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20(*R*)-ginsenoside Rg3, a rare saponin from red ginseng, ameliorates acetaminophen-induced hepatotoxicity by suppressing PI3K/AKT pathway-mediated inflammation and apoptosis

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ABSTRACT

Although ginsenoside Rg3 was isolated as a major component of Korea red ginseng and confirmed to exert potential hepatoprotective effect on acetaminophen (APAP)-induced liver injury via induction of glutathione S-transferase (GST) in vitro, the*in vivo* hepatoprotective effect of Rg3 and the underlying molecular mechanism of action remain unclear. The current study was aimed to explore whether 20(*R*)-Ginsenoside Rg3 (20(*R*)-Rg3) could alleviate acetaminophen-induced liver injury in mice and to determine the involvement of PI3K/AKT signaling pathway. Our findings demonstrated that a single injection of APAP (250 mg/kg) increased the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), tumor necrosis factor-α (TNF-α), and interleukin-1β (IL-1β); such increases were attenuated by pretreatment of mice with 20(*R*)-Rg3 for seven days. The depletion of glutathione (GSH), generation of malondialdehyde (MDA) and the over expression of cytochrome P450 E1 (CYP2E1) and 4-hydroxynonenal (4-HNE) caused by APAP exposure were also inhibited by 20(*R*)-Rg3 pretreatment. Moreover, 20(*R*)-Rg3 pretreatment significantly alleviated APAP-induced apoptosis, necrosis, and inflammatory infiltration in liver tissues. Importantly, 20(*R*)-Rg3 effectively attenuated APAP-induced liver injury in part via activating PI3K/AKT signaling pathway. In summary, 20(*R*)-Rg3 exerted liver protection against APAP-caused hepatotoxicity evidenced by inhibition of oxidative stress and inflammatory response, alleviation of hepatocellular necrosis and apoptosis via activation of PI3K/AKT signaling pathway, showing potential as a novel therapeutic agent to prevent liver damage.

1. Introduction

Acetaminophen (APAP) is a traditional non-steroidal antipyretic-analgesic drug, often implicated in drug induced liver injury(DILI), even acute liver failure [1]. The hepatotoxicity induced by overdose of APAP is initiated by generating the reactive intermediates from drug metabolism such as *N*-acetyl-*p*-benzo quinone imine (NAPQI). Under physiologic circumstances, a high dose of APAP causes liver injury through a toxic metabolic intermediate, NAPQI metabolized by cytochrome P450 enzyme system namely CYP2E1 and CYP3A4 isoenzymes, which is then conjugated to glutathione (GSH) and detoxified to mercapturic acid and eliminated [2]. However, overdoses of APAP rapidly deplete the hepatic GSH, and the remaining NAPQI results in hepatocellular necrosis with high mortality. In addition, when APAP-induced acute liver injury occurred, activated hepatic macrophages will release various pro-inflammatory cytokines, including TNF-α and IL-1β [3]. Activated AKT is a downstream effector of PI3K, which inhibits inflammation and apoptosis by regulating multiple target proteins such as NF-κB and Bcl-2 family [4]. PI3K/AKT signaling pathway is known to regulate the transcriptional activity of NF-κB pathway through phosphorylation and facilitation of I-κBα degradation [5,6]. In fact, previous studies have reported that PI3K/AKT signaling pathway affects early liver regeneration and is involved in the development of APAP-induced ALI [7,8]. Therefore, we hypothesize that PI3K/AKT signaling

Abbreviations: 20(*R*)-Rg3, 20(*R*)-ginsenoside Rg3; ALF, acute liver failure (ALF); AKT, protein kinase B; I-κB, inhibitor kappa B; ALT, alanine aminotransferase; TNF-α, tumor necrosis factor-α; GSH, glutathione; 4-HNE, 4-hydroxynonenal; APAP, acetaminophen; PI3K, phosphatidylinositol-3-kinase; NF-κB, nuclear factor-kappa B; IKK, inhibitor kappa B kinase; AST, aspartate aminotransferase; IL-1β, interleukin-1β; MDA, malondialdehyde; CYP2E1, cytochrome P450 E1

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pathways-mediated inflammation and apoptosis plays an important role in APAP-induced liver injury.

