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The therapeutic detoxification of chlorogenic acid against acetaminophen-induced liver injury by ameliorating hepatic inflammation

Zhiyong Zheng [a], Yuchen Sheng [b], Bing Lu [a], Lili Ji [a,*]

[a] The Shanghai Key Laboratory of Complex Prescription, The MOE Key Laboratory for Standardization of Chinese Medicines, and The SATCM Key Laboratory for New Resources and Quality Evaluation of Chinese Medicines, Institute of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China
[b] Center for Drug Safety Evaluation and Research, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China

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ABSTRACT

Chlorogenic acid (CGA) has been reported to prevent acetaminophen (AP)-induced hepatotoxicity when mice were pre-administered orally with CGA for consecutive 7 days before AP intoxication in our previous study. This study investigated the therapeutic detoxification of CGA against AP-induced hepatotoxicity and the engaged mechanism. The mice were orally administered with CGA (10, 20, 40 mg/kg) at 1 h after given AP (400 mg/kg), and another 3 h later the mice were killed for the following experiments. Results of serum transaminases analysis and histological evaluation demonstrated the detoxification of CGA against AP-induced hepatotoxicity. CGA reduced AP-induced the increased myeloperoxidase (MPO) enzymatic activity and its expression. CGA reduced AP-induced the increased liver expression of toll-like receptor (TLR)-3/4 and MyD88, and the increased phosphorylation of inhibitor of kappa B (IκB) and p65 subunit of nuclear factor κB (NFκB). CGA reduced AP-induced the increased NFκBp65 expression in nucleus. In addition, CGA reduced AP-induced the increased serum levels and liver mRNA expression of tumor necrosis factor alpha (TNFα), interleukin (IL)-1β, IL-6, monocyte chemoattractant protein-1 (MCP-1), and keratinocyte chemoattractant (KC). