



Basic Study

Recq15 protects against lipopolysaccharide/ D-galactosamine-induced liver injury in mice

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Abstract

AIM: To investigate the effects of Recq15 deficiency on liver injury induced by lipopolysaccharide/D-galactosamine (LPS/D-Gal).

METHODS: Liver injury was induced in wild type (WT) or Recq15-deficient mice using LPS/D-Gal, and assessed by histological, serum transaminases, and mortality analyses. Hepatocellular apoptosis was quantified by transferase dUTP nick end labeling assay and Western

blot analysis of cleaved caspase-3. Liver inflammatory chemokine and cytochrome P450 expression was analyzed by quantitative reverse transcription-PCR. Neutrophil infiltration was evaluated by myeloperoxidase activity. Expression and phosphorylation of ERK, JNK, p65, and H2A.X was determined by Western blot. Oxidative stress was evaluated by measuring malondialdehyde production and nitric oxide synthase, superoxide dismutase, glutathione peroxidase, catalase, and glutathione reductase activity.

RESULTS: Following LPS/D-Gal exposure, Recq15-deficient mice exhibited enhanced liver injury, as evidenced by more severe hepatic hemorrhage, higher serum aspartate transaminase and alanine transaminase levels, and lower survival rate. As compared to WT mice, Recq15-deficient mice showed an increased number of apoptotic hepatocytes and higher cleaved caspase-3 levels. Recq15-deficient mice exhibited increased DNA damage, as evidenced by increased γ -H2A.X levels. Inflammatory cytokine levels, neutrophil infiltration, and ERK phosphorylation were also significantly increased in the knockout mice. Additionally, Recq15-deficient mice exhibited increased malondialdehyde production and elevated inducible nitric oxide synthase, superoxide dismutase, glutathione peroxidase, catalase, and glutathione reductase activity, indicative of enhanced oxidative stress. Moreover, CYP450 expression was significantly downregulated in Recq15-deficient mice after LPS/D-Gal treatment.

CONCLUSION: Recq15 protects the liver against LPS/D-Gal-induced injury through suppression of hepatocyte apoptosis and oxidative stress and modulation of CYP450 expression.

Key words: Recq15; Liver injury; Apoptosis; Oxidative stress; CYP450

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Core tip: Wild type and Recq15-deficient mice were intraperitoneally injected with lipopolysaccharide and D-galactosamine (LPS/D-Gal). The aim of the study was to explore the effects of Recq15 deficiency on LPS/D-Gal-induced liver injury. Our findings reveal that Recq15 protects against liver injury *via* inhibition of hepatocyte apoptosis and oxidative stress and regulation of hepatic CYP450 expression levels.

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INTRODUCTION

The RecQ family is a highly conserved group of DNA helicases that play a critical role in DNA replication, recombination, transcription, and repair^[1]. Mammals express five RecQ homologues: RECQL1, BLM, WRN, RECQL4, and RECQL5^[2], which share a conserved helicase domain, but differ in their C- and/or N-terminal domains^[3]. Mutations in BLM, WRN, and RECQL4 are linked to the human genetic disorders Bloom's syndrome (BS), Werner's syndrome (WS), and Rothmund-Thomson's syndrome (RTS), respectively. These disorders are characterized by increased genomic instability and cancer susceptibility^[2]. RECQL5 has not been directly linked to human genetic disease, but has been implicated in DNA double strand break (DSB) repair and DNA transcription^[4-10].

Several lines of evidence suggest that RECQ helicases also play a role in hepatic cell proliferation and metabolism. For example, RECQL1 expression is significantly correlated with histological grade and MIB-1 indices of hepatocellular carcinoma (HCC) development. Silencing RECQL1 expression suppresses HCC cell proliferation both *in vitro* and *in vivo*^[11]. BLM-deficient cells from patients with BS show slower growth and increased irradiation-mediated apoptosis. Deletion of BLM in mice leads to a reduced number of fetal liver cells and increased cell death^[12]. In addition, a recent report has shown that *Wrn*-mutant mice exhibit accelerated typical age-related liver changes, including pseudocapillarization that directly affects hepatic metabolism^[13]. Moreover, WRN regulates the transcription of hepatic cytochrome P450 2B (*CYP2B*) genes, which are involved in the metabolism of various active substances^[14], suggesting that WRN may function in liver metabolism. Hepatocyte cell death and impaired hepatic metabolism are associated with many liver diseases, including chronic and acute liver injury and liver cancer^[15-17]. Previously, we reported that Recq15 deficiency in mice resulted in increased susceptibility to cancers, including liver cancer^[4,6]; however, it remains unknown whether Recq15 also has a role in liver injury.

Lipopolysaccharide/D-galactosamine (LPS/D-Gal) treated mice are a known model of acute liver injury. D-Gal is an amino sugar that blocks RNA synthesis and greatly increases the sensitivity of mice to LPS-induced hepatotoxicity^[18,19]. Using this model, we examined the function of Recq15 in liver injury. We demonstrated that Recq15 protects against LPS/D-Gal-induced liver injury and found that the enhanced liver injury in Recq15-deficient mice occurs due to increased hepatic apoptosis, elevated oxidative stress, and downregulation of CYP450 expression.

MATERIALS AND METHODS

Animals

Male, 6-8-wk-old Recq15-deficient and wild type

Table 1 Primers used in this study

Gene	Forward primer (5'→3')	Reverse primer (5'→3')	Length of product
TNF- α	GAACTGGCAGAAGAGGCACT	AGGGTCTGGGCCATAGAACT	203 bp
IL-6	GAGGATACCACTCCCAACAGACC	AAGTGCATCATCGTTGTTTCATAC	141 bp
Cyp2A4	CGGAAGACGAACGGTGCTTTC	GAGGCTTCCCAGCATCATCTAAGA	123 bp
Cyp2A5	TCGGAAGACGAACGGTGCTTTC	GCTTCCCAGCATCATTCGAAGC	124 bp
Cyp2B9	TGAAGCTTTTCTGCCCTTCT	GTGTGAGCAGCTACCAATG	147 bp
Cyp2B10	GACTTTGGGATGGGAAAGAG	CCAAACACAATGGAGCAGAT	68 bp
GAPDH	ACGGATTGGTCGTATTGGGC	CTCGCTCTGGGAAGATGGTGAT	216 bp

(WT) C57BL/6 mice were used in this study. The Recq15-deficient mice used in this study have been characterized previously^[4-7]. Mice were fed a commercial diet and maintained in a controlled environment at 20-25 °C and 50% \pm 5% relative humidity with a 12:12 h dark-light cycle. All animal studies were approved by the Wenzhou Medical University Institutional Animal Care and Use Committee.

