



Original Article

UMSCs Attenuate LPS/D-GalN-induced Acute Liver Failure in Mice by Down-regulating the MyD88/NF-κB Pathway

Hailing Liao, Siying Du, Ting Jiang, Mengyao Zheng, Zhao Xiang and Jinhui Yang*

Department of Digestive Medicine, The Second Affiliated Hospital of Kunming Medical University, Kunming, Yunnan, China

Received: 9 December 2020 | Revised: 13 January 2021 | Accepted: 23 March 2021 | Published: 22 April 2021

Abstract

Background and Aims: Acute liver failure (ALF) is an inflammatory process of acute liver cell injury. Mesenchymal stem cells (MSCs) are undifferentiated, primitive cells with anti-inflammatory, anti-apoptotic, and multi-directional differentiation abilities. This study aimed to explore the therapeutic mechanism of umbilical cord (U)MSCs in ALF. **Methods:** D-galactosamine (D-GalN) combined with lipopolysaccharide (LPS) was used to establish an ALF model. After model establishment, UMSCs were injected via the tail vein. After UMSC transplantation, the number of mouse deaths was monitored every 12 h. A fully automatic biochemical analyzer was used to detect changes in biochemical analysis. Pathological changes was observed by stained with hematoxylin and eosin. The expression of My D88 was detected by immunohistochemical analysis, quantitative reverse transcription, and western blotting. The expression of NF-κB was detected by quantitative reverse transcription, western blotting. The expression of Bcl-2, Bax were detected by quantitative reverse transcription, western blotting. The expression of TNF-α, IL-1β, IL-6 were detected by enzyme-linked immunosorbent assay. **Results:** The 48-h survival rate of the UMSC-treated group was significantly higher than that of the LPS/D-GalN-exposed group. After 24 h of LPS/D-GalN exposure, UMSCs reduced serum alanine aminotransferase and aspartate aminotransferase levels and improved the liver structure. Western blot and real-time fluorescence quantitative nucleic acid amplification analyses showed that UMSCs decreased MyD88 expression, thereby inhibiting LPS/GalN-induced phosphorylation and degradation of inhibitor of nuclear factor (NF)-κB (IκB). Additionally, NF-κB p65 underwent nuclear translocation, inhibiting the production of the inflammatory factors interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α and played a protective role in ALF by down-regulating the pro-apoptotic gene Bax and up-regulating the anti-apoptotic gene Bcl-2. In summary, these findings indicate that UMSCs play a protective role in LPS/GalN-induced acute liver injury

via inhibition of the MyD88 pathway and subsequent inhibition of NF-κB-mediated cytokine production. **Conclusions:** Through the above mechanisms, UMSCs can effectively reduce LPS/D-GalN-induced ALF, reduce mouse mortality, and restore damaged liver function and damaged liver tissue.

Citation of this article: Liao H, Du S, Jiang T, Zheng M, Xiang Z, Yang J. UMSCs attenuate LPS/D-GalN-induced acute liver failure in mice by down-regulating the MyD88/NF-κB pathway. *J Clin Transl Hepatol* 2021;9(5):690–701. doi: 10.14218/JCTH.2020.00157.

Introduction

Acute liver failure (ALF) refers mainly to interactions among multiple factors that lead to the acute necrosis of liver cells and rapid loss of liver function; in such, liver function is decompensated, and severe liver disease may eventually lead to functional failure. This syndrome¹⁻² is clinically characterized by acute onset, rapid progression, and high mortality. Since 1983, liver transplantation has been recognized as the most effective treatment for ALF.³ However, as living standards have continuously improved, the incidence of ALF has also increased annually. Thus, the gap between the number of patients awaiting transplant and the supply of organs is widening, and the development of a treatment to replace liver transplantation is urgently needed. As early as the 1980s, Arnold Caplan proposed mesenchymal stem cells (MSCs) and MSC-based treatments.⁴ Studies have reported that transplanted MSCs can secrete many cytokines, promote liver tissue repair, inhibit immune cell proliferation and migration to the liver, and regulate liver function and the systemic immune inflammatory response.⁵ Knowledge on stem cell (SC) biology has increased rapidly, opening new avenues for SC-based therapies and the use of SCs as a cell therapy platform for ALF.⁶

SC transplantation therapy has become another important option to improve ALF treatment. SCs are undifferentiated, primitive progenitor cells with the characteristics of self-renewal, proliferation potential, and differentiation potential,⁷ and they can differentiate into multifunctional cells under certain conditions. In addition, studies have shown that SCs have other characteristics, including anti-inflammatory activity, apoptosis resistance, antioxidant activity, immunosuppressive activity, tissue repair capability, and growth factor expression.⁸ In humans, umbilical cord (U)MSCs can be derived from different compartments of the organ, including the amniotic membrane region, Wharton's jelly, perivascular zone⁹

Keywords: Acute liver failure; Stem cells; Inflammation; Signaling pathway.
Abbreviations: ALF, acute liver failure; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Bax, Bcl 2-Associated X Protein; Bcl-2, B-cell lymphoma-2; D-GalN, D-galactosamine; IL-1β, interleukin-1β; IL-6, interleukin-6; IκB, inhibitor of nuclear factor-κB; LPS, lipopolysaccharide; MSCs, mesenchymal stem cells; My D88, Myeloid differentiation factor 88; NF-κB, nuclear factor-κB; P-IκB, phosphorylated-IκB; TBil, total bilirubin; TNF-α, tumor necrosis factor-α; UMSCs, umbilical cord mesenchymal stem cells.

*Correspondence to: Jinhui Yang, Department of Digestive Medicine, The Second Affiliated Hospital of Kunming Medical University, No. 374 Yunnan Burma Road, Wuhua District, Kunming, Yunnan 650031, China. ORCID: <https://orcid.org/0000-0002-5733-0647>. Tel: +86-13608712810, E-mail: yangjinhui111@qq.com

of the vascular wall, and endothelial region in the middle and outer membrane region. Among all MSC groups, UMSCs have a strong proliferative ability. Research shows that UMSCs can maintain a stable doubling time in multiple passages, as the doubling time of the bone mesenchymal stem cells increased significantly after only six passages.¹⁰ Compared to other MSCs, UMSCs are more primitive, and because of their unique gene expression profile, UMSCs produce few teratomas.¹¹ In addition, because the placenta has a barrier, there is a low risk of infection from UMSC transplants, and hence, UMSCs are more suitable for clinical research.

Lipopolysaccharide (LPS) combined with D-galactosamine (D-GalN) is often used to establish animal models of liver failure, which is characterized by the activation of nuclear factor (NF)- κ B and the excessive secretion of inflammatory cytokines/mediating factors, leading to a systemic inflammatory response.¹² Accumulating evidence indicates that various proinflammatory cytokines/mediators, such as tumor necrosis factor (TNF)- α , leukocyte-derived interleukin (IL)-1 β , IL-6, inducible nitric oxide synthase and cyclooxygenase-2, are involved in LPS/GalN-induced liver toxicity.¹³ Therefore, inhibiting the inflammatory response may be an approach for treating liver failure.

Myeloid differentiation factor (MyD88) is an important Toll-like receptor (TLR)/IL-1 receptor superfamily member, and transduction of all TLRs into the cytoplasm by MyD88, in whole or in part, makes it a necessary adaptor protein to activate NF- κ B and the mitogen-activated protein kinase signaling pathway.¹⁴ LPS is recognized by TLR4 expressed by the cell and activates innate immunity through a MyD88-dependent pathway. Studies have shown that after genetic knockout of MyD88 in mice, LPS-induced activity almost completely disappeared. After intraperitoneal injection of high concentrations of LPS, mice can survive for more than 96 h, but all mice without MyD88 gene knockout die within 96 h, indicating that MyD88 plays an important role in LPS activation. NF- κ B is comprised of the p50 and p65 subunits; in addition, the inhibitor of NF- κ B (I κ B) is essential for host defense and mediates expression of the above-mentioned pro-inflammatory mediators and cytokines. In addition, NF- κ B controls apoptosis. Therefore, the inhibition of pro-inflammatory mediators and apoptosis is considered a potential strategy for ALF prevention and treatment. Studies have shown that overactivated MyD88 signaling is a key factor in the development of many immune-mediated diseases, which provides us with new therapeutic areas targeting MyD88 signaling pathways to reduce the intensity of immune responses.¹⁵

On the basis of the characteristics of the LPS/D-GalN model, we suspect that UMSCs may protect liver injury by inhibiting the inflammatory pathway and apoptosis pathway. Therefore, in this study, a mouse model of ALF was established via the administration of D-GalN combined with LPS. The therapeutic effect of UMSCs on ALF was evaluated, and the potential mechanism was revealed.

Methods

Mouse model establishment and cell transplantation

Healthy female BALB/c mice (weight, 20–22 g; age, 6–8 weeks; no specific pathogen grade) were purchased from the Animal Experiment Center of Kunming Medical University (China). Mice were housed in an environmentally controlled room (temperature, 24°C; humidity, 40–80%) under a 12-h dark/12-h light cycle and had free access to food and water. All experiments were approved by the Medical Ethics Committee of Kunming Medical University and were conducted in accordance with the experimental animal care principles of the University. Sixty-eight mice

were randomly divided into three groups, namely, the control group ($n=10$), the LPS/D-GalN group ($n=28$), and the LPS/D-GalN+UMSCs group ($n=28$). D-GalN (900 mg/kg; Sigma-Aldrich, St. Louis, MO, USA) was administered via intraperitoneal injection at 12-h intervals for a total of two times. After the second intraperitoneal injection of D-GalN, LPS (10 μ g/kg; Sigma-Aldrich) was also administered to establish the ALF model. At 24 h after the LPS/D-GalN injection, mice in the LPS/D-GalN + UMSCs group were injected with UMSCs (5×10^6 cells/mouse; Beike Bio, Shenzhen, China) via the tail vein. Mice in the LPS/D-GalN group were not given any treatment. At 12 h, 1 day, 2 days, 5 days, 7 days, 14 days, 21 days, and 28 days after cell transplantation, mice were anaesthetized with 40 mg/kg pentobarbital sodium (Sigma-Aldrich) via intraperitoneal injection, and blood samples and liver tissue samples were collected for histopathological studies and protein detection.

Long-term survival analysis and biochemical analysis

After UMSC transplantation, the number of mouse deaths was monitored every 12 h. At 12 h, 24 h, and 48 h after UMSC transplantation, mice were anaesthetized by the intraperitoneal injection of pentobarbital sodium. Mouse eyeballs were then removed for blood collection. Each blood sample was incubated at room temperature for 30 m and was then centrifuged at 3,000 rpm for 15 m at 4°C. The upper layer of serum was stored at -20°C . A fully automatic biochemical analyzer (Beckman AU-5421; Beckman-Coulter, Brea, CA, USA) was used to detect changes in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin (TBil) levels.

Pathological and immunological analyses

Mice were sacrificed at the designated time points (12 h, 1 day, 2 days, 5 days, 7 days, 14 days, 21 days, and 28 days) after UMSC transplantation. Liver lobes were excised from the same site and fixed with 4% paraformaldehyde (Solarbio, Beijing, China) for 24 h for histological and immunological analyses. Paraffin-embedded liver tissue was sliced into 5- μ m sections and stained with hematoxylin and eosin (HE; Solarbio). Pathological changes in liver tissue were assessed under an optical microscope. For immunohistochemical analysis, the tissue sections were heated in citric acid buffer (0.02 mol/L, pH=5.8) (Solarbio) for antigen retrieval. Bovine serum albumin (5%; Sigma-Aldrich) in phosphate-buffered saline (Solarbio) was used to block non-specific binding. Then, according to the instructions of the reagent manufacturer, the sections were incubated with an anti-MyD88 antibody (Abcam, Cambridge, UK) overnight at 4°C. The sections were then incubated with a horseradish peroxidase-conjugated secondary antibody (Abcam) at 37°C for 1 h and evaluated under an optical microscope. The optical density value was calculated by ImagePro Plus software (Media Cybernetics, Inc., Rockville, MD, USA).

Enzyme-linked immunosorbent assay (referred to as ELISA)

Blood was collected from mouse eyeballs, incubated at room temperature for 30 m and centrifuged at 3,000 rpm for 15 m at 4°C. The supernatant was collected, and ELISA kits (Jiang Lai, Shanghai, China) were used to determine serum TNF- α , IL-1 β , and IL-6 cytokine levels. The absorbance was measured at 450 nm in a microplate reader (ELx800; Bio-Tek, Winooski, VT, USA).

Quantitative reverse transcription (qRT)-PCR

TRIzol Reagent (Solarbio) was used to extract the total RNA from mouse liver tissue, according to the manufacturer's instructions. RNA was reverse transcribed to prepare the cDNA templates, and qRT-PCR was performed in a Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) under the following cycling conditions: initial denaturation at 95°C for 10 s, 40 cycles of a two-step PCR (95°C for 5 s, 60°C for 30 s), denaturation at 95°C for 15 s, annealing at 60°C for 1 m, and denaturation at 95°C for 15 s. β -actin was used as the housekeeping gene to normalize expression data. All expression levels were analyzed by the Δ CT method. Primers were designed by the Shanghai Jierui Biological Engineering Co., Ltd (Shanghai, China). The primer sequences for I κ B were 5' GGTGCAGGAGTGTGGTGG 3', 5' CTGAGTGAGGTAGTATCTGAGGC 3'; those for NF- κ B were 5' GATGTGCATCGCAAGTGG 3', 5' AGAAGTTGAGTTTCGGGTAGGC 3'; those for MyD88 were 5' CCCACTCGCAGTTTGTG 3', 5' CCACCTGTAAAGGCTTCTCG 3'; those for BAX were 5' CAGGATGCGTCACCAAGAA 3', 5' CAGGATGCGTCCACCAAGAA 3'; those for Bcl-2 were 5' GCTACCGTGTGACTTCGC 3', 5' ATCCAGCCTCCGTTATCC 3'; and those for M-actin were 5' TGCTGTCCCTGTATGCCTCT 3', 5' TTTGATGTACGCACGATT 3'.

Western blotting

RIPA lysis buffer (Solarbio) was used to extract protein from liver tissue. A bicinchoninic acid kit (Sigma-Aldrich) was used to determine protein concentrations. Samples containing equal amounts of protein (50 μ g) were subjected to electrophoresis. Proteins were separated on a 10% sodium dodecyl sulfate-polyacrylamide gel and transferred to a polyvinylidene fluoride membrane (Sigma-Aldrich). The membrane was blocked with skim milk (Sigma-Aldrich) and incubated with primary antibodies (Abcam) overnight at 4°C. The next day, the membrane was incubated with Tris-buffered saline containing Tween. After washing, the membrane was incubated with the secondary antibody (Abcam) for 1.5 h at room temperature. Immunoreactions were visualized according to the instructions of the instrument manufacturer (ChemiDoc™ XRS+; Bio-Rad, Hercules, CA, USA), and the intensities of the immunoreactive bands were measured using ImageJ analysis software (National Institutes of Health, Bethesda, MD, USA).

Data analysis

GraphPad Prism version 7.0 (GraphPad Software, San Diego, CA, USA) statistical software was used for statistical analysis. The Kaplan-Meier method with the log rank test was used to analyze survival. Measurement data are expressed as the means \pm standard deviations, and analysis of variance in a random block design was used for comparisons among multiple groups. A p -value < 0.05 was considered to indicate a statistically significant difference.

Results

UMSC transplantation improves survival

Survival analysis is the most straightforward approach to evaluate the effect of UMSCs on ALF. As shown in Figure 1 (panels C) and Table 1, the survival rate of mice was determined every 12 h after UMSC injection. The effective trans-

plantation of UMSCs increased the survival rate by 90% at 48 h, indicating that UMSC transplantation can increase the survival rate of mice with LPS/D-GalN-induced ALF.

UMSC transplantation restores liver function

The serum ALT, AST and TBil levels in mice in the LPS/D-GalN group gradually increased with the time and duration of disease. The serum ALT level in mice in the LPS/D-GalN+UMSCs group peaked 24 h after transplantation (248 \pm 37.3 U/L); the AST and TBil levels peaked 12 h after transplantation (379.3 \pm 7.2 U/L; 53.9 \pm 6.1 mg/dL). Subsequently, the levels of ALT, AST, and TBil in mice in the LPS/D-GalN+UMSCs group gradually decreased with time to 63.3 \pm 2.1 U/L, 214.3 \pm 7.5 U/L, and 29.9 \pm 0.8 mg/dL, respectively, 48 h after transplantation. The ALT, AST, and TBil levels in the LPS/D-GalN+UMSCs group of mice at 12 h, 24 h, and 48 h after UMSC transplantation differed significantly from those in the LPS/D-GalN group of mice ($p < 0.05$; Table 2). This result shows that UMSCs can significantly improve liver function.

UMSC transplantation reduces liver histopathological changes induced by LPS/D-GalN

Liver histopathological analysis revealed the protective effect of UMSCs on LPS/GalN-induced ALF. As shown in Figure 1 (panels A–B), the normal rat liver lobular structure is typical. At 24 h after the injection of D-GalN, the control group lost its normal lobular structure, showing hepatocyte cytoplasmic edema, local regional expansion, and hepatocyte degeneration. The patchy necrosis of hepatocytes was observed, in addition to inflammatory cell infiltration in the necrotic area. At 7 days after transplantation, the LPS/D-GalN+UMSCs group showed a significant recovery in liver structure, resolution of the large areas of degeneration and necrosis, significantly decreased inflammatory cell infiltration, and gradual recovery of the liver lobular structure. Furthermore, we observed significant bile duct hyperplasia in the portal area and normal liver cells around the bile duct. After 28 d, the liver structure basically returned to normal. Hematoxylin-eosin staining showed significant morphological changes in the transplant group compared to the control group ($p < 0.05$).

UMSCs inhibit MyD88 expression

To explore the effect of UMSCs on MyD88, we obtained liver tissues at various time points after LPS/D-GalN induction and UMSC treatment and analyzed them by immunohistochemistry (Fig. 2), Western blotting (Fig. 3 C–E), and real-time fluorescence quantitative nucleic acid amplification and detection (Fig. 3 A–B). The MyD88 expression level increased after LPS/D-GalN induction. However, UMSC treatment significantly inhibited MyD88 expression in a time-dependent manner. Therefore, UMSCs may play a therapeutic role by inhibiting MyD88 expression.

UMSCs inhibit LPS/GalN-induced NF- κ B signaling pathway activation

NF- κ B is the main regulator of LPS/D-GalN-induced liver inflammation. LPS/D-GalN promoted I κ B phosphorylation and degradation (Fig. 4) as well as the nuclear translocation of NF- κ B p65 (Fig. 5), and these effects were significantly inhibited after UMSC transplantation. This pattern shows that the UMSC-mediated inhibition of inflammation effectively

