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Ameliorative effectiveness of allicin on acetaminophen-induced acute liver damage in mice

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ABSTRACT

Acetaminophen (APAP) is commonly prescribed for relieving pain and fever symptoms. However, the clinical use of APAP is accompanied with the side-effect of hepatotoxicity. In this study, we aimed to investigate immediate benefit role of allicin on APAP-induced acute liver damage in mice. The freshly-prepared APAP solution (300 mg/kg, bw) was intragastrically given to mice. The current findings showed that co-treatment of allicin effectively inhibited APAP-induced hepatotoxic effect, as revealed in attenuated hepatocellular pathological impairments and normalized serum liver enzymes (ALT and AST) and inflammatory molecules (TNF-α and IL-6). In addition, the immunoreactive cells of NF-κB-p52 in the liver were reduced following allicin treatment. Furthermore, the intrahepatic mRNAs of NF-κB, TLR4 and proteins of Ikb-α, p-p65 were downregulated. Overall, these preclinical observations elucidate that allicin exerts hepatoprotective effects against APAP-induced hepatic cytotoxicity, possibly through the molecular mechanism of blocking inflammatory stress associated with intrahepatic TLR4/NF-κB pathway.

1. Introduction

As a broad-spectrum of pain reliever or fever reducer, acetaminophen (APAP) is prescribed worldwide (Amitai, 2014). However, overdoses of APAP intake can lead to life-threatening liver damage, even fulminant liver failure. It can be considered as a hazardous risk when the side effect occurs (Ju, 2012). Hepatotoxicity induced by APAP is attributed to the frequent cause of acute liver damage both in the USA and the UK (Lancaster, Hiatt, & Zarrinpar, 2015). Toxicologically, APAP is metabolized primarily by the hepatocytes, in which in turn the metabolites are cytotoxicity (Bunchorntavakul & Reddy, 2013). As one of intermediate byproducts, APAP-generated N-acetyl-p-benzoquinonimine (NAPQI) is toxic, which leads to severe damage to the liver cells (Jan et al., 2014). Acute liver damage represents a pathological condition characterized by hepatocellular inflammatory infiltration (Rolando et al., 2000). However, more details regarding the molecular mechanism of APAP-induced hepatotoxicity has not been completely elucidated.

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Allicin, an organosulphur substance isolated from garlic, possesses wide health benefits, including antibacterial, antifungal, antiprotozoal, and antiviral properties as revealed in basic and clinical researches (Khodavandi, Alizadeh, Aala, Sekawi, & Chong, 2010; Salama et al., 2014; Wu et al., 2015a; Wu, Santos, & Fink-Gremmels, 2015b). Some studies show that allicin has anti-inflammatory and anti-thrombotic activities, and serves as an alternative antioxidant (Ariga & Seki, 2006; Chung, 2006; Li et al., 2015a, 2015b). Based on the advantages conferred by allicin, we propose a possible hypothesis that allicin may retard the APAP-induced acute liver damage via attenuating intrahepatic inflammatory stress. Thus, we implemented relevant experiments that aimed to evaluate the potential benefits of allicin against APAP-induced hepatic injury. Further, the molecular mechanisms warranted to be discussed.

2. Materials and methods

2.1. Reagents used

Allicin (purity >95.0%) was obtained from Ye Yuan Biotechnology Co., Ltd. (Shanghai, China). Acetaminophen (APAP, purity >99.0%) was purchased from Yuanye Biotechnology Co., Ltd. (Shanghai, China). Other materials and equipment will be described in experimental sections.

2.2. Laboratory animals

Healthy male Kun Ming (KM) mice, about 8-week-old with 25 ± 2 g, were provided by the Experimental Animal Centre of Guangxi Medical University (Nanning, China). Experimental procedures were performed in accordance with the protocols authorized by *Institutional Ethical Committee* of Guilin Medical University. Laboratory mice were acclimatized for 7 days ahead of experiments. All mice were maintained and handled throughout the designated conditions with temperature of 22 ± 1 °C, relative humidity of 50 ± 2%, and a 12L:12D cycles. The animals were fed with standard rodent chow (Keao XIeli Food Co. Ltd.; Beijing, China) and water *ad libitum*.