Reagents and antibodies

LPS (*E. coli*, 0111:B4) and D-Gal were purchased from Sigma (St Louis, MO, United States). Caspase-3 (rabbit polyclonal, 1:1000), ERK (rabbit polyclonal, 1:2000), phospho-ERK (rabbit polyclonal, 1:2000), JNK (rabbit polyclonal, 1:1000), phospho-JNK (mouse monoclonal, 1:2000), phospho-p65 (mouse monoclonal, 1:1000), phospho-H2A.X (γ -H2A.X, rabbit polyclonal, 1:1000), β -actin (rabbit polyclonal, 1:2000), and GAPDH (rabbit polyclonal, 1:3000) antibodies were obtained from Cell Signaling Technology (Waltham, MA, United States). Peroxidase-conjugated secondary antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA, United States).

Liver injury induction

Liver injury was induced in 6-8-wk-old male mice *via* an intraperitoneal injection of LPS/D-Gal. For mortality assay, mice were intraperitoneally injected with 20 μ g/kg LPS and 400 mg/kg D-Gal and mortality was recorded for 72 h. To induce acute liver injury, mice were intraperitoneally injected with 10 μ g/kg LPS and 300 mg/kg D-Gal. Mice were sacrificed at 1 and 6 h after LPS/D-Gal administration. Blood and liver samples were collected for further experiments.

Serum analysis

The serum activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was measured with a commercial assay kit (Nanjing Jiancheng Biological Technology, Inc., Nanjing, China). The enzyme activity was expressed as international units per liter (U/L). Serum tumor necrosis factor (TNF)- α and interleukin (IL)-6 levels were measured using commercial assay kits (Nanjing Jiancheng Biological Technology).

Histopathological analysis

Formalin-fixed specimens were embedded in paraffin

and stained with hematoxylin-eosin for conventional morphological evaluation under a light microscope.

Myeloperoxidase activity analysis

Myeloperoxidase (MPO) activity was determined using an MPO detection kit according to the manufacturer's instructions (Nanjing Jiancheng Biological Technology, Inc.).

Oxidation stress analysis

Lipid peroxidation was determined by measuring malondialdehyde (MDA) levels using an assay kit (Beyotime Institute of Biotechnology, Inc., Shanghai, China). The activity of inducible nitric oxide synthase (iNOS), superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), and glutathione reductase (GR) was tested using commercial assay kits (Nanjing Jiancheng Biological Technology, Inc.).

Terminal deoxynucleotidyl transferase dUTP nick end labeling assay

Hepatocellular apoptosis was evaluated by transferase dUTP nick end labeling (TUNEL) assay using the DeadEnd™ Colorimetric TUNEL System (Promega, Madison, WI, United States). The terminal transferase reactions produced a dark-brown precipitate. For each mouse liver section, the number of TUNEL-positive cells in five randomly selected fields was counted.

Quantitative real-time PCR

Total RNA was isolated from liver tissue using TRIZOL reagent (Invitrogen) and was treated with DNase to remove contaminating DNA before cDNA synthesis. RNA (2 μ g) was reverse-transcribed to cDNA with murine leukemia virus (MLV)-reverse transcriptase (Invitrogen). Each cDNA sample was analyzed in triplicate on an ABI 7300 Real-Time Detection system (Applied Biosystems, Foster City, CA, United States) using SYBR Green (Tiangen, Beijing, China). An endogenous housekeeping gene (GAPDH) was used as an internal standard. The primer sequences are shown in Table 1. The primer concentration used in the PCR assay was 0.5 μ mol/L. Cycle conditions were as follows: 95 °C for 2 min followed by 40 cycles of 95 °C for 15 s, 60 °C for 30 s, and 68 °C for 30 s. Relative mRNA quantification was calculated using the comparative threshold cycle (Ct) method. $\Delta\Delta$ Ct was converted to a fold change of expression using the formula $2^{-\Delta\Delta$ Ct}.