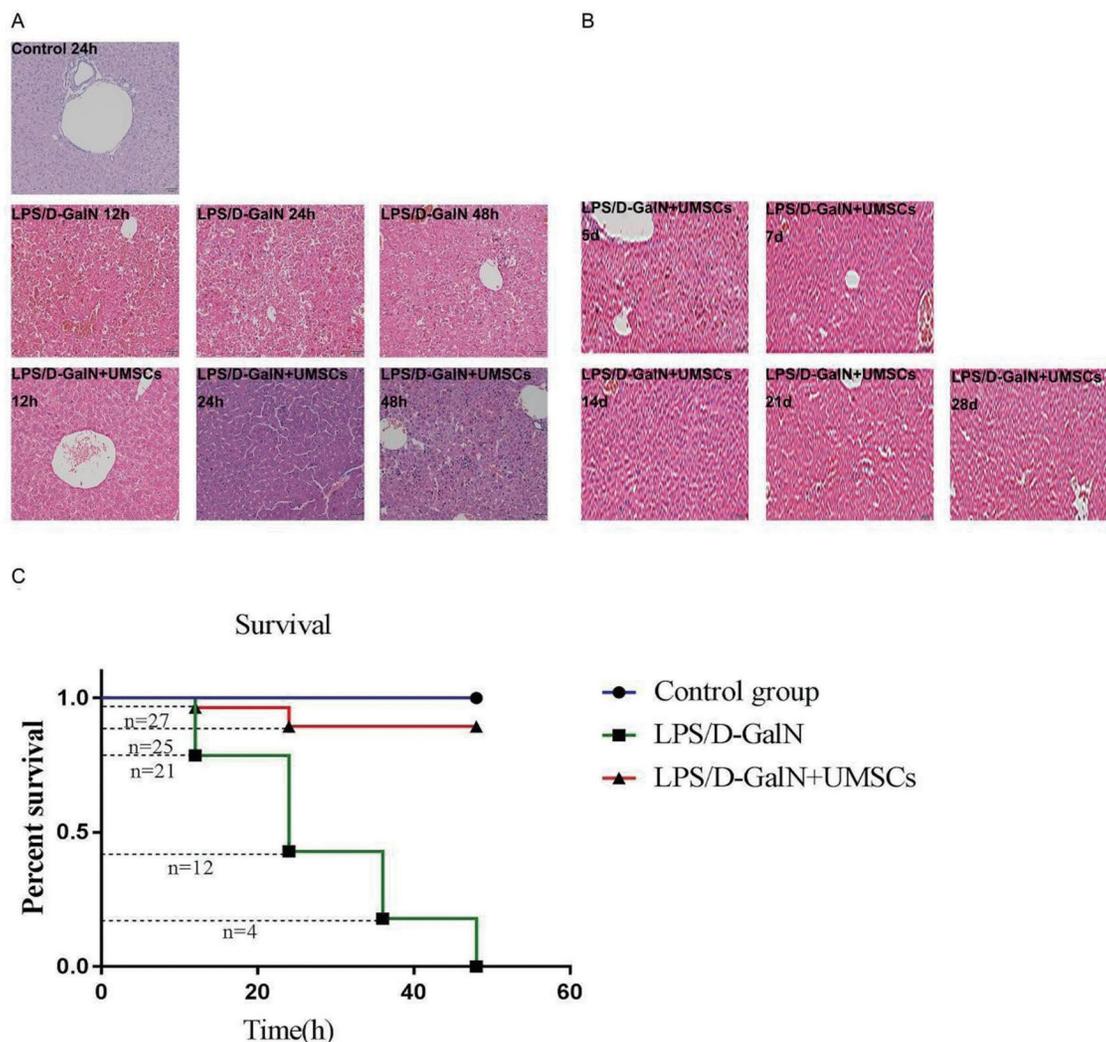


Fig. 1. Liver injury gradually repaired completely after UMSC transplantation. (A–B) Representative histological changes are shown in images of liver tissue from mice in each group (hematoxylin–eosin $\times 100$). (C) Survival curves of the LPS/D-GalN-induced ALF group, control group, and the UMSC treatment group. The survival rate of the LPS/D-GalN+UMSCs group was significantly higher than that of the LPS/D-GalN group at each time point ($n=3/\text{group}$, $p<0.01$). D-GalN, D-galactosamine; LPS, lipopolysaccharide. My D88, Myeloid differentiation factor 88.

blocked NF- κ B signaling pathway activation.

UMSCs inhibit the release of inflammatory factors

The inflammatory cytokines TNF- α , IL-6 and IL-1 β play an important role in liver injury. To further study the anti-inflammatory effect of UMSCs, their effect on serum TNF- α ,

IL-6 and IL-1 β secretion was detected by ELISA (Fig. 6). After LPS/D-GalN treatment, the TNF- α , IL-6 and IL-1 β levels in serum samples increased significantly, indicating that LPS/D-GalN can stimulate the release of these inflammatory mediators. After UMSC transplantation, the levels of these inflammatory cytokines were significantly decreased and gradually returned to normal as the survival time increased. This pattern shows that UMSCs inhibit the LPS/D-

Table 1. The survival rate of mice at each time point

Time	Number of death		Survival rate	
	LPS/D-GalN	LPS/D-GalN+UMSCs	LPS/D-GalN	LPS/D-GalN+UMSCs
12h	6	1	78.6%	96.4%
24h	10	2	42.9%	89.3%
36h	7	0	17.9%	89.3%
48h	5	0	0%	89.3%

Number of deaths and survival rates at each time point in each group ($n=3/\text{group}$, $p<0.01$). D-GalN, D-galactosamine; LPS, lipopolysaccharide.

Table 2. Comparison of serum ALT, AST and TBil levels at different time points.

Group (time point)	AST	ALT	TBil
Control	38.6±2.3	41.1±3.5	9.4±1.9
LPS/D-GalN (12 h)	1,927±16.9*	2,120±35.3*	79.6±1.0*
LPS/D-GalN (24 h)	2,303.7±47.3*	3,143±121.6*	95.3±2.0*
LPS/D-GalN (48 h)	1,369±12.8*	3,653±74.9*	76±4.7*
LPS/D-GalN+UMSCs (12 h)	233.3±13 [#]	379.3±7.2 [#]	53.9±6.1 [#]
LPS/D-GalN+UMSCs (24 h)	248±37.3 [#]	268±23.4 [#]	51.1±4.5 [#]
LPS/D-GalN+UMSCs (48 h)	63.3±2.1 [#]	214.3±7.5 [#]	29.9±0.8 [#]

Data are expressed as means±standard deviations (*n*=3/group): **p*<0.05 vs. control; [#]*p*<0.05 vs. LPS/D-GalN. ALT, alanine aminotransferase; AST, aspartate aminotransferase; D-GalN, D-galactosamine; LPS, lipopolysaccharide; TBil, total bilirubin.

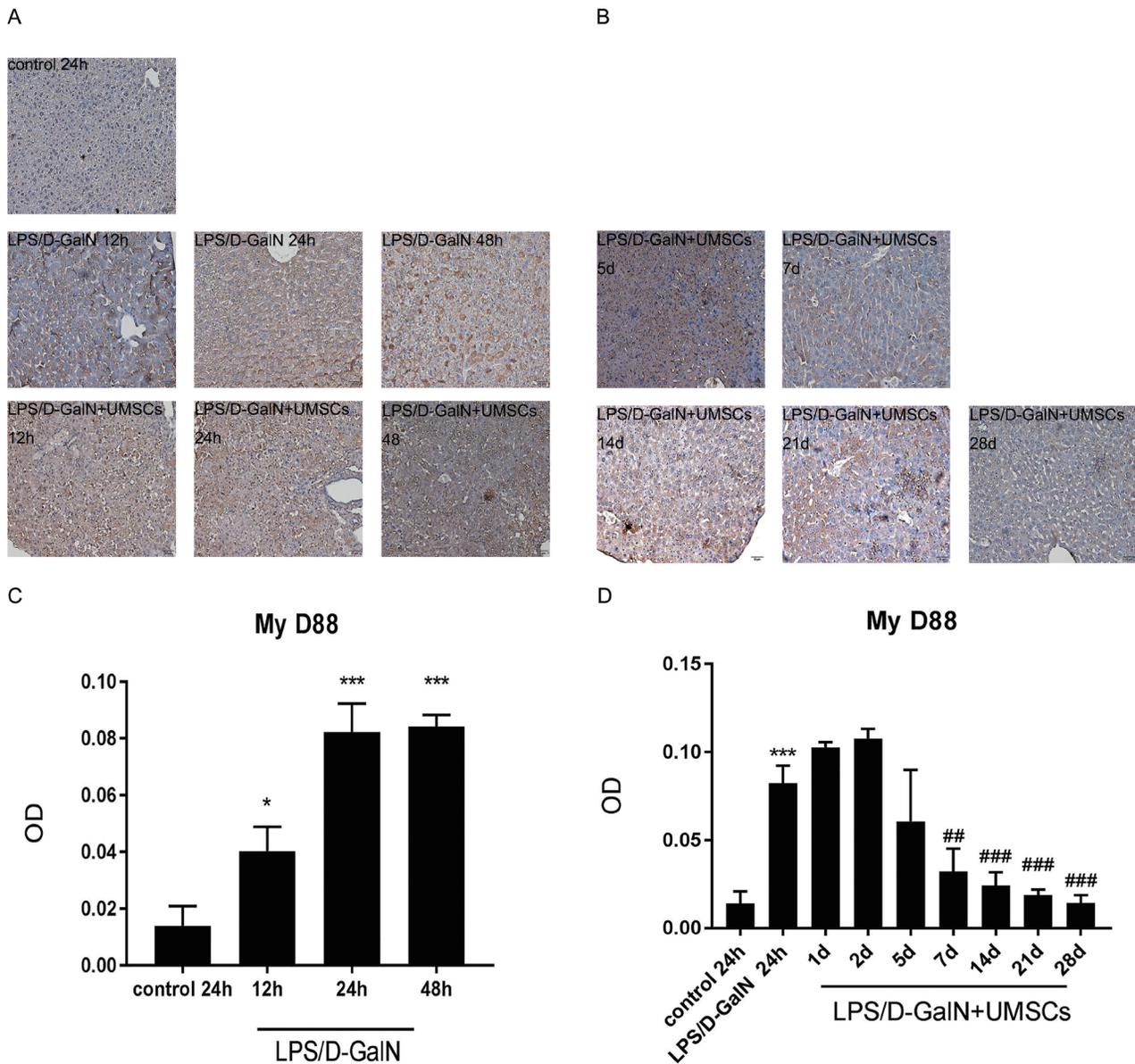


Fig. 2. Protein expression in the liver (immunohistochemistry ×100). The MyD88 expression level increased after LPS/D-GalN induction. At 7 d after transplantation, MyD88 protein expression was significantly inhibited in the LPS/D-GalN+UMSCs group. Data are presented as means±standard deviations (*n*=3/group). **p*<0.05 vs. control; ****p*<0.001 vs. control; ###*p*<0.001 vs. LPS/D-GalN. D-GalN, D-galactosamine; LPS, lipopolysaccharide; MyD88, Myeloid differentiation factor 88.

