

Protective effect of rosiglitazone against acetaminophen-induced acute liver injury is associated with down-regulation of hepatic NADPH oxidases



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HIGHLIGHTS

- RSG pretreatment protects against APAP-induced acute liver injury.
- RSG pretreatment inhibits APAP-induced hepatic cell death.
- RSG alleviates hepatic GSH depletion during APAP-induced liver injury.
- RSG downregulates hepatic NADPH oxidases during APAP-induced liver injury.
- Synthetic PPAR- γ agonists might be effective agents for preventing liver injury.

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ABSTRACT

The peroxisome proliferator-activated receptor gamma (PPAR- γ) is a ligand-activated nuclear receptor that regulates glucose and lipid metabolism. The aim of the present study was to investigate the effects of rosiglitazone (RSG), a synthetic PPAR- γ agonist, on acetaminophen (APAP)-induced acute liver injury. Male CD-1 mice were injected with APAP (300 mg/kg). Some mice were pretreated with RSG (20 mg/kg) 48, 24 and 1 h before APAP injection. As expected, RSG pretreatment alleviated APAP-induced acute liver injury. Moreover, RSG pretreatment attenuated APAP-induced hepatic cell death and improved the survival. Although it did not affect hepatic cytochrome P450 (CYP)2E1 expression, RSG pretreatment attenuated reduction of hepatic glutathione peroxidase (GSH-Px), glutathione reductase (GSH-Rd) and glutathione S-transferase (GST) activities, inhibited upregulation of hepatic nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX)-2 and NOX-4, and alleviated hepatic GSH depletion during APAP-induced acute liver injury. In addition, RSG pretreatment suppressed activation of hepatic nuclear factor kappa B (NF- κ B) and extracellular signal-related kinase (ERK)/mitogen-activated protein kinase (MAPK) signaling during APAP-induced acute liver injury. These results provide a novel mechanistic explanation for RSG-mediated protection against APAP-induced acute liver injury. The present results suggest that synthetic PPAR- γ agonists might be effective agents for preventing the progression of APAP-induced acute liver injury.

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1. Introduction

Acetaminophen (APAP) is a widely used antipyretic and analgesic drug. Although safe at therapeutic doses, APAP overdose induces acute liver injury (Jaeschke et al., 2012a; Beger et al., 2015; Maes et al., 2016). If it is not controlled, APAP-induced acute liver

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injury may progress to acute liver failure with a much high mortality (Lancaster et al., 2015; Goldberg et al., 2015). Increasing evidence demonstrates that cytochrome P450 (CYP) 2E1 and CYP3A, which convert APAP into the reactive metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI), play key roles in APAP-induced acute liver injury (Thummel et al., 1993; Chen et al., 1998; Zaher et al., 1998; Manyike et al., 2000). According to an early report, excess NAPQI depletes hepatic glutathione (GSH), leading to mitochondrial permeability transition pore (MPT), cytosolic leakage of cytochrome c and hepatic cell death (Masubuchi et al., 2005). Several studies reported that hepatic c-Jun N-terminal kinase (JNK) was activated in the pathogenesis of APAP-induced acute liver injury (Gunawan et al., 2006; Latchoumycandane et al., 2007). Recently, several reports showed that hepatic receptor interacting protein (RIP)1 and RIP3 were involved in the process of APAP-induced liver injury (Ramachandran et al., 2013; Zhang et al., 2014; Deutsch et al., 2015; Dara et al., 2015). The damage-associated molecular patterns (DAMP)-evoked hepatic sterile inflammation played important roles in the progression of APAP-induced acute liver injury (Jaeschke et al., 2012b; Huebener et al., 2015).

The peroxisome proliferator-activated receptor gamma (PPAR- γ) is a ligand-activated nuclear receptor that regulates glucose and lipid metabolism (Lefterova et al., 2014; Wang et al., 2014, 2016). Numerous reports indicate that PPAR- γ has an anti-inflammatory activity (Uchimura et al., 2001; Wen et al., 2010; Choo et al., 2015). Rosiglitazone (RSG) is a synthetic PPAR- γ agonist that has been used for the treatment of type II diabetes (Sgarra et al., 2012). Several studies showed that RSG had a potent anti-inflammatory effect (Lee et al., 2005; Wang et al., 2011). Indeed, RSG repressed hepatic inflammation in the pathogenesis of nonalcoholic steatohepatitis (Tahan et al., 2007; Gupta et al., 2010; Torres et al., 2011; Lea et al., 2014). Nevertheless, whether RSG alleviates hepatic sterile inflammation in the progression of APAP-induced liver injury needs to be determined.

The aim of the present study was to investigate the effects of pretreatment with RSG on APAP-induced acute liver injury. We showed that RSG pretreatment protected against the progression of APAP-induced acute liver injury. Although it had little effect on hepatic CYP2E1 expression, RSG pretreatment attenuated reduction of hepatic glutathione peroxidase (GSH-Px), glutathione reductase (GSH-Rd) and glutathione S-transferase (GST) activities, inhibited upregulation of hepatic nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX)-2 and NOX-4, and alleviated hepatic GSH depletion during APAP-induced acute liver injury. Our results provide evidence for roles of PPAR- γ as a regulator of hepatic NADPH oxidases in the progression of APAP-induced acute liver injury. Synthetic PPAR- γ agonists might be effective agents for preventing the progression of APAP-induced acute liver injury.

2. Materials and methods

2.1. Chemicals and reagents

APAP and RSG were purchased from Sigma Chemical Co. (St. Louis, MO). Antibody against CYP2E1 was from Milipore (Temecula, California, USA). Antibodies against NF- κ B p65, ERK, phosphor-ERK (pERK), α -tubulin and Lamin A/C were from Santa Cruz Biotechnologies (Santa Cruz, CA). Antibodies against NOX-2 and NOX-4 were from Abcam (Cambridge, MA). Chemiluminescence (ECL) detection kit was from Pierce Biotechnology (Rockford, IL). All other reagents were purchased from Sigma Chemical Co. (St. Louis, MO) if not otherwise stated.