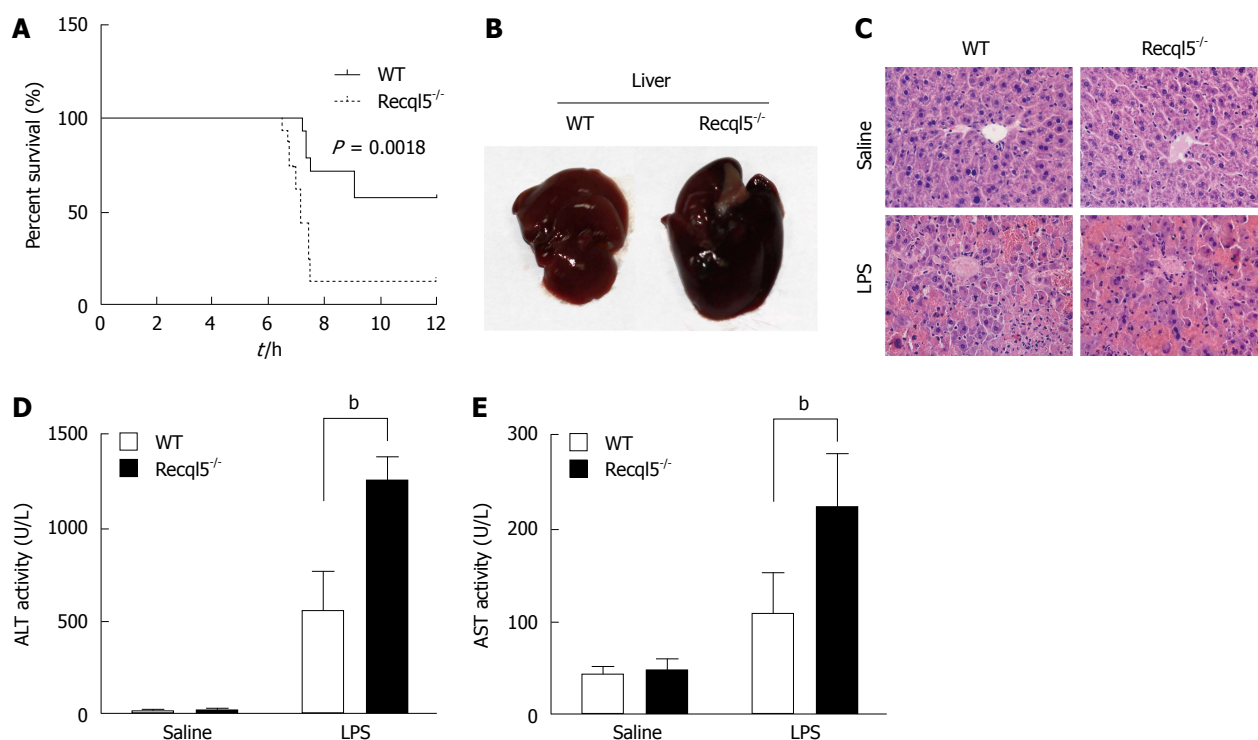


Figure 1 Enhanced lipopolysaccharide/D-Gal-induced liver injury in Recq15-deficient mice. A: Survival curves after lipopolysaccharide (LPS)/D-Gal injection. Wild type (WT) and Recq15-deficient (Recq15^{-/-}) mice were treated with 20 μ g/kg LPS and 400 mg/kg D-Gal (WT, $n = 14$; KO, $n = 16$). Survival curves were created using the Kaplan-Meier method and compared by log-rank (Mantel-Cox) test; B: Images of the whole livers demonstrate the different degree of hemorrhage. WT and Recq15-deficient mice were treated with 300 mg/kg D-Gal and 10 μ g/kg LPS ($n = 4-7$ per group). Livers were removed 6 h after injection; C: HE staining of liver sections 6 h after injection; D: Serum alanine transaminase (ALT) activity 6 h after injection; E: Serum aspartate transaminase (AST) activity 6 h after treatment. ^b $P < 0.01$, WT vs Recq15^{-/-}.

Western blotting

Liver tissue was lysed in lysis buffer [50 mmol/L Tris-HCl pH 7.6, 150 mmol/L NaCl, 1% NP-40 and 0.1% (w/v) SDS] supplemented with a protease/phosphatase inhibitor cocktail (Cell Signaling Technology). After sonication, the lysate was centrifuged at 12000 rpm for 15 min at 4 $^{\circ}$ C, and the supernatant was collected. Proteins were separated using SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes (Bio-Rad, Hercules, CA, United States). After blocking with 5% (w/v) milk in TTBS (TBS plus 0.1% Tween-20), the membranes were incubated with primary antibodies followed by horseradish peroxidase (HRP)-conjugated secondary antibodies. Protein bands were visualized with the Immun-Star HRP chemiluminescence kit (Bio-Rad). For densitometric analysis, Image J software was used.

Statistical analysis

Statistical comparisons were performed using Student's *t*-test or analysis of variance (ANOVA) where appropriate. Data are expressed as the mean \pm SD. Kaplan-Meier survival analysis was performed using the log-rank test. *P*-values less than 0.05 were considered significant.

RESULTS

Enhanced liver injury in Recq15-deficient mice

To investigate the role of Recq15 in liver injury, we

first examined the effect of Recq15 deficiency on mouse mortality after LPS shock. Following injection of 20 μ g/kg LPS and 400 mg/kg D-Gal, the mortality was significantly increased in Recq15-deficient mice as compared to WT mice (Figure 1A). Lethal shock in D-Gal/LPS-treated mice is characterized by acute liver injury^[20]. To further elucidate the direct effects of Recq15 on liver injury, we used a low-dose LPS/D-Gal model (10 μ g/kg LPS and 300 mg/kg D-Gal). Liver morphology analysis^[21] showed that the liver of Recq15-deficient mice was swollen and exhibited more bleeding on the surface, as compared to the liver of WT mice, indicating that there was more severe liver hemorrhage in knockout mice after treatment (Figure 1B). These data were further confirmed by HE staining (Figure 1C). Moreover, the serum ALT and AST levels, two well-established biochemical markers of hepatocellular damage, were significantly increased in Recq15-deficient mice 6 h after injection (Figure 1D and E). Together, these results indicate that Recq15 has a protective role in liver injury induced by LPS/D-Gal.

Increased hepatocellular apoptosis and DNA damage in Recq15-deficient mice

Hepatocyte apoptosis is considered a main cause of liver injury in the LPS/D-Gal model^[22]. Thus we evaluated whether Recq15 deficiency affected hepatocyte apoptosis. The TUNEL assay showed that the number of apoptotic hepatocytes was significantly

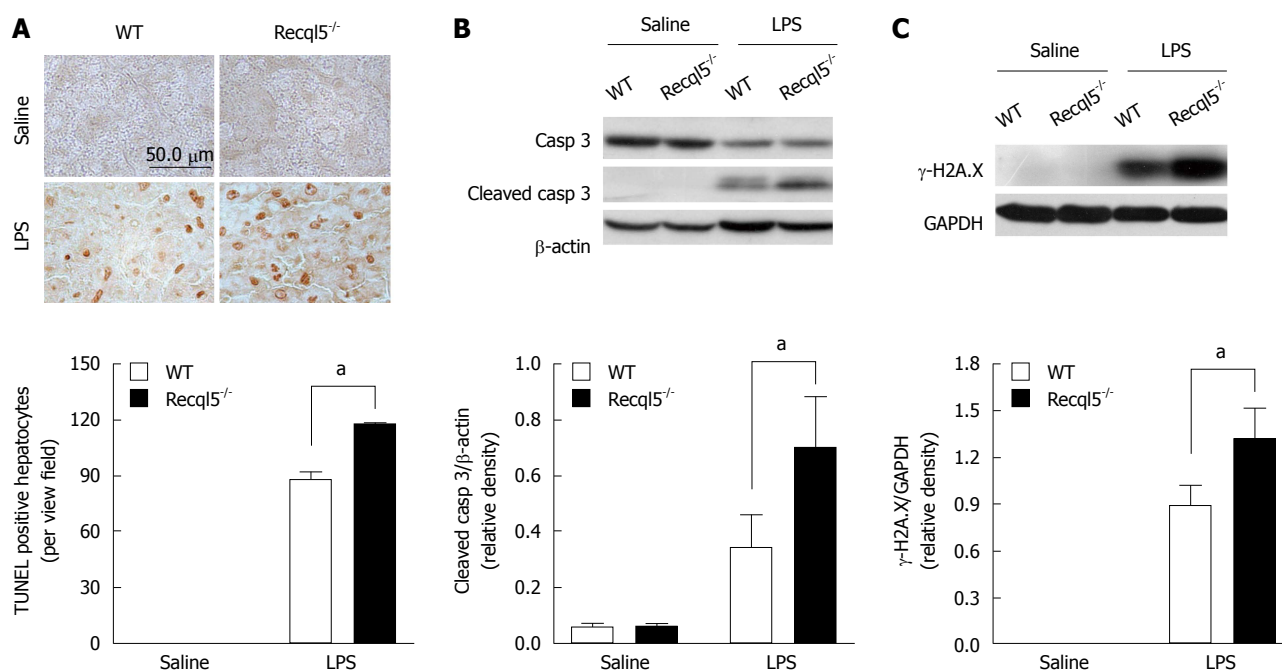


Figure 2 Enhanced hepatocellular apoptosis in Recq15-deficient mice after lipopolysaccharide/D-Gal treatment. A: TUNEL assay for apoptotic hepatocytes in wild type (WT) and Recq15-deficient (Recq15^{-/-}) mice after lipopolysaccharide (LPS)/D-Gal injection; B: Western blot analysis of cleaved caspase-3 protein levels in the livers of WT and Recq15-deficient mice in response to LPS/D-Gal; C: Western blot analysis of γ -H2A.X levels in the livers of WT and Recq15-deficient mice in response to LPS/D-Gal. ^a*P* < 0.05, WT vs Recq15^{-/-}.