Ginsenoside Rg3 (G-Rg3), one of the most famous component isolated from red ginseng, is responsible for most of the pharmacological actions of ginseng, including immunomodulation, anti-cancer, and anti-oxidative activities [9]. Previous studies have reported that G-Rg3 significantly suppressed TNF-αexpression and NF-κB activation to inhibit inflammation response and oxidative stress through the activation of AKT [10,11]. A report by Gum et al. indicated that G-Rg3, isolated from Korean red ginseng as the major component, exerted a potential protective effect against APAP-induced liver injury in vitro [12]. G-Rg3 significantly increased the protein and mRNA expression levels of glutathione S-transferase A2 (GSTA2) in H4IIE cells, whereas ginsenoside Rc and Rg1 with the same concentration did not show these increases. Simultaneously, they also reported the ameliorative effects of G-Rg3 on NAPQI-induced hepatotoxicity in a rat model [13]. Although these reported results indicated that oxidative stress was involved in the NAPQI-induced liver injury, few studies have focused on the exact molecular mechanism through thatG-Rg3 improved APAP-induced liver toxicity in vivo. It's worthy to note that G-Rg3 exists in two epimers, 20(*S*)-ginsenoside *R*g3 (20(*S*)-*R*g3) and 20(*R*)-ginsenoside *R*g3 (20(*R*)-Rg3), in *Panax ginseng*. Interestingly, most of published literatures provided more emphasis on the pharmacological activities of 20(*S*)-*R*g3, whereas few attentions were paid to that of 20(R)-Rg3. In fact, 20(*R*)-Rg3 has been used clinically as an anticancer drug for many years in China. In recent years, 20(*R*)-Rg3 was reported to bemore powerful in anti-tumor, anti-oxidation, and promoting immune response [14,15]. The chemical structure of 20 (R)-Rg3 is shown in Fig. 1.

Here in this study, the effects and possible mechanisms of 20(*R*)-Rg3 action against APAP-induced liver injury were investigated in mouse.

2. Materials and methods

2.1. Chemical compounds and reagents

20(*R*)-Rg3with purity of 97.5% was isolated from black ginseng as described in our previous work [16]. APAP was purchased from Aladdin Biotech Co., Ltd. (Shanghai, China). Hematoxylin and eosin (H &E) dyes, ALT, AST, MDA, and GSH assay kits were purchased from Nanjing Jiancheng Institute of Biotechnology (Nanjing China). TNF-α and IL-1β ELISA kits were purchased from the R&D company (Minneapolis, MN,USA). Hoechst 33,258 dye kit was bought from Beyotime Biotechnology Co., Ltd. (Shanghai, China). TUNEL apoptosis detection kits were bought from Roche Applied Science (Shanghai, China). DyLight 488-SABC immunofluorescence staining kit was purchased in BOSTER Biological Technology Co., Ltd. (Wuhan China). The rabbit monoclonal anti-mouse CYP2E1, 4-HNE PI3K, p-PI3K, AKT, p-AKT, NF-κB p65, p-NF-κB p65, Bax, Bcl-2, GAPDH, IKKα, p-IKKα, IKKβ, p-IKKβ, I-κBα, and p-I-κBα/β antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA). The remaining reagents were analytical pure, and provided by Beijing Chemical Works (Beijing, China).

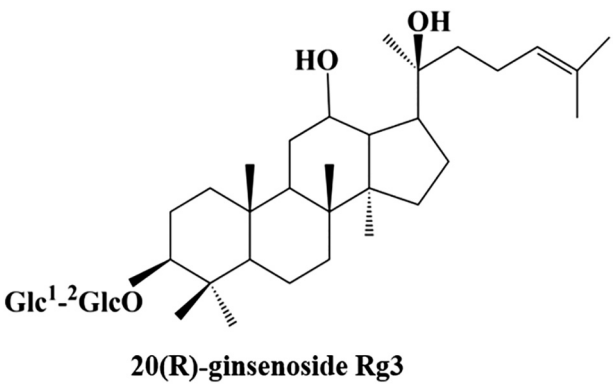

Fig. 1. Chemical structures of 20(*R*)-ginsenoside Rg3.