2.2. Animals and treatments

Male CD-1 mice (6–8 week-old, 28–30 g) were purchased from Beijing Vital River (Beijing, China). The animals were allowed free access to food and water at all times and were maintained on a 12-h light/dark cycle in a controlled temperature (20–25 °C) and humidity (50 \pm 5%) environment for a period of 1 week before use. Sixty mice were divided into 10 groups (six mice each group). In APAP alone group, mice were intraperitoneally (i.p.) injected with APAP (300 mg/kg). The doses of APAP used in the present study referred to our previous study (Wang et al., 2013). In APAP + RSG group, mice were administered with RSG (20 mg/kg, dissolved in PBS) by gavage at 48, 24 and 1 h before APAP (300 mg/kg). The doses of RSG used in the present study referred to others (Tao et al., 2010). In control group, mice were i.p. injected with PBS. In RSG alone group, mice were administered with RSG (20 mg/kg) by gavage at 48, 24 and 1 h before NS injection. Mice were sacrificed at different time points (0, 0.5, 1, 4 and 24 h) after APAP injection. Serum samples were collected for measurement of biochemical parameters. Some liver samples were collected and frozen immediately in lipid nitrogen for measurement of GSH and malonaldehyde (MDA), real-time RT-PCR and immunoblot. Some liver samples were fixed in neutral-buffered formalin for histological examination and terminal dUTP nick-end labeling (TUNEL) assay. For survival assay, twenty mice were divided into two groups. In APAP group, mice were i.p. injected with APAP (300 mg/kg). In RSG + APAP group, mice were orally administered with RSG (20 mg/kg) at 48, 24 and 1 h before APAP (300 mg/kg). Animal death was observed until 7 d after APAP. This study was approved by the association of laboratory animal sciences and the center for laboratory animal sciences at Anhui medical university (permit number: 15-0011). All procedures on animals followed the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.3. Evaluation of liver injury

Serum alanine aminotransferase (ALT) was measured using commercially available assay kits according to the manufacturer's instructions. Liver tissues were fixed in 4% formalin and embedded in paraffin according to the standard procedure. Paraffin embedded tissues were cut 5 μ m thick and stained with hematoxylin and eosin (H&E) for morphological analysis. To quantify the extent of necrosis, the percentage of necrosis was estimated by measuring the necrotic area relative to the entire histological section, and an analysis of the area was performed with NIH ImageJ software (<http://rsb.info.nih.gov/ij/>).

2.4. Immunoblots

Hepatic nuclear extracts or hepatic lysates were separated electrophoretically by SDS-PAGE and transferred to a polyvinylidene fluoride membrane. For hepatic nuclear extracts, the membranes were incubated for 2 h with NF- κ B p65 antibody. For hepatic lysates, the membranes were incubated for 2 h with following antibodies: CYP2E1, ERK, pERK, NOX-2 and NOX-4. For nuclear protein, Lamin A/C was used as a loading control. For total proteins, α -tubulin was used as a loading control. After washes in DPBS containing 0.05% Tween-20 four times for 10 min each, the membranes were incubated with secondary antibody for 2 h. The membranes were washed for four times in DPBS containing 0.05% Tween-20 for 10 min each, followed by signal development using an ECL detection kit.

2.5. Isolation of total RNA and real-time RT-PCR

Total RNA in liver tissue was extracted using TRIzol (Life Technologies, Grand Island, NY, USA). The purity of RNA was assessed according to the ratio of absorbance at 260 nm and 280 nm. RNase-free DNase-treated total RNA (1.0 μ g) was reverse-transcribed with AMV (Promega; Madison, WI, USA). Real-time PCR was performed with Light Cycler[®] 480 SYBR Green I Kit (Roche Diagnostics GmbH, Mannheim, Germany) using genetic-specific primers as listed in supplemental Table 1. The amplification reactions were carried out on a LightCycler[®] 480 Instrument (Roche Diagnostics GmbH, Mannheim, Germany) with an initial hold step (95 °C for 10 min) and 45 cycles of a three-step PCR (95 °C for 15 s, 60 °C for 15 s, 72 °C for 20 s). The comparative C_T-method (Hoebeek et al., 2007; Ferlini and Rimessi, 2012) was used to determine the amount of target, normalized to an endogenous reference and relative to a calibrator ($2^{-\Delta\Delta C_T}$) using the Lightcycler 480 software (Roche, version 1.5.0). All RT-PCR experiments were performed in triplicate.

2.6. Terminal dUTP nick-end labeling (TUNEL) staining

For measurement of hepatic nuclear DNA strand breaks, paraffin-embedded sections were stained with the TUNEL technique using an in situ apoptosis detection kit (Promega Madison, WI) according to the manufacturer's protocols. Sections were counterstained with hematoxylin. TUNEL-positive

cells were counted in twelve randomly selected fields from each slide at a magnification of $\times 200$. The percentage of TUNEL-positive hepatocytes was analyzed in six liver sections from six different mice.

2.7. Hepatic GSH metabolic enzyme activities

Liver tissues were homogenized in 50 mM phosphate buffer (pH 7.4) and centrifuged at 3200g for 20 min at 4 °C. Glutathione peroxidase (GSH-Px) activity was measured according to method as described by others (Paglia and Valentine, 1967). GSH-Px activity was expressed as U/mg protein/min. Hepatic GSH-Rd activity was analyzed following NADPH oxidation at 340 nm in the presence of GSSG. Hepatic GSH-Rd activity was expressed as U/mg protein/min. Glutathione S-transferase (GST) activity was determined using method as described by others (Habig and Jakoby, 1981). Briefly, GST activity was measured by determining the rate of conjugate formation between GSH and 1-chloro-2,4-nitrobenzene (CDNB) at 340 nm. Hepatic GST activity was expressed as U/mg protein/min. All enzyme activities were determined at 25 °C. The concentration of protein was measured by the BCA assay (Pierce, Rockford, IL, USA).