increased in Recq15-deficient mice, as compared to WT mice after LPS/D-Gal administration (Figure 2A). In agreement with this observation, Western blot analysis showed that the cleaved caspase-3 levels were significantly elevated in Recq15-deficient mice (Figure 2B). Given the important role of Recq15 in genomic stability, we assumed that LPS/D-Gal treatment would result in elevated DNA damage in Recq15-deficient mice, which, in turn, would trigger apoptosis. Indeed, Western blot showed that the level of γ -H2A.X, a biomarker of DNA damage, was significantly increased in Recq15-deficient mice (Figure 2C). Together, these results suggest that Recq15 deficiency results in increased LPS/D-Gal-induced DNA damage and hepatocyte apoptosis, thereby inducing liver injury.

Increased inflammatory response in Recq15-deficient mice

The release of pro-inflammatory cytokines is involved in liver injury stimulated by LPS/D-Gal^[18,22]. Among these, cytokines TNF- α and IL-6 are key mediators of hepatocyte apoptosis. To examine whether Recq15 deficiency could alter TNF- α and IL-6 expression, we measured their hepatic mRNA levels. As compared with WT mice, the mRNA levels of TNF- α and IL-6 were significantly elevated in Recq15-deficient mice at 1 and 6 h, respectively. Similar results were found for serum TNF- α and IL-6 levels (Figure 3C and D). Consistent with these data, the neutrophil infiltration, which can be triggered by TNF- α signaling^[21], was significantly increased in knockout mice, as evaluated by MPO activity (Figure 3E). LPS/D-Gal-induced secretion of

inflammatory cytokines is primarily dependent on the activation of the mitogen activated protein kinase (MAPK) and nuclear factor (NF)- κ B pathways^[18,23]. We then tested whether deletion of Recq15 affected these pathways. Our results showed that the phosphorylation of extracellular signal-related kinase (ERK) was significantly increased in Recq15-deficient mice, whereas there was no significant difference in c-Jun N-terminal kinase (JNK) and p65 phosphorylation between Recq15-deficient mice and control mice (Figure 3F), suggesting that Recq15 deficiency results in ERK activation.

Elevated oxidative stress in Recq15-deficient mice

Oxidative stress is a known contributor to LPS/D-Gal-induced liver injury^[24]. To investigate the effects of Recq15 deficiency on oxidative stress, we measured several parameters involved in this process, including MDA production and iNOS, SOD, GPX, CAT and GR activity. Our data showed that the levels of MDA, an end product of lipid peroxidation, were significantly increased in Recq15-deficient mice, as compared to WT mice (Figure 4A). Furthermore, there was a significant increase in iNOS activity in Recq15-deficient mice, indicative of enhanced NO production (Figure 4B). Additionally, SOD, GPX, CAT, and GR activity in Recq15-deficient mice was significantly reduced (Figure 4C-F). These results suggest that Recq15 deficiency leads to increased LPS/D-Gal-mediated oxidative stress.