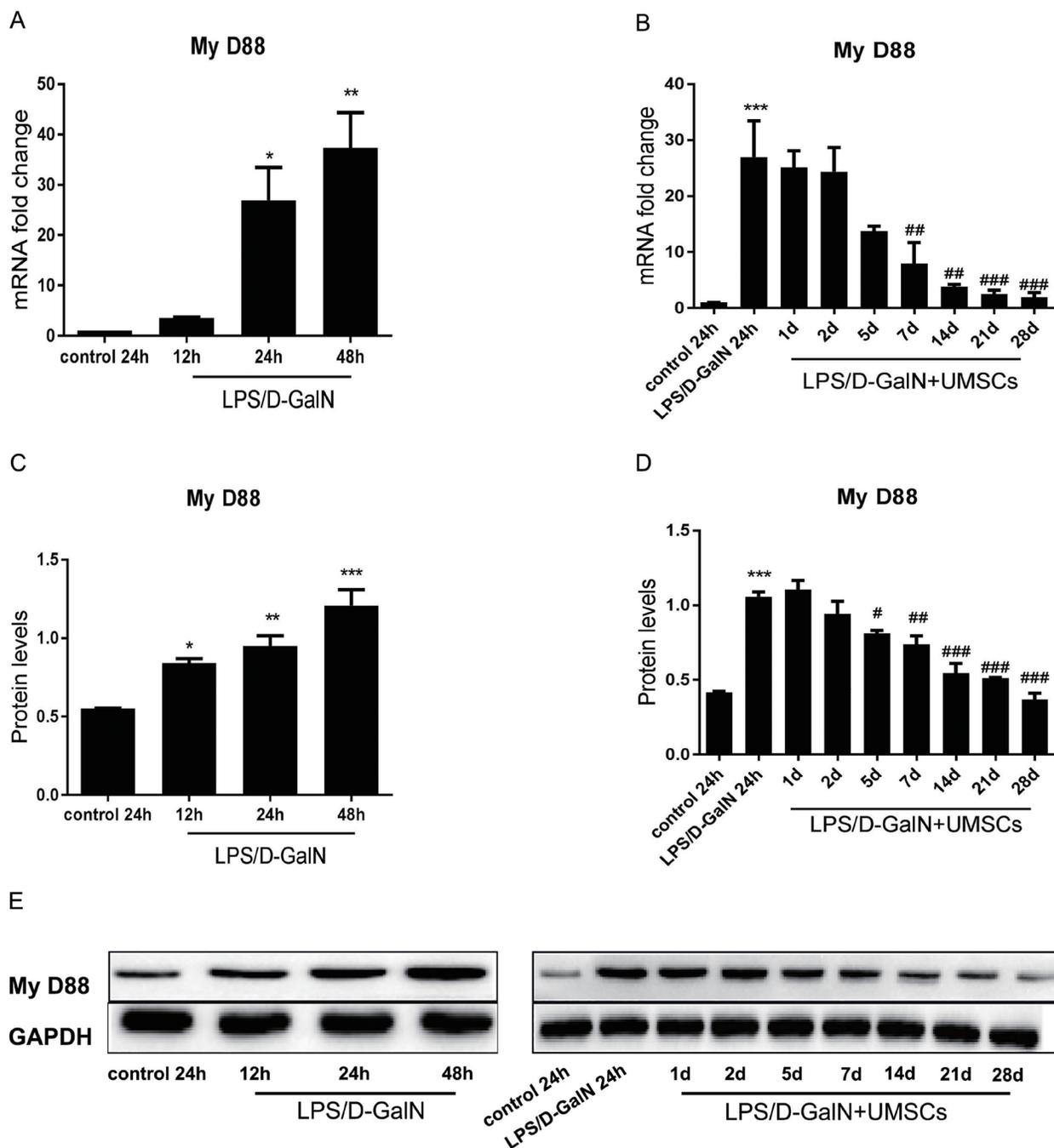


Fig. 3. mRNA (A–B) and DNA (C–E) expression of MyD88 in different groups. Data are presented as means±standard deviations ($n=3$ /group). * $p<0.05$ vs. control; ** $p<0.01$ vs. control; *** $p<0.001$ vs. control; # $p<0.05$ vs. LPS/D-GalN; ## $p<0.01$ vs. LPS/D-GalN; ### $p<0.001$ vs. LPS/D-GalN. D-GalN, D-galactosamine; LPS, lipopolysaccharide; My D88, Myeloid differentiation factor 88.

GalN-induced production of inflammatory cytokines.

UMSCs inhibit the expression of apoptosis-related proteins

To explore the inhibitory effect of UMSCs on LPS/D-GalN-induced hepatocyte apoptosis, we evaluated the expression of apoptosis-related signaling proteins by western blot anal-

ysis and qRT-PCR. In the LPS/D-GalN group, the expression of Bax increased, while that of Bcl-2 decreased (Fig. 7). In contrast, in the UMSC-treated group, Bax was down-regulated, and Bcl-2 was up-regulated.

Discussion

The liver is an important metabolic tissue and plays an im-

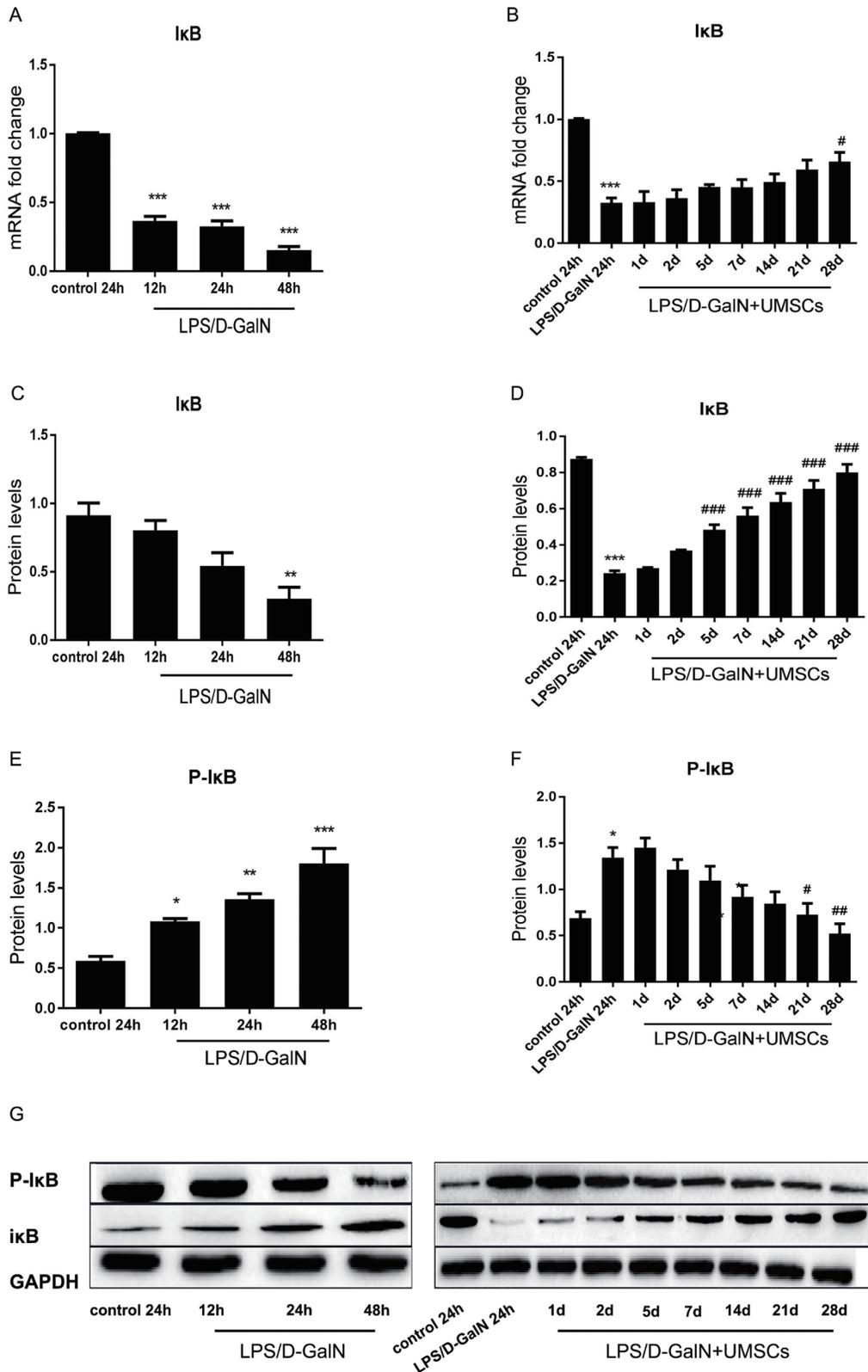


Fig. 4. UMSC therapy inhibited IkB phosphorylation and degradation, mRNA (A–B) and DNA (C–D) expression of IkB in different groups, and DNA expression (E–F, G) of P-IkB in different groups. Data are presented as means±standard deviations (*n*=3/group). **p*<0.05 vs. control; ***p*<0.01 vs. control; ****p*<0.001 vs. control; #*p*<0.05 vs. LPS/D-GalN; ##*p*<0.01 vs. LPS/D-GalN; ###*p*<0.001 vs. LPS/D-GalN. D-GalN, D-galactosamine; IkB, inhibitor of nuclear factor-κB; LPS, lipopolysaccharide; P-IkB, phosphorylated-IkB; UMSCs, umbilical cord mesenchymal stem cells.

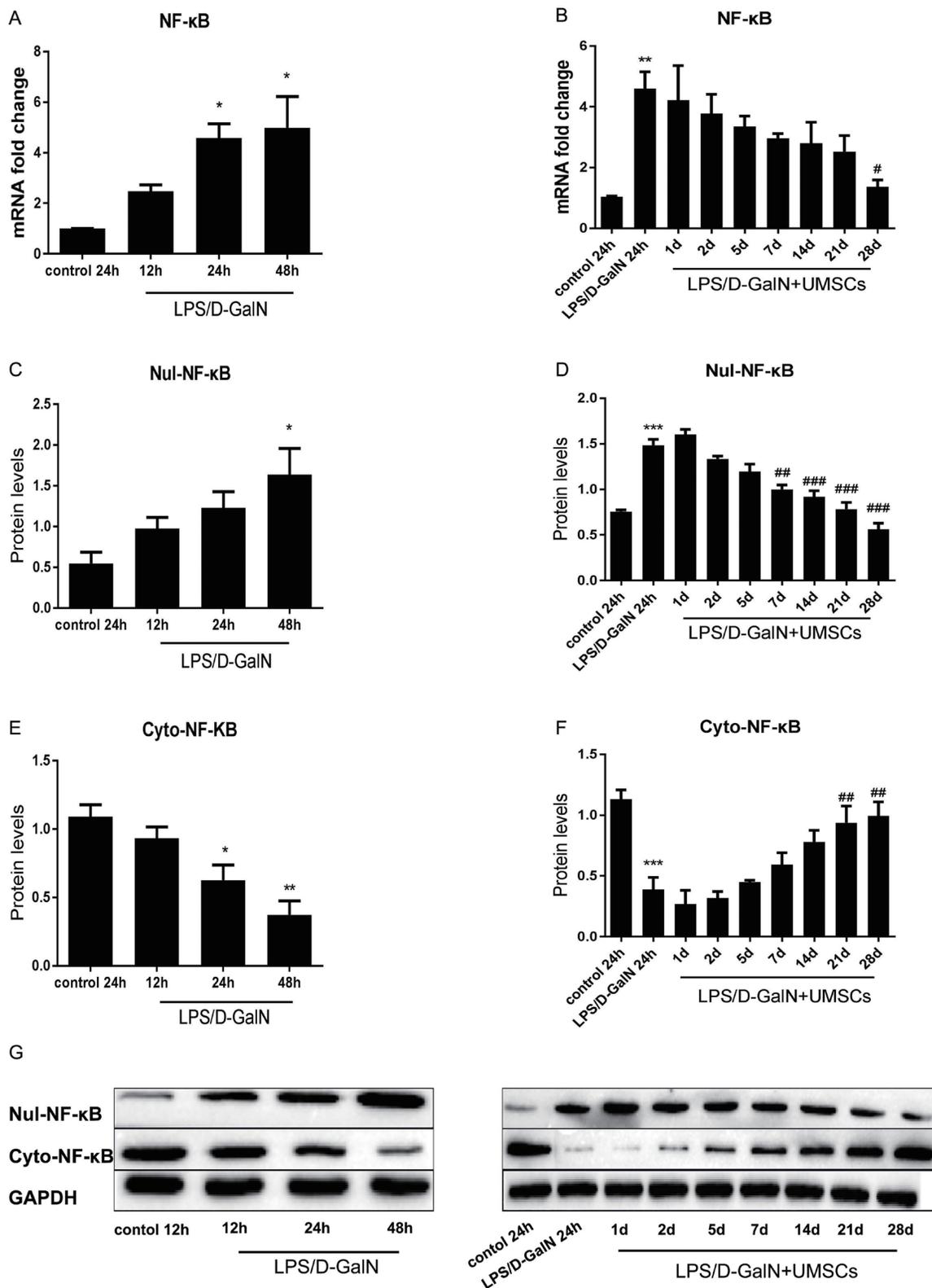


Fig. 5. UMSC therapy inhibited nuclear translocation of NF-κB p65. mRNA (A–B) of NF-κB in different groups, DNA expression (C–D, G) of Nul-NF-κB in different groups, and DNA expression (E–F, G) of Cyto-NF-κB in different groups. Data are presented as means±standard deviations ($n=3/\text{group}$). * $p<0.05$ vs. control; ** $p<0.01$ vs. control; *** $p<0.001$ vs. control; # $p<0.05$ vs. LPS/D-GalN; ## $p<0.01$ vs. LPS/D-GalN; ### $p<0.001$ vs. LPS/D-GalN. D-GalN, D-galactosamine; LPS, lipopolysaccharide; NF-κB, nuclear factor-κB; Nul-NF-κB, nuclear levels of NF-κB; UMSCs, umbilical cord mesenchymal stem cells.