2.8. Biochemical parameters

For preparation of hepatic homogenates, 100 mg of liver tissue was homogenized on ice in 1 ml of homogenization buffer (50 mM

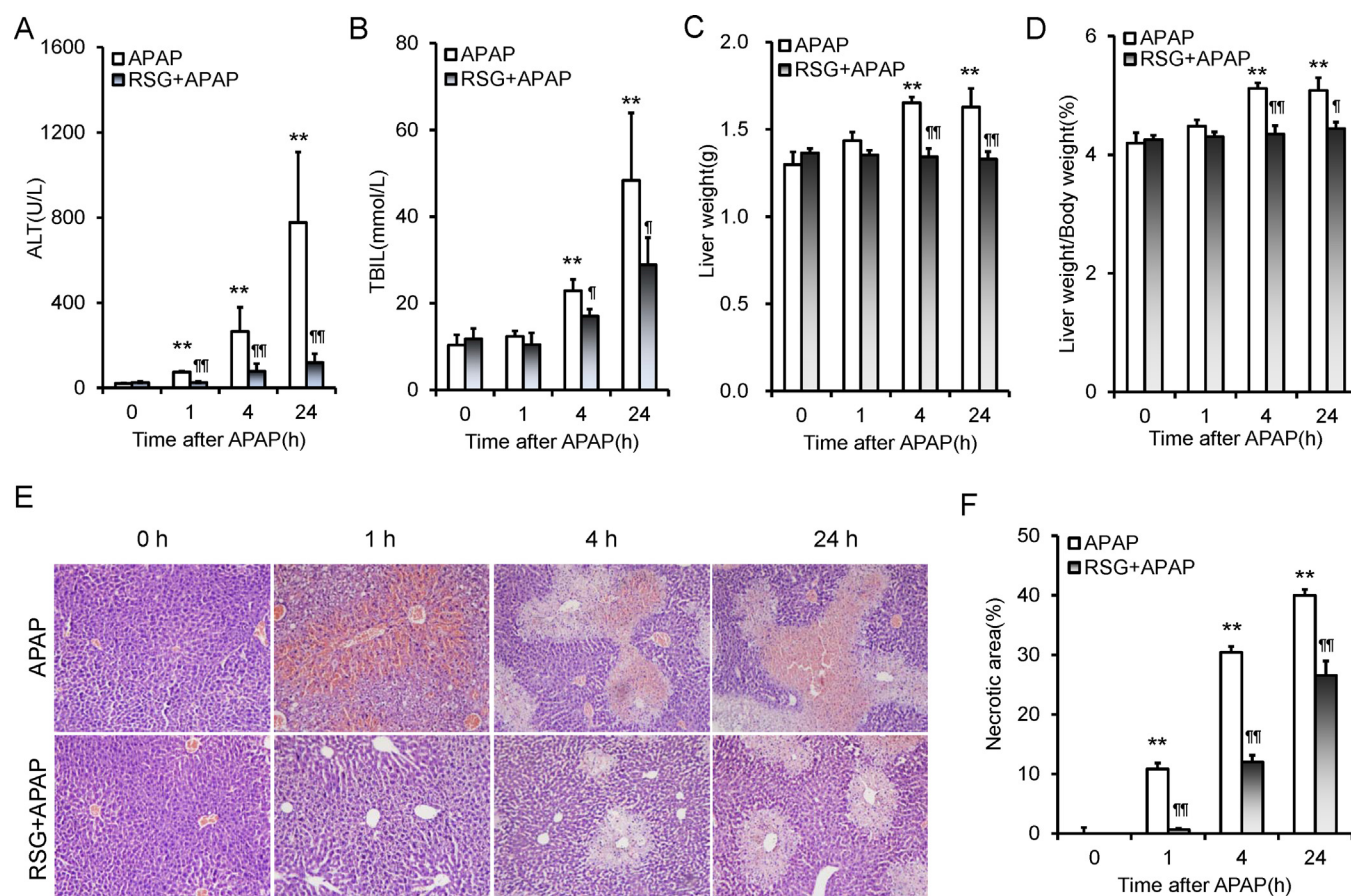


Fig. 1. Effects of RSG pretreatment on APAP-induced acute liver injury. Mice were i.p. injected with APAP (300 mg/kg). In RSG + APAP group, mice were administered with RSG (20 mg/kg) by gavage at 48, 24 and 1 h before APAP. Serum and liver samples were collected 1, 4 and 24 h after APAP. (A) Serum ALT and (B) TBIL were measured. (C) Absolute liver weight and (D) relative liver weight were analyzed. (E) Representative photomicrographs of liver histology were shown (H&E, magnification: 100 \times). (F) Necrotic area was analyzed. All data were expressed as means \pm SE (n = 6). ** P < 0.01 as compared with control group (0 time point). ∇P < 0.05, $\nabla\nabla P$ < 0.01 as compared with APAP group at the same time point.

Tris–HCl, 180 mM KCl, 10 mM EDTA, pH 7.4). Hepatic malondialdehyde (MDA) was determined according to others (Ohkawa et al., 1979). Hepatic MDA level was expressed as nmol/mg protein. Hepatic GSH content was measured as described by others (Anderson, 1985). Hepatic GSH content was expressed as nmol/mg protein. Serum C-reactive protein (CRP) was measured using Latex enhanced immunoturbidimetric assay kit (Beijing Leadman Biochemistry Co., Ltd, Beijing, China) by Hitachi 7600-020 Automatic biochemical analyzer in the Laboratory of Anhui No.2 Province's hospital.

2.9. Statistical analysis

All data were expressed as means \pm SE. SPSS 13.0 statistical software was used for statistical analysis. All statistical tests were two-sided using an alpha level of 0.05. ANOVA and the Student–Newmann–Keuls post hoc test were used to determine differences among different groups.