Decreased CYP450 expression in Recq15-deficient mice

CYP450s are important for the metabolism of a variety

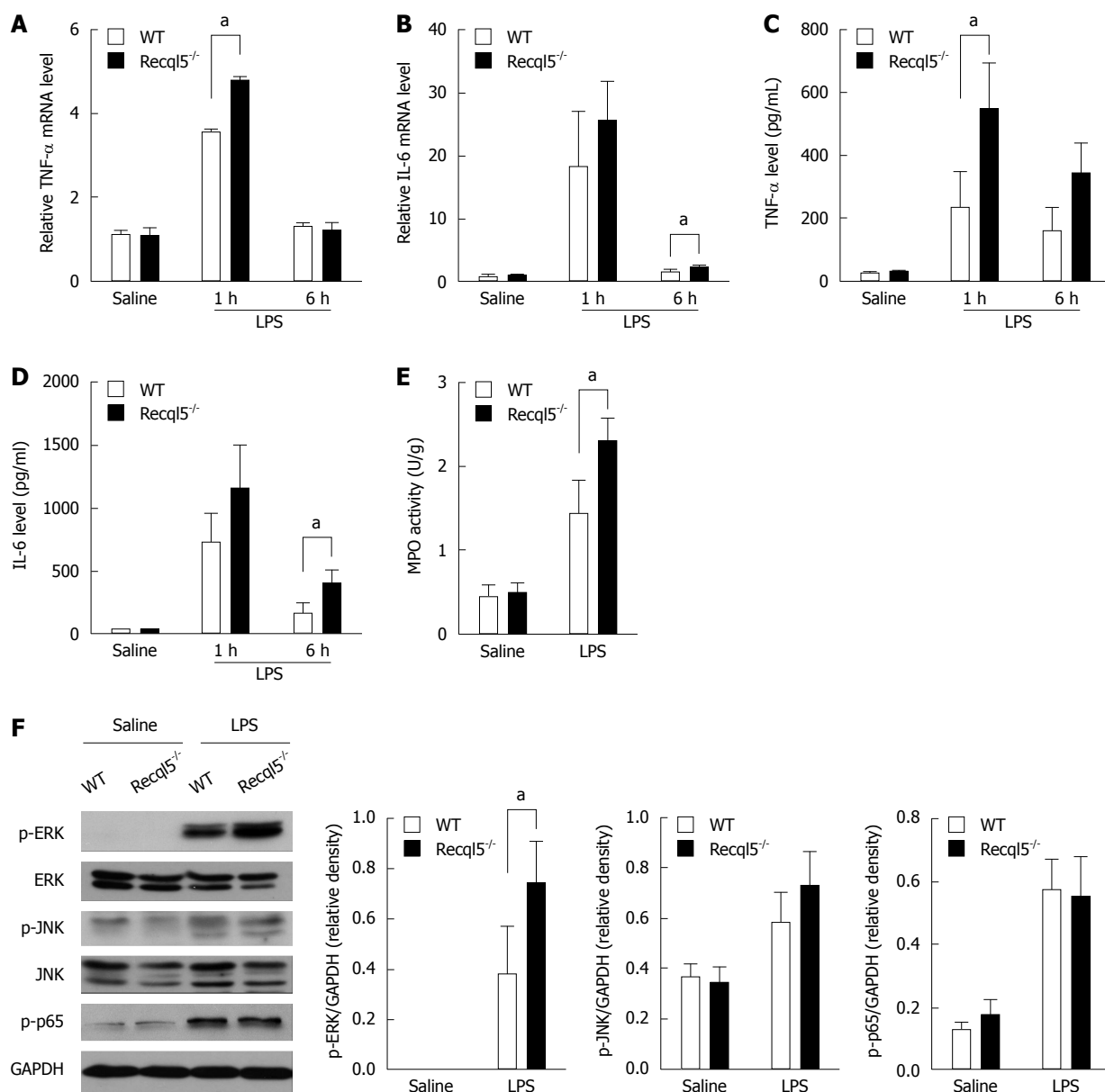


Figure 3 Enhanced inflammatory responses in *Recq15*-deficient mice following lipopolysaccharide/D-Gal exposure. TNF- α (A) and IL-6 (B) mRNA expression in WT and *Recq15*-deficient (*Recq15*^{-/-}) mice 1 and 6 h after LPS/D-Gal exposure ($n = 4-7$ per group). Serum TNF- α (C) and IL-6 (D) levels in WT and *Recq15*-deficient mice 1 and 6 h after LPS/D-Gal administration; E: Hepatic MPO activity in WT and *Recq15*-deficient mice following LPS/D-Gal challenge; F: Western blot analysis of ERK1/2, JNK, and p65 expression and phosphorylation in the livers of WT and *Recq15*-deficient mice after LPS/D-Gal treatment. ^a $P < 0.05$, WT vs *Recq15*^{-/-}.

of endogenous and exogenous substrates^[25,26]. It has been reported that reduced CYP450 gene expression is associated with enhanced liver injury^[27]. Therefore, we examined whether *Recq15* deficiency could alter CYP450 expression. The mRNA levels of four CYP450 members were detected, including CYP2A4, CYP2A5, CYP2B9 and CYP2B10, which are regulated by LPS as reported previously^[28-30]. Our results showed that, following LPS/D-Gal exposure, mRNA levels of CYP2A4, CYP2A5, CYP2B9, and CYP2B10 were significantly reduced in *Recq15*-deficient mice, as compared to WT mice (Figure 5). These data indicate that *Recq15* deficiency results in the downregulation of CYP450 expression, which may impair LPS and/or D-Gal disposition.

DISCUSSION

In the present study, we demonstrated that *Recq15* has a protective role against LPS/D-Gal-induced liver injury, as *Recq15*-deficient mice exhibited increased hepatic hemorrhage, elevated serum aminotransferase levels, and decreased survival rate. LPS/D-Gal-induced liver injury is a well-established experimental animal model of acute hepatic failure. The outcomes of this model are associated with increased hepatocyte apoptosis, inflammation, and oxidation^[18,31,32]. First, we speculated that *Recq15* deficiency might increase hepatocyte apoptosis, which could lead to enhanced liver damage. Indeed, TUNEL assays and Western blot confirmed an increase in hepatocyte apoptosis

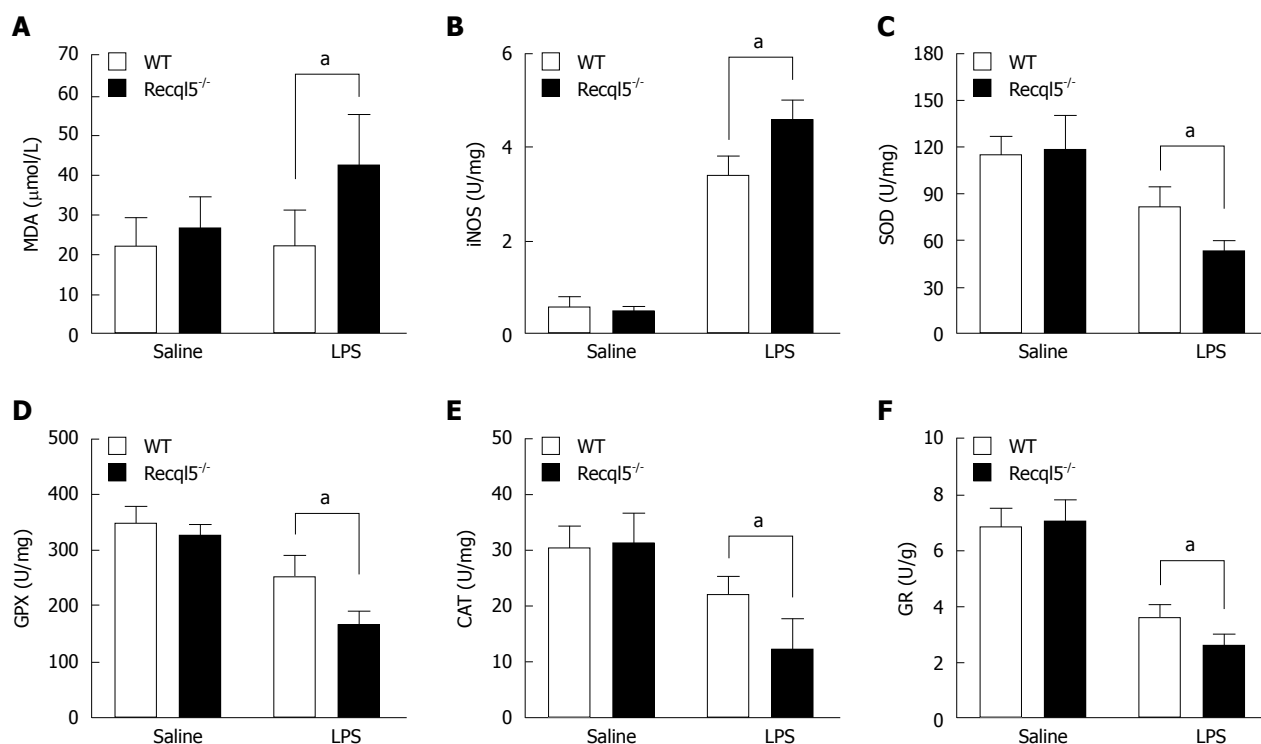


Figure 4 Enhanced oxidative stress in Recq15-deficient mice after lipopolysaccharide/D-Gal treatment. A: Serum MDA contents in WT and Recq15-deficient (Recq15^{-/-}) mice 6 h after lipopolysaccharide (LPS)/D-Gal injection. Liver iNOS (B), SOD (C), GPX (D), CAT (E), and GR (F) activity in WT and Recq15-deficient mice 6 h after LPS/D-Gal administration. *n* = 4-7 per group. ^a*P* < 0.05, WT vs Recq15^{-/-}.

in Recq15-deficient mice. Moreover, consistent with the role of Recq15 in genomic stability, we found that Recq15-deficient liver exhibited increased DNA damage, which has been recognized as an inducer of apoptosis^[33]. Our data suggest that Recq15 is a regulator of cell apoptosis. In agreement with these findings, a previous study suggested that BLM also regulates cell apoptosis, and that BLM deficiency results in increased cell death and apoptosis, which is associated with p53 dysfunction^[12].