Acute liver damage mice were established following previous descriptions (Tsukada & Suematsu, 2012). In brief, mice were intragastrically administered with 300 mg/kg APAP solution dissolved in pre-heated saline. After APAP intake for 1.5 h, mice were intraperitoneally injected with allicin doses (15, 30 mg/kg, bw) solubilized in 0.5% Tween-80 emulsifier. In parallel, the mice in vehicle and lesioned controls received 0.5% Tween-80 emulsifier alone. Six mice were assigned in each group. After 24 hours, mice were sacrificed via anaesthesia with 10% chloral hydrate (0.04 ml/10 g) on the designated dates. Blood samples were harvested through cardiocentesis, and livers were isolated until further assay.

2.3. Serum enzymes analysis

Concentrations of serum enzymes reflected by ALT, AST were determined using the commercial reagents (Nanjing Jiancheng Bioengineering Institute, China) according to the manufacturer's manual.

2.4. Serum cytokines assay

Serum levels of inflammatory molecules, such as TNF-α, IL-6, were measured by the enzyme-linked immunosorbent assay (ELISA) kits (Cusabio, Wuhan, China) according to the user's guide.

2.5. Histopathological inspections

Paraformaldehyde (PFA), 4%-fixed paraffin-embedded liver samples were prepared as 5 μm slices for use. Briefly, liver sections were subjected to haematoxylin and eosin (H&E) stain. The samples were then imaged for further pathological assessment by a pathologist. Liver-injured condition was screened and analyzed, in which the area of necrosis/inflammation was evaluated and calculated as previously described (Su, Chen, Wei, Chen, & Wu, 2014; Wu et al., 2015a, 2015b).

In addition, the experimental steps of electron microscope associated with hepatic ultrastructure were described according to a previous study (Wu et al., 2013). This experiment aimed to investigate the pathophysiological changes followed by APAP-toxicity and the potential benefits of allicin administration, respectively.

2.6. RNA extraction and real-time PCR

Hepatocellular total RNA was isolated by the commercial Trizol reagent (Life Technologies, Carlsbad, CA, USA). When the purity was being examined, the fresh RNA was used for synthesis of first-strand complementary DNA (cDNA) by a biochemical kit (Life Technologies). PT-PCR procedures were conducted via a 7500 Fast Real-Time System (Applied Biosystems, Foster City, CA, USA). Each primer sequence (forward or reverse) was showed below: NF-κB forward primer, 5′ GAA CGA TAA CCT TTG CAG GC 3′, antisense primer: 5′ TTT CGA TTC CGC TAT GTG TG 3′ (130 bp); TLR4 forward primer, 5′ CAT GGA TCA GAA ACT CAG CAA AGT C 3′, antisense primer: 5′ CAT GCC ATG CCT TGT CTT CA 3′ (179 bp); actin sense primer: 5′ TGT GTC CGT CGT GGA TCT GA 3′, antisense primer: 5′ TTG CTG TTG AAG TCG CAG GAG 3′ (150 bp). Briefly, real-time PCR program was implemented with 30 cycles, including 94 °C for 10 min, annealing at 56 °C for 30 s, elongation at 72 °C for 60 s, extension at 72 °C for 10 min. In addition, final data regarding relative expression of each gene were yielded through numerical calculation to the mouse actin and normalized to the control using the 7500 Sequence Detection System Software (Applied Biosystems).

2.7. Immunohistochemical stains

Overall, these experimental protocols were performed according to the manufacturer's manual (Boster, Wuhan, China) and previous descriptions (Li et al., 2015a, 2015b). The liver sections were dewaxed and rehydrated via stepwise concentrations of ethanol and xylene, respectively. After blocking with 5% bovine serum albumin (BSA) for 1 h, the slices were exposed to primary rabbit–anti-mouse NF-κB-p52 antibody (1:400; overnight; Boster, Wuhan, China) prior to incubation with anti-rabbit secondary antibody (1:1000; 37 °C, 1 h; Boster, Wuhan, China) using a horseradish peroxidase (HRP) conjugated compact polymer system. Diaminobenzidine (DAB) was used