3. Results

3.1. RSG pretreatment protects against APAP-induced acute liver injury

APAP-induced acute liver injury was evaluated. As expected, serum ALT level was only slightly increased at 1 h after APAP and continued to rise from 4 h to 24 h after APAP (Fig. 1A). Serum TBIL level was slightly elevated until 4 h after APAP and markedly increased at 24 h after APAP (Fig. 1B). The absolute and relative

liver weights were elevated until 4 h after APAP and remained increased at 24 h after APAP (Fig. 1C and D). Histopathology observed a characteristic centrilobular necrosis in liver of APAP-treated mice (Fig. 1E). Necrotic area was only 5.0% at 1 h after APAP, 20% at 4 h after APAP, and up to 32% at 24 h after APAP (Fig. 1F). The effects of RSG pretreatment on APAP-induced acute liver injury were then analyzed. As shown in Fig. 1A and B, RSG pretreatment alleviated APAP-induced increase in serum ALT and TBIL. Moreover, RSG pretreatment attenuated APAP-induced elevation of absolute and relative liver weights (Fig. 1C and D). In addition, RSG pretreatment alleviated APAP-induced hepatic centrilobular necrosis (Fig. 1E and F).

3.2. RSG pretreatment improves survival and inhibits APAP-induced hepatic cell death

APAP-induced hepatic cell death was measured by TUNEL assay. As expected, only a few of TUNEL+ cells were observed at 1 h after APAP. The number of TUNEL+ cells was significantly elevated until 4 h after APAP and continued increased at 24 h after APAP (Fig. 2A and B). Survival test showed that only 50% (5/10) mice were alive until 7 d after APAP (Fig. 2C). Of interest, the number of hepatic TUNEL+ cells was significantly reduced when mice were pretreated with RSG (Fig. 2A and B). Correspondingly, the survival rate was increased to 80% (8/10) when mice were pretreated with RSG (Fig. 2C).

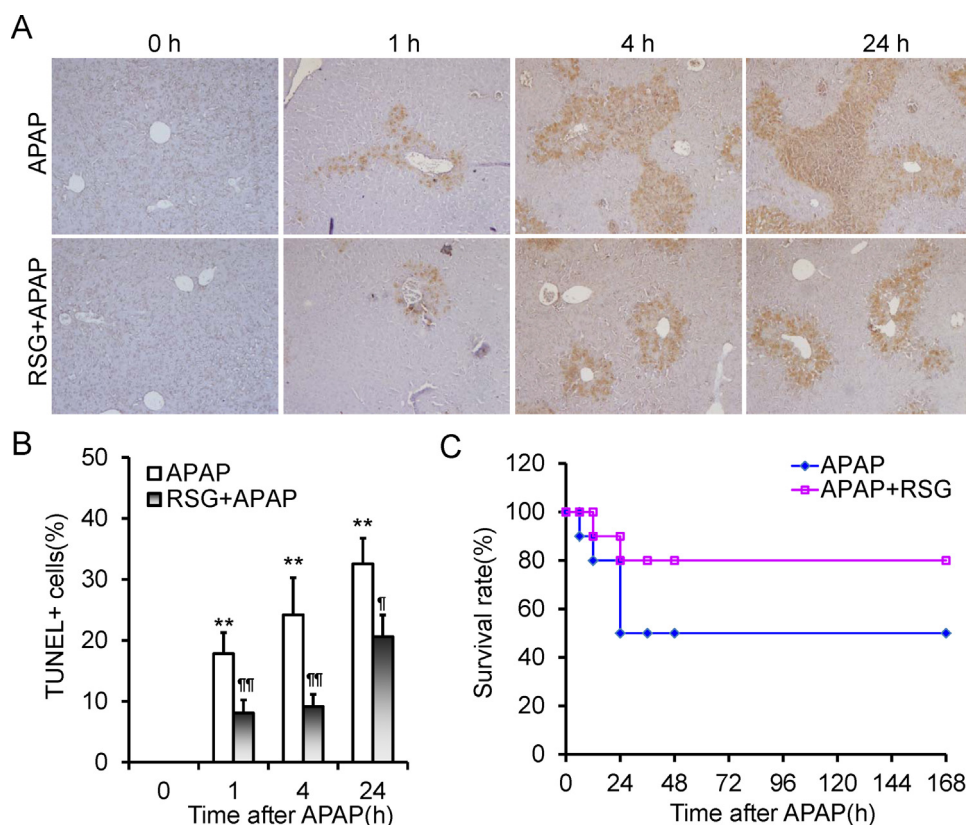


Fig. 2. Effects of RSG pretreatment on hepatic cell death and mortality during APAP-induced acute liver injury. (A and B) Mice were i.p. injected with APAP (300 mg/kg). In RSG + APAP group, mice were with RSG (20 mg/kg) by gavage at 48, 24 and 1 h before APAP. Hepatic cell death was measured using TUNEL assay at 1, 4 and 24 h after APAP injection. (A) Representative photomicrographs from different groups were shown. (B) TUNEL+ cells were analyzed. All data were expressed as means \pm SE (n = 6). ** P < 0.01 as compared with control group (0 time point). ∇P < 0.05, $\nabla\nabla P$ < 0.01 as compared with APAP group at the same time point. (C) Twenty mice were divided into two groups. In APAP group, mice were i.p. injected with APAP (300 mg/kg). In RSG + APAP group, mice were orally administered with RSG (150 mg/kg) at 48, 24 and 1 h before APAP (300 mg/kg). Animal death was observed until 7 d after APAP.

3.3. RSG pretreatment alleviates hepatic GSH depletion and lipid peroxidation during APAP-induced acute liver injury

The effects of RSG pretreatment on APAP-induced hepatic GSH depletion were evaluated. As shown in Fig. 3A, hepatic GSH content was significantly reduced at 1 h after APAP and remained decreased at 4 h after APAP injection. Interestingly, RSG pretreatment significantly attenuated APAP-induced hepatic GSH depletion (Fig. 3A). The effects of RSG pretreatment on hepatic lipid peroxidation during APAP-induced acute liver injury were then analyzed. As shown in Fig. 3B, the level of hepatic MDA, a marker of lipid peroxidation, was significantly elevated at 1 h after APAP injection. Interestingly, RSG pretreatment significantly attenuated hepatic lipid peroxidation during APAP-induced acute liver injury (Fig. 3B).