TNF- α and IL-6 are two proximal mediators of hepatotoxicity in several models of liver damage, including LPS/D-Gal^[34,35]. TNF- α -induced hepatocyte apoptosis may be an early causal event during LPS/D-Gal-induced liver injury^[32]. We found that LPS/D-Gal upregulated TNF- α and IL-6 in Recq15-deficient mice. Moreover, it has been reported that LPS activates various signaling pathways, including MAPK and NF- κ B, thereby inducing the production of inflammatory cytokines^[18]. In line with these data, we observed an upregulation in ERK phosphorylation in knockout mice following LPS/D-Gal challenge. Together, our data suggest that, in the LPS/D-Gal model, Recq15 deficiency activates ERK signaling, resulting in inflammatory cytokine production and subsequently, hepatocyte apoptosis and damage.

Oxidative stress is associated with damage to a wide range of molecules, including lipids, proteins, and nucleic acids, and may play a crucial role in LPS/D-Gal-stimulated liver injury. For example, treatment with

antioxidants significantly reduces LPS/D-Gal-induced liver injury in mice, whereas inhibition of antioxidant enzyme activity enhances liver damage^[24,36]. Oxidative stress can be triggered by increased free radical production and/or decreased antioxidant activity^[37]. We found that MDA and NO production, indicative of free radicals, were significantly increased in Recq15-deficient mice. In contrast, the activity of the antioxidant enzymes SOD, GPX, CAT and GR was significantly reduced in mice deficient in Recq15. These data suggest that Recq15 deficiency results in an imbalance between free radical generation and antioxidant defenses, thereby enhancing oxidative stress-induced liver injury.

CYP450 oxidases are the predominant enzymes involved in Phase I detoxification. Downregulation of CYP450 increases the risk of liver damage after hepatotoxin exposure^[27,38]. We found that Recq15 deficiency resulted in reduced expression of CYP2A4, CYP2A5, CYP2B9, and CYP2B10, indicative of impaired LPS and/or D-Gal disposition, which might further aggravate liver injury. The mechanism by which Recq15 regulates CYP450 expression remains unknown. It has been shown that WRN regulates CYP2B transcription by forming complex with the Wrn binding site within the CYP2B promoter^[14]. Further investigations are required to figure out whether Recq15 regulates CYP450 expression in the same manner as WRN.

In summary, the current study showed for the first time that Recq15 protects against liver injury induced

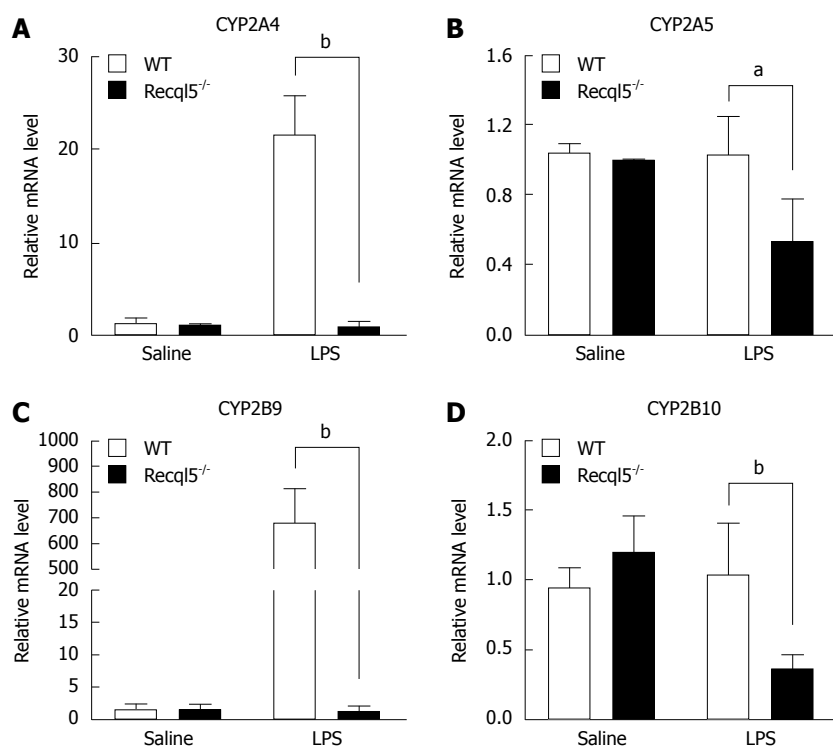


Figure 5 Downregulation of CYP450 in Recq15-deficient mice after lipopolysaccharide/D-Gal administration. WT and Recq15-deficient (Recq15^{-/-}) mice were injected with lipopolysaccharide (LPS)/D-Gal or saline, and livers were harvested at 6 h ($n = 4-7$ per group). RNA was isolated from the livers, and mRNA levels of CYP2A4 (A), CYP2A5 (B), CYP2B9 (C), and CYP2B10 (D) were measured by real-time quantitative PCR and normalized to GAPDH mRNA levels. ^a $P < 0.05$, ^b $P < 0.01$, WT vs Recq15^{-/-}.

by LPS/D-Gal. The protective effect of Recq15 is attributed to the inhibition of hepatocyte apoptosis and oxidative stress, as well as the regulation of CYP450 expression. Our findings indicate a hepatoprotective role for Recq15 in liver injury.

COMMENTS

Background

RecQ helicases are regulators of genomic integrity and play important roles in hepatic cell proliferation and metabolism. Deficiency in one of the RecQ family member, Recq15, results in elevated susceptibility to various types of cancers, including liver cancer; however, the possible function of Recq15 in liver injury remains unknown.

Research frontiers

Recq15 is a member of the RecQ helicase family that plays critical roles in DNA replication, recombination, transcription, and repair. This study analyzes the possible functions of Recq15 in liver injury.

Innovations and breakthroughs

The study showed for the first time that Recq15 has a protective role against liver injury induced by LPS/D-Gal. The protection is mediated via the suppression of apoptosis, DNA damage, oxidative stress and CYP450 expression.

Applications

This study will help to improve the readers' understanding of the mechanisms involved in acute liver injury and may represent a novel target for the acute liver failure therapy.

Terminology

Oxidative stress is a term used to describe the condition of oxidative damage

resulting when the critical balance between free radical generation and antioxidant defenses is unfavorable.

