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Hepatoprotective effects exerted by *Poria Cocos* polysaccharides against acetaminophen-induced liver injury in mice

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ABSTRACT

Our study was to investigate the potential pharmacological activity of *Poria Cocos* polysaccharides (PCP) against acetaminophen (APAP)-induced liver injury in mice. PCP-dosed mice were used to conducting biochemical assays of serological liver enzyme (ALT), lactate dehydrogenase (LD), inflammatory cytokines (TNF-α, IL-6), and immunoassays for functional proteins in the livers. Consequently, APAP-exposed mice resulted in elevated levels of ALT, LD, TNF-α, IL-6 in sera. Interestingly, PCP-dosed mice exhibited reduced ALT, LD and inflammatory cytokines in blood. Inflammatory infiltration and cell death in liver tissue were decreased following by PCP treatments. Furthermore, immunofluorescence staining showed that AKR7A, c-Jun, Bcl-2-positive cells were increased in PCP-dosed livers in mice, while Bax-labeled cells were decreased. In addition, hepatocellular down-regulated NF-κBp65, IkBα expressions were observed dose-dependently in PCP-dosed livers in mice. Taken together, the current findings indicate that *Poria Cocos* polysaccharides exert pharmacological bioeffects against APAP-induced liver injury in mice, and the underlying molecular mechanism is associated to suppressing inflammatory response and apoptosis in liver cells.

1. Introduction

Chemical-induced liver damage is linked to hepatic toxicity, in which drug-induced liver injury (DILI) is a common cause of acute and chronic liver injuries [1]. More and more manufactured medications have been reported to induce DILI, and it is the major reason for medication to be withdrawn from the market [2]. In addition, DILI accounts for 5% of all hospital admissions and 50% of all acute liver injuries [3]. Acetaminophen (APAP) is a drug for managing pain and fever, however, overdose treatment is the major reason of drug-induced acute liver failure and liver disease worldwide [4]. Damaging to liver cells is induced by APAP-based toxic metabolite NAPQI (*N*-acetyl-*p*-benzoquinone imine) produced by cytochrome P-450 enzymes in liver cells [5]. Scientific evidences for APAP-dosed treatment may manifest a potential liver healthy risk [6]. In addition, our previous studies suggest that APAP-induced the liver cytotoxicity is related to molecular mechanisms of apoptosis and inflammatory stress, and the adverse effect of perinatal exposure to APAP on hepatic glucose metabolism in offspring mice is linked to disturbance of insulin-dependent AKT signaling pathway [7,8]. Taken together, suppression of APAP-induced DILI may improve drug safety and also relieve stresses for patients. Therefore, developing alternative strategy to reduce APAP-associated DILI warrants to be urgent currently.

Poria cocos is a polyporaceae fungus that is used widely as a medicinal mushroom in Chinese medicine. Indications for use in the traditional Chinese medicine include promoting urination and invigorating the spleen functions [9,10]. Botanical extracts of *Poria Cocos* polysaccharides (PCP) have been prescribed in clinical adjuvant therapy for cancers in China, benefiting from efficacious immunoregulation [11]. Due to its promising pharmacological activities, we extrapolated that PCP might possess effective benefits against APAP-induced DILI. In this study, APAP-induced DILI in mice was employed to assess hepatoprotective effects of PCP through a series of biological and chemical assays. And underlying molecular mechanisms would be discussed, respectively.

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2. Materials and methods

2.1. Imperative materials

Over-the-counter APAP were purchased from Sinopharm Group Guangdong Med-World Pharmaceutical Co. Ltd. (Foshan, China). In brief, PCP was provided by Hunan Butian Pharmaceutical Co., Ltd. (Batch No. 20170103), and additional experimental materials chemicals were indicated as follows.

2.2. Animal experiments

Male Kunming mice (6–7 weeks, 20 ± 2 g) were provided by the Experimental Animal Centre at Guilin Medical University (Guilin, China). All mice were housed in animal room with the designated conditions of temperature of 25 ± 2 °C, relative humidity of 60 ± 10%, and 12 h light/dark cycle. All mice were allowed to access to filtered water and rodent fodder ad libitum. This study was approved by the Institutional Animal Care and Use Committee at Guilin Medical University.

As depicted in our previous study [12], APAP-induced DILI in mice was treated with 300 mg/kg (drug/body weight) APAP by oral gavage every 3 days within 14 days. In addition, the APAP-exposed mice were randomly assigned to 3 groups (n = 10). Mice in APAP group were set as model control. Mice in PCP-dosed groups (200 mg/kg, 400 mg/kg) were treated with PCP daily for 14 days. Mice in APAP-free group were intragastrically given with the same volume of normal saline for 14 days.

2.3. Sample handlings

At experimental endpoint, all mice were sacrificed by cervical dislocation before blood collection. Sera were prepared from blood with centrifugation of 3500 rpm at 4 °C for 15 min. Liver samples were removed instantly on ice, and parts of the livers were fixed by 4% paraformaldehyde for histopathological stains and the others were stored at −80 °C for immunoblotting tests.