3.4. RSG pretreatment attenuates reduction of hepatic GSH metabolic enzyme activities and inhibits up-regulation of hepatic NADPH oxidases during APAP-induced acute liver injury

The effects of RSG pretreatment on hepatic GSH metabolic activities during APAP-induced acute liver injury were analyzed. As expected, activities of hepatic GSH-Px, GSH-Rd and GST, three hepatic GSH metabolic enzymes, were markedly reduced at 1 h after APAP injection (Fig. 4A–C). Of interest, RSG pretreatment significantly attenuated reduction of hepatic GSH-Rd and GST activities during APAP-induced acute liver injury (Fig. 4A and B). The effects of RSG pretreatment on APAP-induced up-regulation of NADPH oxidases were then analyzed. As shown in Fig. 4D and E, hepatic NOX-2 was elevated, slightly at 1 h after APAP and obviously from 4 h to 24 h after APAP injection. Moreover, hepatic NOX-4 was also elevated, beginning at 1 h after APAP and remaining increased from 4 h to 24 h after APAP injection (Fig. 4E and F). Of interest, RSG pretreatment inhibited hepatic NOX-2 and NOX-4 up-regulation during APAP-induced acute liver injury (Fig. 4D–F).

3.5. RSG pretreatment inhibits activation of hepatic NF- κ B and ERK pathways during APAP-induced acute liver injury

The effects of RSG pretreatment on hepatic NF- κ B signaling during APAP-induced acute liver injury were analyzed. As expected, hepatic nuclear NF- κ B p65 level was elevated, only

slightly at 1 h after APAP and markedly from 4 h to 24 h after APAP (Fig. 5A and B). RSG pretreatment significantly attenuated hepatic NF- κ B activation during APAP-induced acute liver injury (Fig. 5A and B). The effects of RSG pretreatment on hepatic ERK/MAPK signaling during APAP-induced acute liver injury were then evaluated. As expected, hepatic pERK level was elevated, only slightly at 1 h after APAP and markedly from 4 h to 24 h after APAP injection (Fig. 5C and D). RSG pretreatment attenuated hepatic ERK phosphorylation during APAP-induced acute liver injury (Fig. 5C and D).

3.6. RSG pretreatment inhibits inflammatory mediators during APAP-induced acute liver injury

The effects of RSG pretreatment on inflammatory mediators during APAP-induced acute liver injury were analyzed. As expected, serum CRP, a systemic inflammatory mediator, was significantly elevated (supplemental Fig. 1A). Although APAP had little effect on hepatic COX-2 expression (Supplemental Fig. 1D), hepatic TNF- α , an inflammatory cytokine, and KC, an inflammatory chemokine, were significantly upregulated at 24 h after APAP injection (supplemental Fig. 1B and C). Of interest, RSG pretreatment inhibited up-regulation of inflammatory mediators during APAP-induced acute liver injury (supplemental Fig. 1A–C).

3.7. RSG pretreatment does not influence hepatic CYP2E1 expression at early stage of APAP-induced acute liver injury

The effects of RSG pretreatment on hepatic CYP2E1, the key metabolic enzyme for APAP, were analyzed at 0.5 h after APAP. As shown in Fig. 6, there was no significant difference on hepatic CYP2E1 level among different groups.

4. Discussion

The present study investigated the effects of pretreatment with RSG, a synthetic PPAR- γ agonist, on APAP-induced acute liver injury. We showed that RSG pretreatment markedly attenuated APAP-induced elevation of serum ALT and TBIL levels. Moreover, RSG pretreatment alleviated APAP-induced characteristic centrilobular necrosis in liver. In addition, RSG pretreatment reduced the mortality and improved the survival. It is widely accepted that hepatic cell death is an important event during APAP-induced

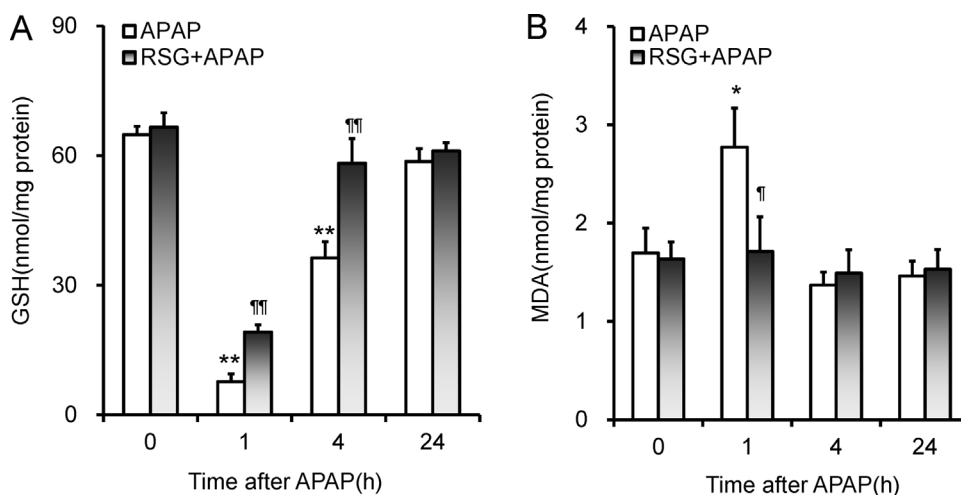


Fig. 3. Effects of RSG pretreatment on hepatic GSH and MDA during APAP-induced acute liver injury. Mice were i.p. injected with APAP (300 mg/kg). In RSG + APAP group, mice were administered with RSG (20 mg/kg) by gavage at 48, 24 and 1 h before APAP (300 mg/kg). (A) Hepatic GSH content was measured at 1, 4 and 24 h after APAP injection. (B) Hepatic MDA level was measured at 1, 4 and 24 h after APAP injection. All data were expressed as means \pm SE (n = 6). *P < 0.05, **P < 0.01 as compared with control group (0 time point). *P < 0.05, **P < 0.01 as compared with APAP group at the same time point.