Peer-review

This is an interesting study investigating the role of Recq15, a member of the RecQ helicase family, in liver injury. The authors found that Recq15 protects against LPS/D-Gal-induced liver injury. They further showed that this effect is attributed to inhibition of hepatocyte apoptosis and oxidative stress, as well as to downregulation of CYP450.

REFERENCES

- 1 **Croteau DL**, Popuri V, Opresko PL, Bohr VA. Human RecQ helicases in DNA repair, recombination, and replication. *Annu Rev Biochem* 2014; **83**: 519-552 [PMID: 24606147 DOI: 10.1146/annurev-biochem-060713-035428]
- 2 **Chu WK**, Hickson ID. RecQ helicases: multifunctional genome caretakers. *Nat Rev Cancer* 2009; **9**: 644-654 [PMID: 19657341 DOI: 10.1038/nrc2682]
- 3 **Popuri V**, Tadokoro T, Croteau DL, Bohr VA. Human RECQL5: guarding the crossroads of DNA replication and transcription and providing backup capability. *Crit Rev Biochem Mol Biol* 2013; **48**: 289-299 [PMID: 23627586 DOI: 10.3109/10409238.2013.792770]
- 4 **Hu Y**, Lu X, Luo G. Effect of Recq15 deficiency on the intestinal tumor susceptibility of Apc(min) mice. *World J Gastroenterol* 2010; **16**: 1482-1486 [PMID: 20333788]
- 5 **Hu Y**, Lu X, Zhou G, Barnes EL, Luo G. Recq15 plays an important role in DNA replication and cell survival after camptothecin treatment. *Mol Biol Cell* 2009; **20**: 114-123 [PMID: 18987339 DOI: 10.1091/mbc.E08-06-0565]
- 6 **Hu Y**, Raynard S, Sehorn MG, Lu X, Bussen W, Zheng L, Stark JM, Barnes EL, Chi P, Janscak P, Jasin M, Vogel H, Sung P, Luo G. RECQL5/Recq15 helicase regulates homologous recombination and suppresses tumor formation via disruption of Rad51 presynaptic filaments. *Genes Dev* 2007; **21**: 3073-3084 [PMID: 18003859]

