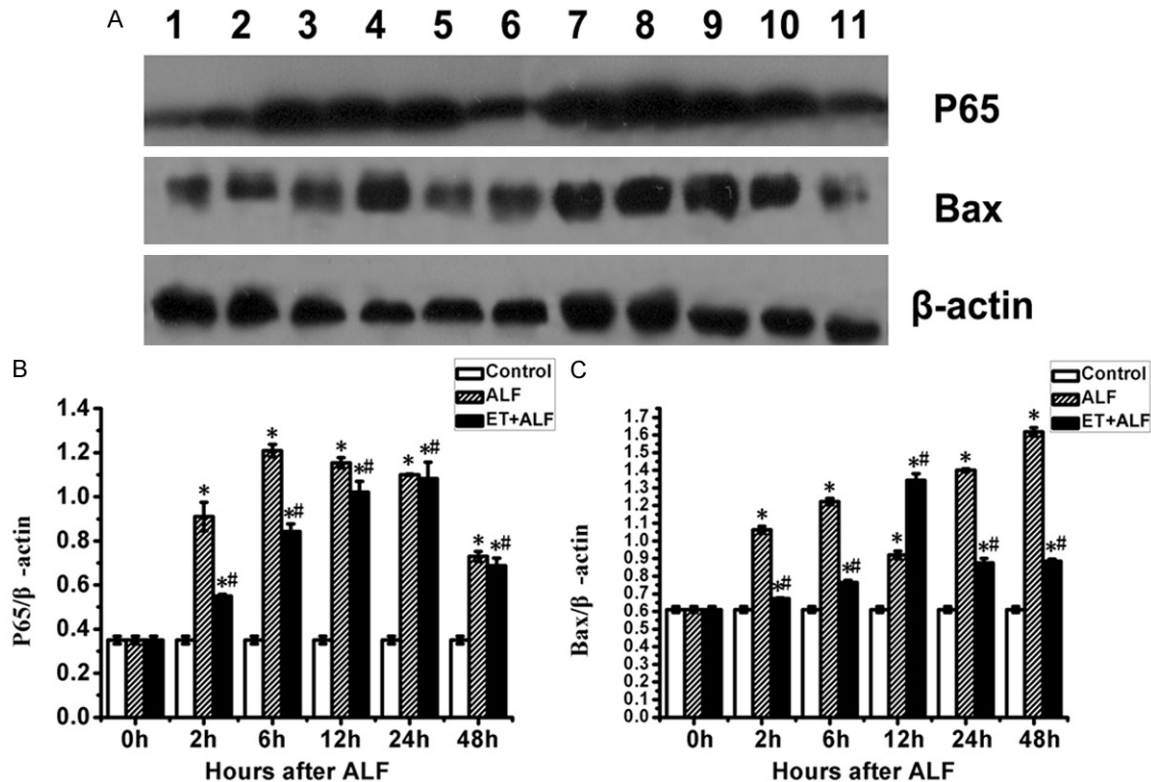


Figure 7. Effect of LPS pretreatment on the changes of P65 (B) and Bax (C) protein expression in liver of rat. A. 1: The control group; 2: ET 2 h group; 3: ET 6 h group; 4: ET 12 h group; 5: ET 24 h group; 6: ET 48 h group; 7: ALF 2 h group; 8: ALF 6 h group; 9: ALF 12 h group; 10: ALF 24 h group; 11: ALF 48 h group. Liver samples were collected at indicated time points after D-GalN/LPS (800 mg/kg D-GalN + 8 μ g LPS) injection to determine the protein level of P65 and Bax by Western blot. Data represent means $\pm$ SD. * P < 0.05 (n = 6). #significant compared to each subgroup, P < 0.05.

had shown that STAT1-deficient mice could abolish D-GalN/LPS-induced liver injury [30, 31]. Our study demonstrated that after injection of D-GalN/LPS, STAT1 was rapidly activated and highly induced liver damage, IL-6 and STAT3 in ALF group increased at 2 hour and peaked 12 hour, but those proteins in ET + ALF group were increased slightly at 2 hour. Those data indicated prior injection of rat with a low dose of LPS might reduce the levels of TNF- α and IL-6 then reduced the activation of STAT1 and STAT3 in the liver, which was involved in promoting liver regeneration. Suppressors of cytokine signaling (SOCS) proteins function as feedback inhibitors of the JAK/STAT signaling pathway, terminating innate and adaptive immune responses [32-34]. In our previous study, we observed in ET + ALF group hepatic SOCS1 and SOCS3 mRNA and proteins elevated higher than ALF group, which indicated SOCSs may involve in the mechanism of LPS pretreatment in ALF.