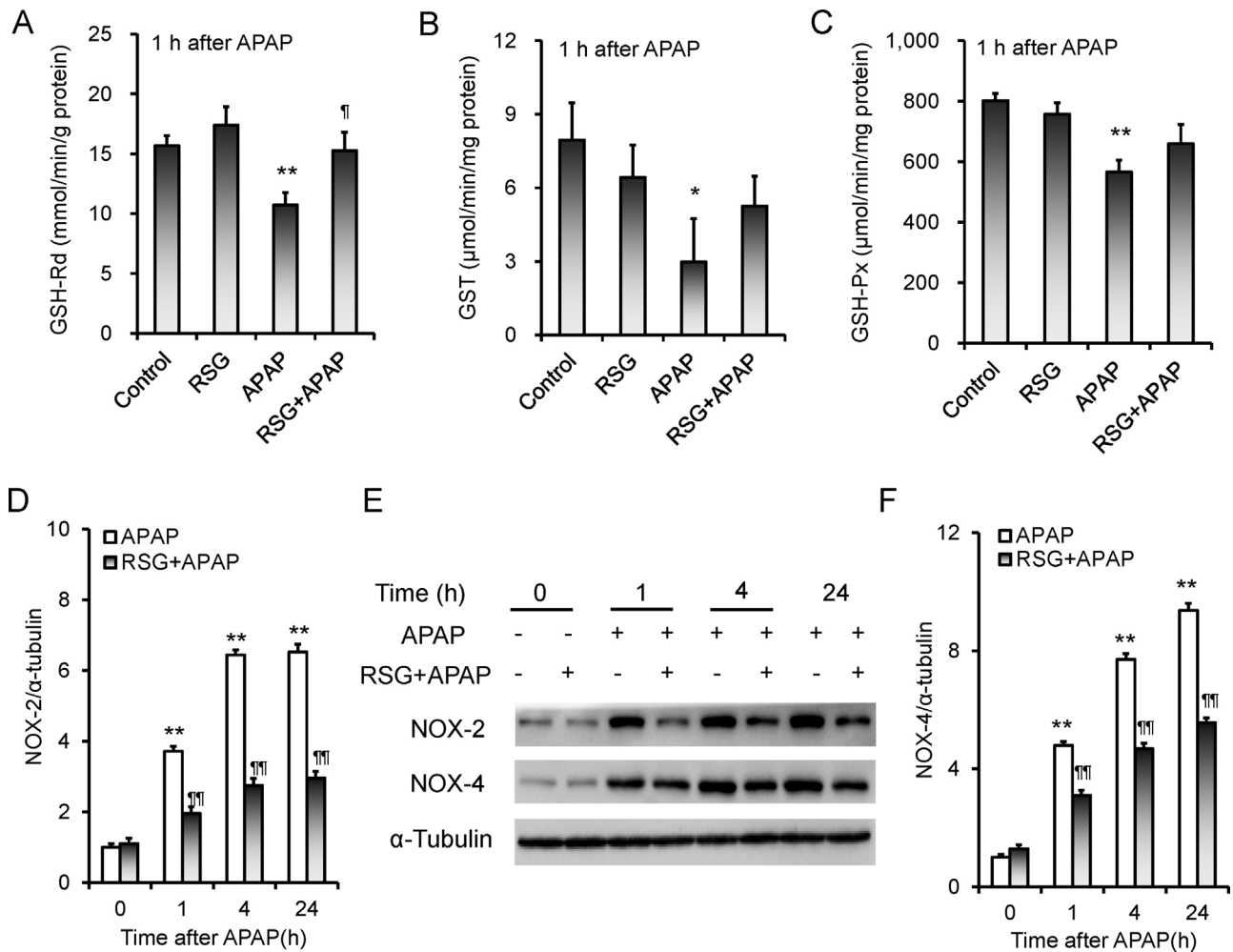


Fig. 4. Effects of RSG pretreatment on hepatic GSH metabolic enzyme activities and hepatic NADPH oxidases during APAP-induced acute liver injury. Mice were i.p. injected with APAP (300 mg/kg). In RSG + APAP group, mice were administered with RSG (20 mg/kg) by gavage at 48, 24 and 1 h before APAP (300 mg/kg). (A–C) Hepatic GSH metabolic enzyme activities were measured at 1 h after APAP injection. (A) GSH-Rd; (B) GST; (C) GSH-Px. (D–F) Hepatic NOX-2 and NOX-4 were measured using immunoblot. (E) Representative gels for NOX-2 (upper panel), NOX-4 (middle panel) and α -tubulin (lower panel) were shown. Quantitative analysis of scanning densitometry for (D) NOX-2 and (F) NOX-4 was performed. All data were expressed as means \pm SE (n = 6). * P < 0.05, ** P < 0.01 as compared with control group (0 time point). ¶ P < 0.05, ¶¶ P < 0.01 as compared with APAP group at the same time point.

acute liver injury (Gujral et al., 2002; Zhang et al., 2014). The present study showed that the number of hepatic dead cells, as determined by TUNEL assay, was slightly elevated 1 h after APAP injection and obviously increased from 4 h to 24 h after APAP injection. Of interest, the number of hepatic dead cells was reduced when mice were pretreated with RSG before APAP injection. These results suggest that RSG pretreatment prevents the progression of APAP-induced acute liver injury.