2.2. Animals and treatment

Male ICR mice (weighting 20–25 g) were purchased from Changchun YISI Experimental Animal Co., Ltd., with Certificate No.: SCXK (JI) 2016-0003. The mice were supplied with standard laboratory diet and water ad libitum at a temperature 25 ± 2 °C with a 12-h light/dark cycle (lights on 6:00 AM to 6:00 PM) and 60 ± 10% humidity. All mice were adapted to the environment at least one week before formal experiment. All experimental procedures were strictly in accordance with the Regulations of Experimental Animal Administration issued by the Ethical Committee for Laboratory Animals at Jilin Agricultural University (Permit No. ECLA-JLAU-17065).

The mice were randomly divided into 4 groups: (1) Normal control group treated with CMC-Na, (2) APAP-treated group (250 mg/kg), (3) APAP/20(*R*)-Rg3-treated group (250/10 mg/kg), (4) APAP/20(*R*)-Rg3-treated group (250/20 mg/kg). Mice were pretreated by gavages with 20(*R*)-Rg3 (10,20 mg/kg per day) for 7 days continuously. On the seventh day, mice were injected with a single dose of APAP (250 mg/kg) after pretreatment of 20(R)-Rg3 for 1 h. After 24 h, all mice were killed immediately and dissected by cervical dislocation, and blood was collected and centrifuged at 3000*g* for 10 min. Serum was taken from the supernatant and stored at −80 °C until analysis. In the meantime, spleen and liver tissues were collected and weighted to calculate the organ indices. Then, part of the liver samples were fixed in 10% formalin solution (m/v) for at least 24 h, and embedded in paraffin for tissue sections, the remaining tissue was rapidly frozen in liquid nitrogen and stored at −80 °C for preparation of homogenate.

2.3. Biochemical marker assay

The activities of AST and ALT in serum and the hepatic levels of GSH and MDA in liver tissues were quantified by commercial available kits as mentioned above (Nanjing Jiancheng Bioengineering Institute, Nanjing China) according to the manufactures' protocols.

The contents of TNF-α and IL-1β in serum were determined using ELISA assay kits according to the manufactures' protocols (R&D, Minneapolis, MN, USA). The absorbance was measured at 450 nm in an ELISA reader (Bio-Rad, California, USA).

2.4. H&E, TUNEL and Hoechst 33,258 staining

For evaluating changes of the liver tissues including central vein congestion, inflammatory cell infiltration, hepatocyte necrosis, and cell apoptosis, the fresh hepatic samples were immersed in 10% formaldehyde for over 24 h. Then liver tissues were paraffin-embedded and sectioned in 5 μm thickness on a Leica Rotary Microtome. Liver sections were stained with H&E for tissue morphology using light microscope (Leica, DM750, Solms, Germany). TUNEL staining was performed with TUNEL apoptosis detection kits from Roche Applied Science (Shanghai, China). The 5 μm-thickness sections were stained using the Hoechst 33,258 solution with 10 μg/mL as described previous [17]. Image-Pro plus 6.0 software (Media Cybernetics, Maryland, and USA) was applied to evaluate the degree of liver apoptosis through quantifying the fragmented and condensed staining.

2.5. Immunofluorescence staining

Immunofluorescence staining was carried out as previously described with minor modifications [17]. Immunofluorescence analysis was executed on liver tissues, to evaluate the levels of protein expression of CYP2E1 and 4-HNE in APAP-induced hepatotoxicity. Specifically, the 5-μm-thick paraffin sections were fixed with a series of xylene and aqueous alcohol solutions for deparaffinizing and rehydrating.