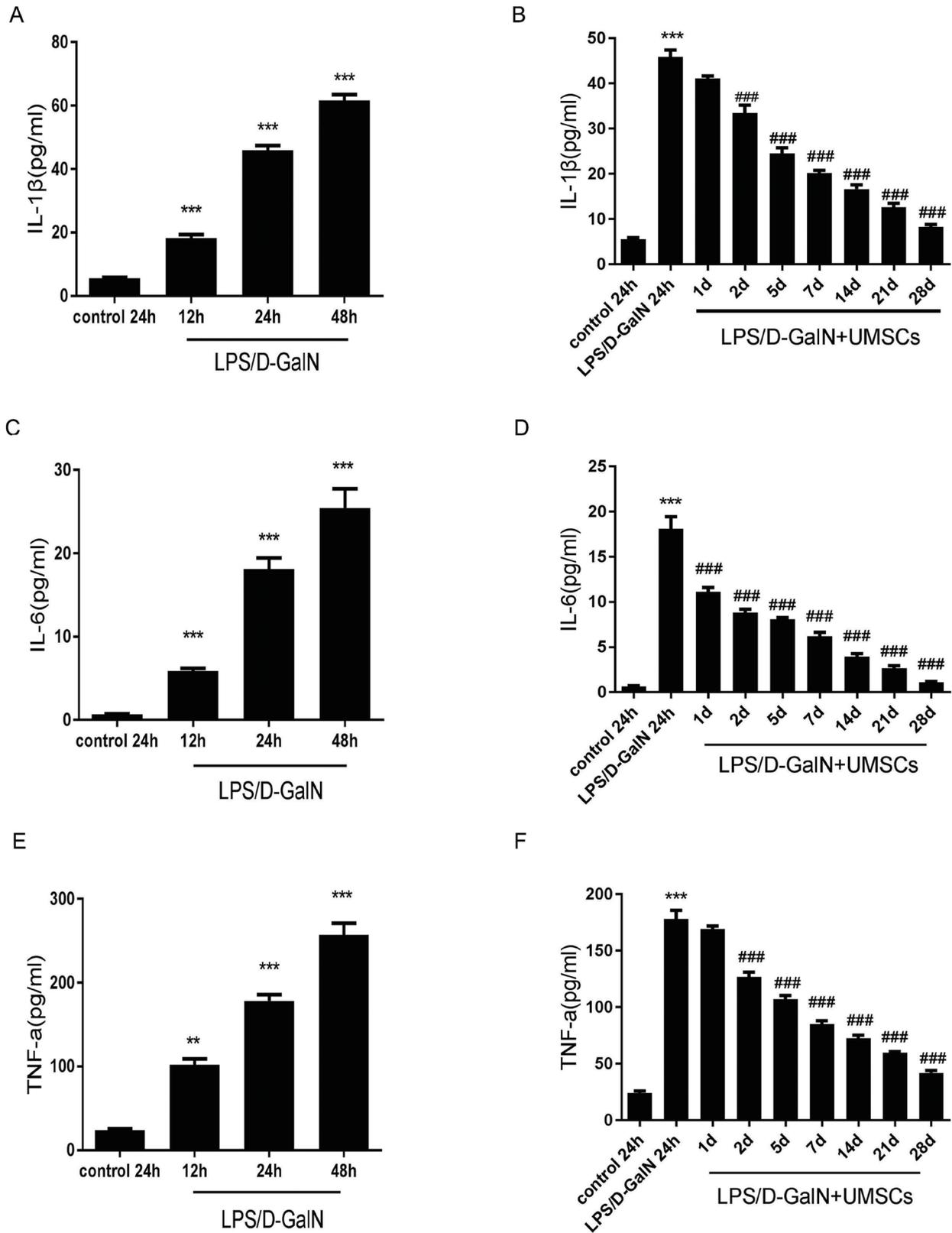


Fig. 6. Effects of UMSCs on inflammatory mediators IL-1β (A-B), IL-6 (C-D), and TNF-α (E-F) in LPS/D-GaIN-induced ALF. Data are expressed as means±standard deviations (*n*=3/group). ***p*<0.01 vs. control; ****p*<0.001 vs. control; ###*p*<0.001 vs. LPS/D-GaIN. D-GaIN, D-galactosamine; IL-1β, interleukin-1β; IL-6, interleukin-6; LPS, lipopolysaccharide; TNF-α, tumor necrosis factor-α; UMSCs, umbilical cord mesenchymal stem cells.