An early report demonstrated that HMGB1/TLR4-activated neutrophil (PMN) NADPH oxidases, which released excess reactive oxygen species (ROS), were involved in the process of hemorrhagic shock/resuscitation-induced liver injury (Fan et al., 2007). The present study showed that hepatic NOX-2 and NOX-4, two subunits of NADPH oxidases, were elevated 1 h after APAP injection and remained increased from 4 h to 24 h after APAP injection, when hepatic damage was further exacerbated. Thus, we speculate that NADPH oxidases-sourced ROS might contribute, at least partially, to the progression of APAP-induced acute liver injury. An early in vitro study found that RSG repressed release of NADPH oxidase-sourced ROS in endothelial cells (Ceolotto et al., 2007). Additional in vitro report showed that RSG attenuated hyperglycemia-induced Nox-4 up-regulation in endothelial cells (Williams et al., 2012). The present study investigated the effects of RSG

pretreatment on hepatic NADPH oxidases during APAP-induced liver injury. Our results showed that RSG pretreatment inhibited APAP-induced up-regulation of hepatic NOX-2 and NOX-4. Moreover, RSG pretreatment alleviated hepatic GSH depletion in the progression of APAP-induced acute liver injury. These results suggest that RSG-mediated protection against APAP-induced acute liver injury is, at least partially, associated with down-regulation of hepatic NOX-2 and NOX-4. The present study also analyzed the effects of RSG pretreatment on hepatic GSH metabolic activities. Our results showed that RSG pretreatment significantly attenuated reduction of hepatic GSH metabolic activities during APAP-induced acute liver injury. These results provide a novel mechanistic explanation for RSG-mediated protection against APAP-induced acute liver injury.

Increasing evidence demonstrates that DAMP-evoked hepatic sterile inflammation plays important roles in the progression of APAP-induced acute liver injury (Antoine et al., 2009; Dragomir et al., 2011; Jaeschke et al., 2012b; Wang et al., 2013; Huebener et al., 2015). Indeed, APAP-evoked hepatic cell death induces release of DAMPs, such as HMGB1, mitochondrial DNA and nuclear DNA fragments, which could activate Kupffer cells and induce hepatic inflammatory response (Martin-Murphy et al., 2010). Numerous studies indicate that PPAR- γ has an anti-inflammatory

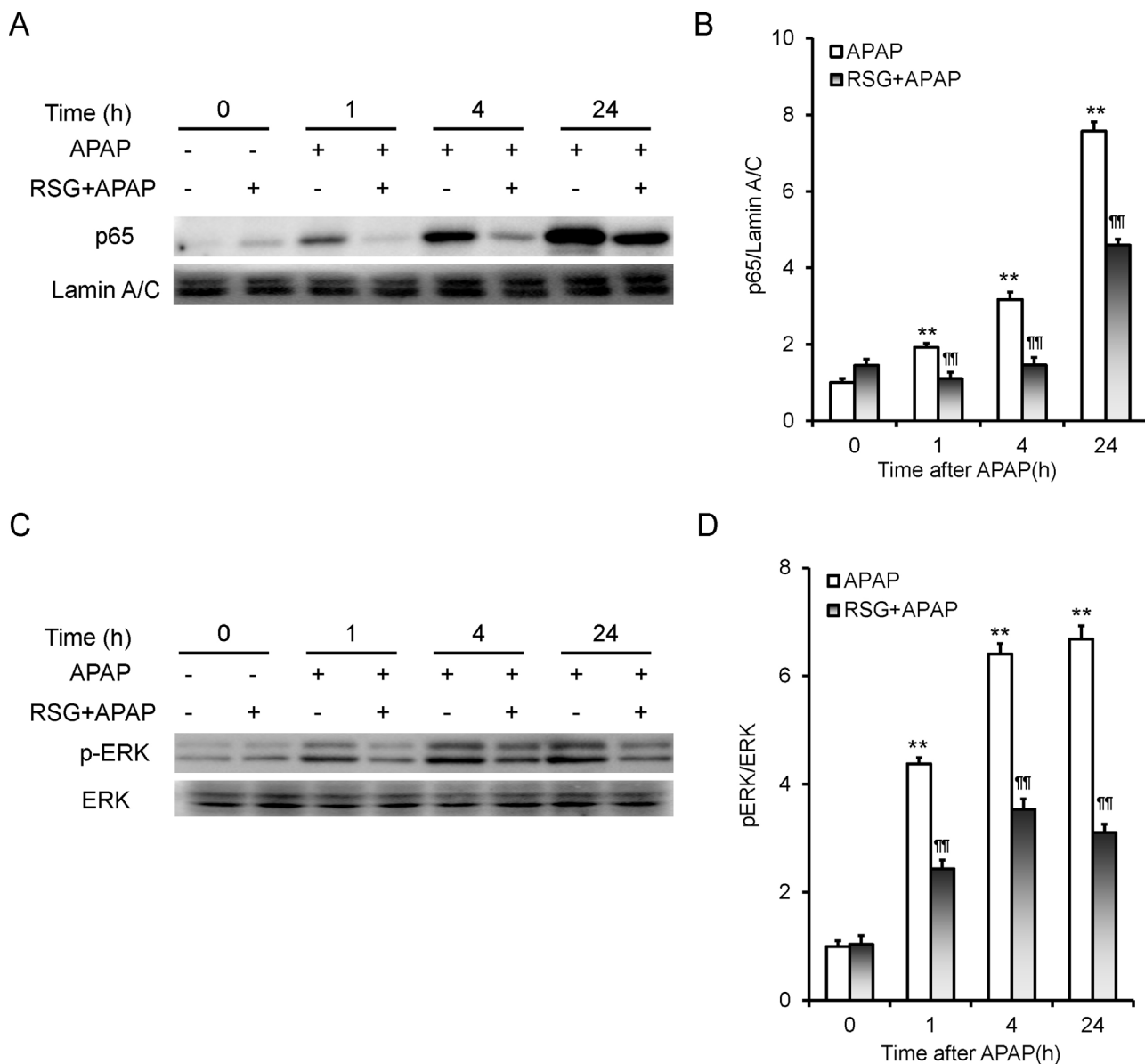


Fig. 5. Effects of RSG pretreatment on hepatic NF- κ B and MAPK/ERK pathways during APAP-induced acute liver injury. Mice were i.p. injected with APAP (300 mg/kg). In RSG + APAP group, mice were administered with RSG (20 mg/kg) by gavage at 48, 24 and 1 h before APAP (300 mg/kg). (A and B) Hepatic nuclear NF- κ B p65 subunit was measured using immunoblot. (A) Representative gels for p65 (upper panel) and Lamin A/C (lower panel) were shown. (B) Quantitative analysis of scanning densitometry for p65 was performed. (C and D) Hepatic pERK and ERK were measured using immunoblot. (C) Representative gels for pERK (upper panel) and ERK (lower panel) were shown. (D) Quantitative analysis of scanning densitometry for pERK/ERK was performed. All data were expressed as means \pm SE (n = 6). ** P < 0.01 as compared with control group (0 time point). ¶¶ P < 0.01 as compared with APAP group at the same time point.