To conclude, our results suggested that LPS pretreatment has potent protection against D-GalN/LPS-induced ALF. It significantly decreased the high lethality, ameliorated the liver injuries, inhibited hepatocytes apoptosis and reduced hepatic inflammatory responses including down-regulating the level of STAT1, STAT3 and up-regulating the level of SOCS1, SOCS3. Those protective effects might be involved in suppression of NF- κ B and Bax activation, which inhibited TNF- α and IL-6 production. Therefore, LPS pretreatment might be considered as a promising new strategy to regulate the inflammatory response in improving the prognosis of ALF and prolong life in patients with liver damage.

Acknowledgements

This work was supported by the Zhejiang Provincial Natural Science Foundation of China (Y12H030022).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Ming-Qin Lu, Department of Infection and Liver Diseases, Liver Research Center, The First Affiliated Hospital of Wenzhou Medical University, Nanbaixiang Street, Wenzhou, Zhejiang, P. R. China. E-mail: lmq0906@163.com

References

- [1] O'Grady JG, Schalm SW and Williams R. Acute liver failure: redefining the syndromes. *Lancet* 1993; 342: 273-275.
- [2] Nakama T, Hirono S, Moriuchi A, Hasuike S, Nagata K, Hori T, Ido A, Hayashi K, Tsubouchi H. Etoposide prevents apoptosis in mouse liver with D-galactosamine/lipopolysaccharide-induced fulminant hepatic failure resulting in reduction of lethality. *Hepatology* 2001; 33: 1441-1450.
- [3] Galle PR. Apoptosis in liver disease. *J Hepatol* 1997; 27: 405-412.
- [4] Lee WM. Acute liver failure. *N Engl J Med* 1993; 329: 1862-1872.
- [5] Schmidt LE and Larsen FS. Prognostic implications of hyperlactatemia, multiple organ failure, and systemic inflammatory response syndrome in patients with acetaminophen-induced acute liver failure. *Crit Care Med* 2006; 34: 337-343.
- [6] Ogushi I, Iimuro Y, Seki E, Son G, Hirano T, Hada T, Tsutsui H, Nakanishi K, Morishita R, Kaneda Y, Fujimoto J. Nuclear factor kappa B decoy oligodeoxynucleotides prevent endotoxin-induced fatal liver failure in a murine model. *Hepatology* 2003; 38: 335-344.
- [7] Sakamoto S, Okanoue T, Itoh Y, Nakagawa Y, Nakamura H, Morita A, Daimon Y, Sakamoto K, Yoshida N, Yoshikawa T, Kashima K. Involvement of Kupffer cells in the interaction between neutrophils and sinusoidal endothelial cells in rats. *Shock* 2002; 18: 152-157.
- [8] Leist M, Gantner F, Bohlinger I, Tiegs G, Germann PG and Wendel A. Tumor necrosis factor-induced hepatocyte apoptosis precedes liver failure in experimental murine shock models. *Am J Pathol* 1995; 146: 1220-1234.
- [9] Helal GK. Thymoquinone supplementation ameliorates acute endotoxemia-induced liver dysfunction in rats. *Pak J Pharm Sci* 2010; 23: 131-137.
- [10] Roller J, Laschke MW, Scheuer C and Menger MD. Heme oxygenase (HO)-1 protects from lipopolysaccharide (LPS)-mediated liver injury by inhibition of hepatic leukocyte accumulation and improvement of microvascular perfusion. *Langenbecks Arch Surg* 2010; 395: 387-394.
- [11] Eipel C, Bordel R, Nickels RM, Menger MD and Vollmar B. Impact of leukocytes and platelets in mediating hepatocyte apoptosis in a rat model of systemic endotoxemia. *Am J Physiol Gastrointest Liver Physiol* 2004; 286: G769-776.
- [12] Biswas SK and Lopez-Collazo E. Endotoxin tolerance: new mechanisms, molecules and clinical significance. *Trends Immunol* 2009; 30: 475-487.
- [13] Cavaillon JM and Adib-Conquy M. Bench-to bedside review: endotoxin tolerance as a model of leukocyte reprogramming in sepsis. *Crit Care* 2006; 10: 233.
- [14] Cross AS. Endotoxin tolerance-current concepts in historical perspective. *J Endotoxin Res* 2002; 8: 83-98.
- [15] Galanos C, Freudenberg MA and Reutter W. Galactosamine-induced sensitization to the lethal effects of endotoxin. *Proc Natl Acad Sci U S A* 1979; 76: 5939-5943.
- [16] Hishinuma I, Nagakawa J, Hirota K, Miyamoto K, Tsukidate K, Yamanaka T, Katayama K, Yamatsu I. Involvement of tumor necrosis factor-alpha in development of hepatic injury in galactosamine-sensitized mice. *Hepatology* 1990; 12: 1187-1191.
- [17] Ferlito M, Romanenko OG, Ashton S, Squadrito F, Halushka PV and Cook JA. Effect of cross-tolerance between endotoxin and TNF-alpha or IL-1beta on cellular signaling and mediator production. *J Leukoc Biol* 2001; 70: 821-829.
- [18] Bohlinger I, Leist M, Gantner F, Angermuller S, Tiegs G and Wendel A. DNA fragmentation in mouse organs during endotoxic shock. *Am J Pathol* 1996; 149: 1381-1393.
- [19] Qiu Z, Kwon AH, Tsuji K, Kamiyama Y, Okumura T and Hirao Y. Fibronectin prevents D-galactosamine/lipopolysaccharide-induced lethal hepatic failure in mice. *Shock* 2006; 25: 80-87.
- [20] Shi DW, Zhang J, Jiang HN, Tong CY, Gu GR, Ji Y, Summah H, Qu JM. LPS pretreatment ameliorates multiple organ injuries and improves survival in a murine model of polymicrobial sepsis. *Inflamm Res* 2011; 60: 841-849.
- [21] Lin M, Rippe RA, Niemela O, Brittenham G and Tsukamoto H. Role of iron in NF-kappa B activation and cytokine gene expression by rat hepatic macrophages. *Am J Physiol* 1997; 272: G1355-1364.
- [22] Gong X, Luo FL, Zhang L, Li HZ, Wu MJ, Li XH, Wang B, Hu N, Wang CD, Yang JQ, Wan JY. Tetrandrine attenuates lipopolysaccharide-induced fulminant hepatic failure in D-galactosamine-sensitized mice. *Int Immunopharmacol* 2010; 10: 357-363.
- [23] Banerjee J and Ghosh S. Bax increases the pore size of rat brain mitochondrial voltage-dependent anion channel in the presence of