2.4. Serological determinations

Serous contents of ALT, LD were calculated using biochemical testing kits (Nanjing Jiancheng Bioengineering Institute, China), following by booklet' procedures. And circulating levels of cytokines (TNF-α and IL-6) were measured using the commercial ELISA kits (Shanghai Elisa Biotech Co., Ltd., China), following by manual' procedures [13].

2.5. Morphological inspections

Paraffin-embedded liver sections were stained with hematoxylin and eosin (HE) and DAPI dye, and histopathological changes were screened and imaged under an optical microscope (BX53F, Olympus, Japan). The conditions of hepatocellular inflammatory infiltration and cell death were assessed from different viewing fields in sections.

Immunohistochemically, dewaxed liver sections were blocked with 5% bovine serum albumin (BSA) at 37 °C for 1 h, followed by incubation of diluted primary NF-κBp65, IkBα antibodies (1:50; Wuhan Boster, China; Santa Cruz, USA). Subsequently, the sections were incubated with manufacture-based SABC solutions (Wuhan Boster, China) before staining with diaminobenzidine dye and counterstaining with hematoxylin. Finally, the sections were mounted and imaged using an optical microscope (BX53F, Olympus, Japan) [14,15].

2.6. Immunofluorescence staining protocols

In brief, dewaxed liver sections were blocked with 5% BSA buffer at 37 °C for 1 h, followed by incubated with primary antibodies of AKR7A, Bcl-2, Bax, c-Jun (1:50, Beyotime Biotechnology, China) for overnight at 4 °C. The liver sections were co-incubated with related IgG H&L Alexa Fluor® 488 and 536 (1:100, Abcam, USA) for 2 h at 4 °C. And the DAPI buffer (Abcam, USA) was specifically stained for cell nucleus before further imaging and data analysis [16].

2.7. Western blot analysis

The total proteins of mouse liver were extracted with lysis buffer/protease inhibitor (1 mM, Beyotime Biotechnology, China) prior to protein contents being measured. The 20 μg protein from each sample was electrophoresed by 10% SDS-PAGE gel, followed by blotting to PVDF membrane. The membrane was blocked with non-fat milk solution (Yili Dairy Industry, China) before incubating with primary antibodies of NF-κBp65, IkBα (1:1000, Wuhan Boster, China; Santa Cruz, USA) overnight at 4 °C. The PVDF membrane was further incubated with horseradish peroxidase-conjugated secondary antibodies (1:4000, Beyotime Biotechnology, China) for 1 h at 37 °C. The optical band were imaged and analyzed by respective Molecular Imager and Image Lab™ Software (Bio-Rad, MD, USA) [17].

2.8. Statistical analysis

All data were analyzed using GraphPad Prism 7.0 software. Differences between two groups were compared through the analysis of variance (ANOVA) followed by a *Student's t-test* for two comparisons. The final data were denoted as the mean ± standard deviation (SD). The comparable readings of <0.05 were considered significantly.

3. Results

3.1. Biological benefits of PCP on APAP-exposed mice

As shown in body and liver weights, APAP-exposed mice resulted in reduced weight loss in body and increased liver weight when compared to these in APAP-free mice ($P < 0.05$). APAP-exposed mice with PCP cotreatment contributed to dose-dependent elevation of body mass in mice ($P < 0.05$). In serological tests, APAP-exposed mice showed increased levels of ALT, LD in comparison with nontreated controls ($P < 0.05$). Interestingly, PCP-cotreated mice contributed to reduction of these enzymes contents in sera dose-dependently ($P < 0.05$) (Fig. 1).

3.2. PCP reduced inflammatory cytokines in APAP-exposed mice

Notably, increased contents of TNF-α, IL-6 in sera were detected in APAP-exposed mice, in which abnormal levels showed greater than those in APAP-free mice ($P < 0.05$). Following by PCP cotreatment, elevated inflammatory cytokines of TNF-α, IL-6 were inhibited dose-dependently. The reduced concentrations showed significantly when compared to these in APAP controls ($P < 0.05$) (Fig. 2).

3.3. PCP suppressed hepatocellular histopathological changes induced by APAP

To characterize the cytohistological changes of liver cells in APAP-exposed mice, routine stains were conducted. Observations from HE staining exhibited that APAP-free mice had organized cell distribution, intact cytoskeleton and non-inflammatory impairment, accompanied with plenty cell counts as revealed in DAPI staining. In contrast, APAP-exposed livers showed disordered cell organization, impaired cytoskeleton, necrosis, and resultant inflammatory infiltration. Reduced cell numbers were observed using DAPI staining. Interestingly, PCP-cotreated mice contributed to inhibition of APAP-induced hepatotoxicity, characterized by reduced inflammatory/necrotic cells, improved morphology/cytoskeleton in liver cells, increased cell counts (Fig. 3).