activity. An early report found that PPAR- γ agonist down-regulated hepatic inflammatory cytokines and adhesion molecules during ischemia/reperfusion-induced acute liver injury (Akahori et al., 2007). A recent study showed that PPAR- γ agonist repressed hepatic inflammatory cytokines during cyclophosphamide-induced liver injury (El-Sheikh and Rifaai, 2014). The present study showed that hepatic NF- κ B, an important inflammatory signaling, was activated in the progression of APAP-induced acute liver injury. Moreover, hepatic ERK/MAPK, another inflammatory pathway, was activated during APAP-induced acute liver injury. According to an early study, the activated liver X receptor suppressed LPS-evoked NF- κ B activation in macrophages (Joseph et al., 2003). Additional study found that several agonists of pregnane X receptor suppressed TNF- α -activated NF- κ B signaling in human hepatocytes (Zhou et al., 2006). Recently, two reports

showed that VitD3-activated vitamin D receptor suppressed LPS-induced NF- κ B activation in placenta and kidney (Chen et al., 2015; Xu et al., 2015). PPAR- γ is a nuclear receptor and highly expressed in the liver (Boelsterli and Bedoucha, 2002). The present study showed that RSG pretreatment repressed activation of hepatic NF- κ B and ERK/MAPK in the progression of APAP-induced acute liver injury. Moreover, RSG pretreatment inhibited up-regulation of hepatic inflammatory cytokines and chemokines during APAP-induced acute liver injury. Thus, the present study does not exclude that RSG pretreatment protects against APAP-induced acute liver injury through PPAR- γ -mediated anti-inflammatory activity.

The present study investigated whether RSG by itself could alter APAP metabolism. As expected, RSG pretreatment did not influence hepatic CYP2E1, a key enzyme for APAP metabolism. These results suggest that RSG-mediated protection against APAP-

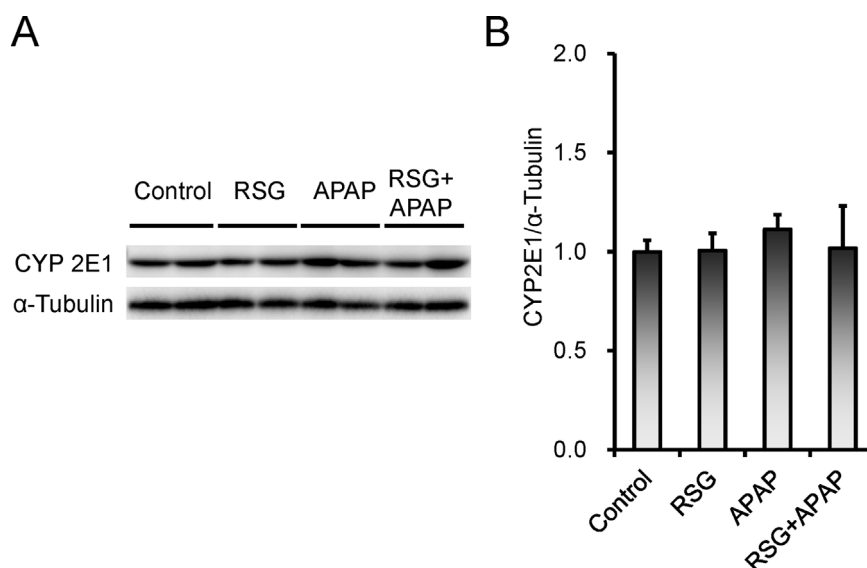


Fig. 6. Effects of RSG pretreatment on hepatic CYP2E1 expression at early stage of APAP-induced acute liver injury. Mice were i.p. injected with APAP (300 mg/kg). In RSG + APAP group, mice were administered with RSG (20 mg/kg) by gavage at 48, 24 and 1 h before APAP (300 mg/kg). Hepatic CYP2E1 was measured using immunoblot at 0.5 h after APAP. (A) Representative gels for CYP2E1 (upper panel) and α-tubulin (lower panel) were shown. (B) Quantitative analysis of scanning densitometry for CYP2E1 was performed. All data were expressed as means \pm SE (n = 6).

induced acute liver injury is not attributed to its inhibition of APAP metabolism. However, the present study has several limitations. First, the present study did not analyze the effects of RSG pretreatment on the reactive metabolite NAPQI. Second, the present study did not explore the mechanism of PPAR- γ -mediated down-regulation of hepatic NOX-2 and NOX-4 in the progression of APAP-induced acute liver injury. Third, the present study did not investigate the mechanism through which RSG inhibits APAP-evoked hepatic cell death. Thus, additional work is necessary to explore the mechanism by which RSG inhibits APAP-induced NADPH oxidases and hepatic cell death. In addition, antioxidant effect of RSG needs to be demonstrated in different models of acute liver injury.

In summary, the present study investigated the effects of pretreatment with RSG on APAP-induced acute liver injury. Our results showed that RSG pretreatment protected mice from APAP-induced acute liver injury. Although it had little effect on hepatic CYP2E1 expression, RSG pretreatment attenuated reduction of hepatic glutathione peroxidase (GSH-Px), glutathione reductase (GSH-Rd) and glutathione S-transferase (GST) activities, inhibited upregulation of hepatic nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX)-2 and NOX-4, and alleviated hepatic GSH depletion during APAP-induced acute liver injury. Our results provide evidence for roles of PPAR- γ as an important regulator of hepatic NADPH oxidases in the progression of APAP-induced acute liver injury. Thus, synthetic PPAR- γ agonists might be effective agents for preventing the progression of APAP-induced acute liver injury.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.toxlet.2016.11.012>.

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