- tBid. *Biochem Biophys Res Commun* 2004; 323: 310-314.
- [24] Fisher JE, McKenzie TJ, Lillegard JB, Yu Y, Juskewitch JE, Nedredal GI, Brunn GJ, Yi ES, Malhi H, Smyrk TC, Nyberg SL. Role of Kupffer cells and toll-like receptor 4 in acetaminophen-induced acute liver failure. *J Surg Res* 2013; 180: 147-155.
- [25] Wang N, Wang Z, Sun H, Shi X, Zhang Y and Liu Q. Augmenter of liver regeneration improves therapeutic effect of hepatocyte homotransplantation in acute liver failure rats. *Int Immunopharmacol* 2013; 15: 325-332.
- [26] Best J, Dolle L, Manka P, Coombes J, van Grunsven LA and Syn WK. Role of liver progenitors in acute liver injury. *Front Physiol* 2013; 4: 258.
- [27] Yang Q, Shi Y, He J and Chen Z. The evolving story of macrophages in acute liver failure. *Immunol Lett* 2012; 147: 1-9.
- [28] Henning JR, Graffeo CS, Rehman A, Fallon NC, Zambirinis CP, Ochi A, Barilla R, Jamal M, Deutsch M, Greco S, Ego-Osuala M, Bin-Saeed U, Rao RS, Badar S, Quesada JP, Acehan D, Miller G. Dendritic cells limit fibroinflammatory injury in nonalcoholic steatohepatitis in mice. *Hepatology* 2013; 58: 589-602.
- [29] Wang H, Lafdil F, Kong X and Gao B. Signal transducer and activator of transcription 3 in liver diseases: a novel therapeutic target. *Int J Biol Sci* 2011; 7: 536-550.
- [30] Lee HJ, Oh YK, Rhee M, Lim JY, Hwang JY, Park YS, Kwon Y, Choi KH, Jo I, Park SI, Gao B, Kim WH. The role of STAT1/IRF-1 on synergistic ROS production and loss of mitochondrial transmembrane potential during hepatic cell death induced by LPS/d-GalN. *J Mol Biol* 2007; 369: 967-984.
- [31] Siebler J, Wirtz S, Klein S, Protschka M, Blessing M, Galle PR, Neurath MF. A key pathogenic role for the STAT1/T-bet signaling pathway in T-cell-mediated liver inflammation. *Hepatology* 2003; 38: 1573-1580.
- [32] Kundu K, Dutta K, Nazmi A and Basu A. Japanese encephalitis virus infection modulates the expression of suppressors of cytokine signaling (SOCS) in macrophages: Implications for the hosts' innate immune response. *Cell Immunol* 2013; 285: 100-110.
- [33] Inagaki-Ohara K, Kondo T, Ito M and Yoshimura A. SOCS, inflammation, and cancer. *JAKSTAT* 2013; 2: e24053.
- [34] Kulkarni RM, Kutcher LW, Stuart WD, Carson DJ, Leonis MA and Waltz SE. Ron receptor-dependent gene regulation in a mouse model of endotoxin-induced acute liver failure. *Hepato-biliary Pancreat Dis Int* 2012; 11: 383-392.