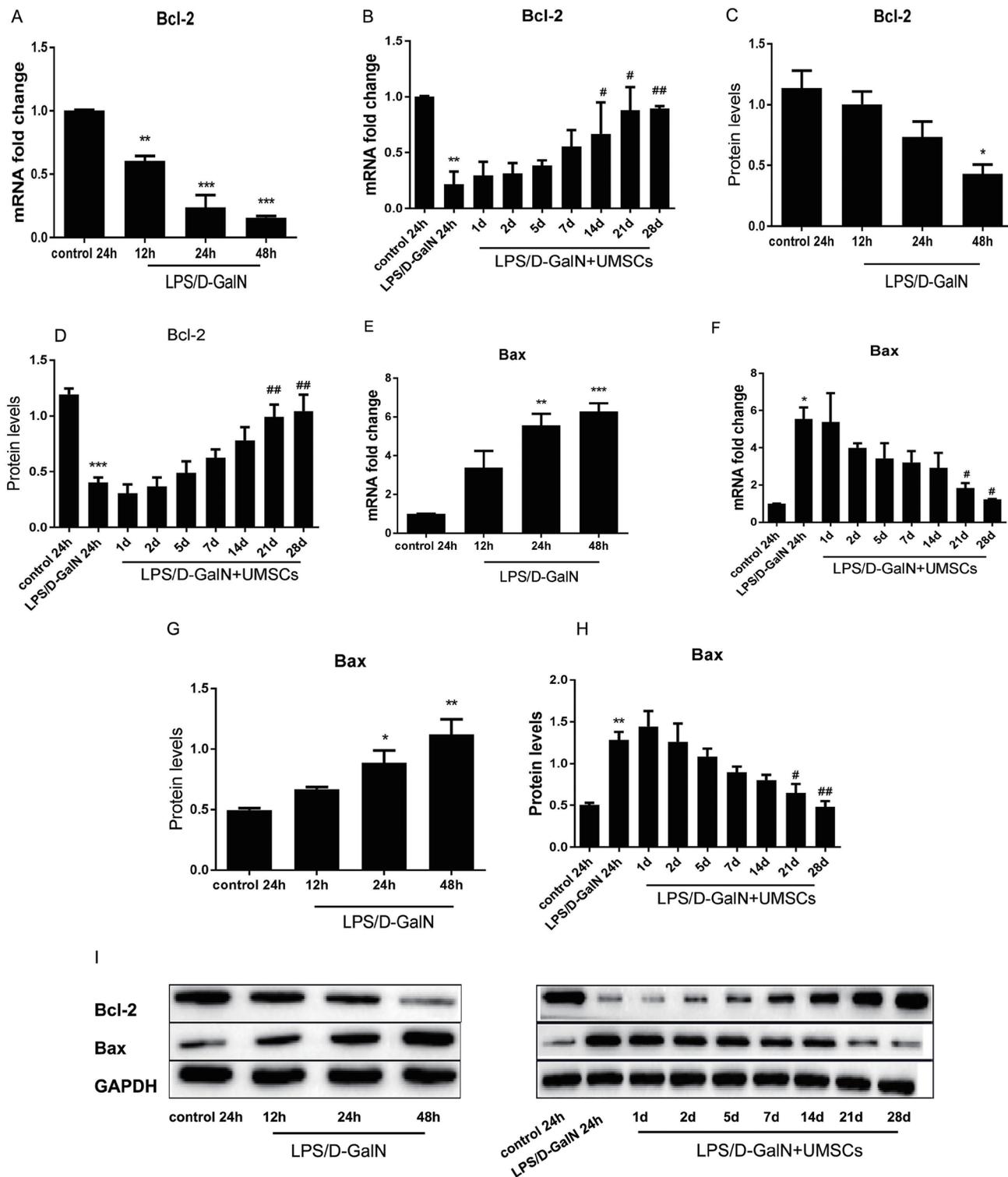


Fig. 7. Effect of UMSCs on apoptosis-related proteins in LPS/D-GalN-induced ALF. mRNA and DNA expressions of Bax in different groups (A–D, I), and mRNA and DNA expressions of Bcl-2 in different groups (E–H, I). These data are expressed as means±standard deviations ($n=3$ /group). * $p<0.05$ vs. control; ** $p<0.01$ vs. control; *** $p<0.001$ vs. control; # $p<0.05$ vs. LPS/D-GalN; ## $p<0.01$ vs. LPS/D-GalN; ### $p<0.001$ vs. LPS/D-GalN. Bax, Bcl 2-Associated X Protein; Bcl-2, B-cell lymphoma-2; D-GalN, D-galactosamine; LPS, lipopolysaccharide; UMSCs, umbilical cord mesenchymal stem cells.

portant role in maintaining balance and health. ALF is a life-threatening clinical syndrome characterized by rapid devel-

opment and high mortality. Finding a clinical method that can effectively treat ALF is an important challenge.

LPS/D-GalN-induced ALF is a well-established experimental model, and inflammation is an important pathogenic mechanism of LPS/D-GalN-induced ALF.¹² A number of studies have shown that UMSCs have the potential for self-renewal and multi-lineage differentiation into terminal cells of many tissues and organs and can participate in immunomodulation.¹⁶ Therefore, it was an important objective of our study to explore whether UMSCs can exhibit anti-inflammatory activity and anti-apoptosis characteristics in ALF. In this study, we investigated the protective effect and the underlying mechanism of UMSCs against ALF in LPS/D-GalN-induced mice with an inflammatory response and apoptosis. The ALF model was successfully established by the intraperitoneal injection of LPS/D-GalN. This model presented a substantial liver injury with obvious changes in histopathological and biochemical parameters. Our results showed the histopathological changes after LPS treatment, including loss of normal lobular structure and showing hepatocyte cytoplasmic edema, local regional expansion, hepatocyte degeneration, necrotic areas filled with inflammatory cells and red blood cells, and inflammatory cell infiltration. Moreover, the biochemical markers of ALT, AST and TBil increased significantly in the LPS/D-GalN group. Liver injury was gradually repaired completely after UMSC transplantation, and there was a marked decrease in the levels of ALT, AST and TBil.

MyD88 is an important TLR/IL-1 receptor superfamily member. LPS is recognized by TLR4 expressed by the cell and activates innate immunity through a MyD88-dependent pathway. Recent studies have shown that the MyD88/NF- κ B signaling pathway plays a key role in inflammation. During this process, MyD88 is activated and induces a cytoplasmic signaling cascade, which leads to the activation of NF- κ B signaling molecules, in turn leading to excessive Kupffer cell activation.¹⁷ Excessive MyD88 signaling pathway activation can lead to various inflammation-related diseases. Studies have shown that treatment with MyD88 inhibitors or knockdown of MyD88 can reduce inflammatory cell infiltration and protect liver cells against apoptosis, improving the survival rate of mice with acute liver injury.¹⁸ NF- κ B is a downstream signaling molecule of MyD88 and an upstream regulator of various inflammation-related genes.¹⁹ NF- κ B is retained in an inactive form in the cytoplasm of hepatocytes that interact with I κ B inhibitors. However, some stimulants, such as LPS, proinflammatory cytokines, viruses and other substances, can trigger NF- κ B activation. The IKK complex is phosphorylated and catalyzes the phosphorylation of I κ B, which is followed by its ubiquitination, resulting in its proteasomal degradation. Then, the I κ B/NF- κ B complex dissociates, resulting in the nuclear translocation of active NF- κ B. Through immunohistochemical staining, qRT-PCR and western blot assay, our present study determined that the MyD88/NF- κ B signaling pathway was successfully activated by LPS. LPS/D-GalN-induced I κ B phosphorylation and degradation and increased NF- κ B p65 nuclear translocation, while UMSCs attenuated these effects. These results indicate that UMSCs partially suppress the MyD88/NF- κ B signaling pathway activity by inhibiting I κ B phosphorylation.

Inflammatory mediators play an important role in LPS/D-GalN-induced ALF. In the liver, LPS first binds to LPS-binding protein, is then transferred to TLR4, and is finally expressed on the surface of Kupffer cells. Activated Kupffer cells can mediate hepatitis progression by secreting TNF- α and other proinflammatory cytokines.²⁰ TNF- α is an important inflammatory mediator associated with LPS/GalN-induced liver injury and may induce hepatocyte apoptosis, which in turn leads to organ failure.²¹ In addition, TNF- α may trigger an inflammatory cascade and induce the production of other cytokines, including IL-1 β and IL-6.²² Previous studies have reported that inhibiting TNF- α synthesis inhibits cytokine production and reduces liver damage.²³ Our results suggest that UMSCs can significantly reduce the production of the

inflammatory factors TNF- α , IL-1 β and IL-6 by down-regulating the expression of upstream regulators of inflammatory factors after transplantation.

Moreover, previous studies have shown that MyD88 overexpression does not immediately induce a strong apoptotic response.²⁴ However, after 2–3 days, in cells with high expression of MyD88, the apoptotic response becomes apparent. Therefore, we suspect that after LPS stimulation, the MyD88-induced apoptotic pathway can become activated. Through qRT-PCR and western blot assay, our findings indicated that after LPS/D-GalN stimulation, the expression of the antiapoptotic protein Bcl-2 was significantly down-regulated and that of the proapoptotic protein Bax was up-regulated. However, after UMSC transplantation, this effect was significantly reversed.

In summary, this study suggests that in the mouse model of LPS/D-GalN-induced ALF, UMSCs can reduce liver damage by suppressing inflammatory mediator release and apoptosis. Mechanistically, this effect is achieved via MyD88/NF- κ B signaling inhibition, which finally exerts a therapeutic effect on ALF. Therefore, we believe that UMSCs are a potential and valuable therapeutic alternative for ALF.

Acknowledgments

The corresponding author wishes to thank Zhiqian Xue for providing unlimited motivation, I will love you forever.

Funding

This research was supported by the National Natural Science Foundation of China (Grant No. 40117026).

Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Study design (HL, JY), performance of experiments (HL, SD), analysis and interpretation of data (HL, SD, TJ, MZ, ZX), manuscript writing (SD, TJ, MZ), critical revision of the manuscript (SD, TJ, MZ, ZX), technical or material support (JY).

Data sharing statement

No additional data are available.

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