- 7 **Hu Y**, Lu X, Barnes E, Yan M, Lou H, Luo G. Recq15 and Blm RecQ DNA helicases have nonredundant roles in suppressing crossovers. *Mol Cell Biol* 2005; **25**: 3431-3442 [PMID: 15831450]
- 8 **Ramamoorthy M**, May A, Tadokoro T, Popuri V, Seidman MM, Croteau DL, Bohr VA. The RecQ helicase RECQL5 participates in psoralen-induced interstrand cross-link repair. *Carcinogenesis* 2013; **34**: 2218-2230 [PMID: 23715498 DOI: 10.1093/carcin/bgt183]
- 9 **Paliwal S**, Kanagaraj R, Sturzenegger A, Burdova K, Janscak P. Human RECQ5 helicase promotes repair of DNA double-strand breaks by synthesis-dependent strand annealing. *Nucleic Acids Res* 2014; **42**: 2380-2390 [PMID: 24319145 DOI: 10.1093/nar/gkt1263]
- 10 **Saponaro M**, Kantidakis T, Mitter R, Kelly GP, Heron M, Williams H, Söding J, Stewart A, Svestrup JQ. RECQL5 controls transcript elongation and suppresses genome instability associated with transcription stress. *Cell* 2014; **157**: 1037-1049 [PMID: 24836610 DOI: 10.1016/j.cell.2014.03.048]
- 11 **Futami K**, Ogasawara S, Goto H, Yano H, Furuichi Y. RecQL1 DNA repair helicase: A potential tumor marker and therapeutic target against hepatocellular carcinoma. *Int J Mol Med* 2010; **25**: 537-545 [PMID: 20198302]
- 12 **Kaneko H**, Fukao T, Kasahara K, Yamada T, Kondo N. Augmented cell death with Bloom syndrome helicase deficiency. *Mol Med Rep* 2011; **4**: 607-609 [PMID: 21567087 DOI: 10.3892/mmr.2011.484]
- 13 **Cogger VC**, Svistounov D, Warren A, Zykova S, Melvin RG, Solon-Biet SM, O'Reilly JN, McMahon AC, Ballard JW, De Cabo R, Le Couteur DG, Leibel M. Liver aging and pseudocapillarization in a Werner syndrome mouse model. *J Gerontol A Biol Sci Med Sci* 2014; **69**: 1076-1086 [PMID: 24149428 DOI: 10.1093/geronola/glt169]
- 14 **Lachaud AA**, Auclair-Vincent S, Massip L, Audet-Walsh E, Lebel M, Anderson A. Werner's syndrome helicase participates in transcription of phenobarbital-inducible CYP2B genes in rat and mouse liver. *Biochem Pharmacol* 2010; **79**: 463-470 [PMID: 19737542 DOI: 10.1016/j.bcp.2009.09.002]
- 15 **Lehmann K**, Tschuor C, Rickenbacher A, Jang JH, Oberkofler CE, Tschopp O, Schultze SM, Raptis DA, Weber A, Graf R, Humar B, Clavien PA. Liver failure after extended hepatectomy in mice is mediated by a p21-dependent barrier to liver regeneration. *Gastroenterology* 2012; **143**: 1609-1619.e4 [PMID: 22960658 DOI: 10.1053/j.gastro.2012.08.043]
- 16 **Waidmann O**, Köberle V, Bettinger D, Trojan J, Zeuzem S, Schultheiß M, Kronenberger B, Piiper A. Diagnostic and prognostic significance of cell death and macrophage activation markers in patients with hepatocellular carcinoma. *J Hepatol* 2013; **59**: 769-779 [PMID: 23792028 DOI: 10.1016/j.jhep.2013.06.008]
- 17 **Degli Esposti D**, Hamelin J, Bosselut N, Saffroy R, Sebah M, Pommier A, Martel C, Lemoine A. Mitochondrial roles and cytoprotection in chronic liver injury. *Biochem Res Int* 2012; **2012**: 387626 [PMID: 22745910 DOI: 10.1155/2012/387626]
- 18 **Wang X**, Zhang L, Wei Z, Zhang X, Gao Q, Ma Y, Liu X, Jiang Y, Liu X, Guo C, Wang X. The inhibitory action of PDCD4 in lipopolysaccharide/D-galactosamine-induced acute liver injury. *Lab Invest* 2013; **93**: 291-302 [PMID: 23295647 DOI: 10.1038/labinvest.2012.174]
- 19 **Shang Y**, Liu Y, Du L, Wang Y, Cheng X, Xiao W, Wang X, Jin H, Yang X, Liu S, Chen Q. Targeted expression of uncoupling protein 2 to mouse liver increases the susceptibility to lipopolysaccharide/galactosamine-induced acute liver injury. *Hepatology* 2009; **50**: 1204-1216 [PMID: 19637283 DOI: 10.1002/hep.23121]
- 20 **Lehmann V**, Freudenberg MA, Galanos C. Lethal toxicity of lipopolysaccharide and tumor necrosis factor in normal and D-galactosamine-treated mice. *J Exp Med* 1987; **165**: 657-663 [PMID: 3819645]
- 21 **González-Terán B**, Cortés JR, Manieri E, Matesanz N, Verdugo Á, Rodríguez ME, González-Rodríguez Á, Valverde ÁM, Martín P, Davis RJ, Sabio G. Eukaryotic elongation factor 2 controls TNF- α translation in LPS-induced hepatitis. *J Clin Invest* 2013; **123**: 164-178 [PMID: 23202732 DOI: 10.1172/JCI65124]
- 22 **Jing Y**, Ai Q, Lin L, Dai J, Jia M, Zhou D, Che Q, Wan J, Jiang R, Zhang L. Protective effects of garcinol in mice with lipopolysaccharide/D-galactosamine-induced apoptotic liver injury. *Int Immunopharmacol* 2014; **19**: 373-380 [PMID: 24560905 DOI: 10.1016/j.intimp.2014.02.012]
- 23 **Chen L**, Ren F, Zhang H, Wen T, Piao Z, Zhou L, Zheng S, Zhang J, Chen Y, Han Y, Duan Z, Ma Y. Inhibition of glycogen synthase kinase 3 β ameliorates D-GalN/LPS-induced liver injury by reducing endoplasmic reticulum stress-triggered apoptosis. *PLoS One* 2012; **7**: e45202 [PMID: 23028846 DOI: 10.1371/journal.pone.0045202]
- 24 **Lu J**, Chen YP, Wan R, Guo CY, Wang XP. Protective effects of ulinastatin on acute liver failure induced by lipopolysaccharide/D-galactosamine. *Dig Dis Sci* 2012; **57**: 399-404 [PMID: 22001939 DOI: 10.1007/s10620-011-1927-0]
- 25 **Ingelman-Sundberg M**. Human drug metabolising cytochrome P450 enzymes: properties and polymorphisms. *Naunyn Schmiedebergs Arch Pharmacol* 2004; **369**: 89-104 [PMID: 14574440]
- 26 **Korzekwa K**. Enzyme kinetics of oxidative metabolism: cytochromes P450. *Methods Mol Biol* 2014; **1113**: 149-166 [PMID: 24523112 DOI: 10.1007/978-1-62703-758-7_8]
- 27 **Xie Y**, Hao H, Wang H, Guo C, Kang A, Wang G. Reversing effects of lignans on CCl4-induced hepatic CYP450 down regulation by attenuating oxidative stress. *J Ethnopharmacol* 2014; **155**: 213-221 [PMID: 24910408 DOI: 10.1016/j.jep.2014.05.016]
- 28 **Shah P**, Guo T, Moore DD, Ghose R. Role of constitutive androstane receptor in Toll-like receptor-mediated regulation of gene expression of hepatic drug-metabolizing enzymes and transporters. *Drug Metab Dispos* 2014; **42**: 172-181 [PMID: 24194512 DOI: 10.1124/dmd.113.053850]
- 29 **Li-Masters T**, Morgan ET. Effects of bacterial lipopolysaccharide on phenobarbital-induced CYP2B expression in mice. *Drug Metab Dispos* 2001; **29**: 252-257 [PMID: 11181491]
- 30 **De-Oliveira AC**, Poça KS, Totino PR, Paumgartten FJ. Modulation of cytochrome P450 2A5 activity by lipopolysaccharide: low-dose effects and non-monotonic dose-response relationship. *PLoS One* 2015; **10**: e0117842 [PMID: 25635819 DOI: 10.1371/journal.pone.0117842]
- 31 **Weber MA**, Schnyder-Candrian S, Schnyder B, Quesniaux V, Poli V, Stewart CL, Ryffel B. Endogenous leukemia inhibitory factor attenuates endotoxin response. *Lab Invest* 2005; **85**: 276-284 [PMID: 15702085]
- 32 **Kuhla A**, Eipel C, Abshagen K, Siebert N, Menger MD, Vollmar B. Role of the perforin/granzyme cell death pathway in D-Gal/LPS-induced inflammatory liver injury. *Am J Physiol Gastrointest Liver Physiol* 2009; **296**: G1069-G1076 [PMID: 19264954 DOI: 10.1152/ajpgi.90689.2008]
- 33 **Roos WP**, Kaina B. DNA damage-induced cell death by apoptosis. *Trends Mol Med* 2006; **12**: 440-450 [PMID: 16899408]
- 34 **Liu J**, Wu KC, Lu YF, Ekuase E, Klaassen CD. Nrf2 protection against liver injury produced by various hepatotoxicants. *Oxid Med Cell Longev* 2013; **2013**: 305861 [PMID: 23766851 DOI: 10.1155/2013/305861]
- 35 **Vandendriessche B**, Goethals A, Simats A, Van Hamme E, Brouckaert P, Cauwels A. MAPK-activated protein kinase 2-deficiency causes hyperacute tumor necrosis factor-induced inflammatory shock. *BMC Physiol* 2014; **14**: 5 [PMID: 25185746 DOI: 10.1186/s12899-014-0005-1]
- 36 **Jia M**, Jing Y, Ai Q, Jiang R, Wan J, Lin L, Zhou D, Che Q, Li L, Tang L, Shen Y, Zhang L. Potential role of catalase in mice with lipopolysaccharide/D-galactosamine-induced fulminant liver injury. *Hepatol Res* 2014; **44**: 1151-1158 [PMID: 23941578 DOI: 10.1111/hepr.12220]
- 37 **Aksu B**, Ayvaz S, Aksu F, Karaca T, Cemek M, Ayaz A, Demirtaş S. Effects of sphingosylphosphorylcholine against oxidative stress and acute lung injury induced by pulmonary contusion in rats. *J Pediatr Surg* 2015; **50**: 591-597 [PMID: 25840069 DOI: 10.1016/

j.jped Surg.2014.06.007]
38 **Liu YF**, Zha BS, Zhang HL, Zhu XJ, Li YH, Zhu J, Guan XH, Feng ZQ, Zhang JP. Characteristic gene expression profiles in

the progression from liver cirrhosis to carcinoma induced by diethylnitrosamine in a rat model. *J Exp Clin Cancer Res* 2009; **28**: 107 [PMID: 19638242 DOI: 10.1186/1756-9966-28-107]

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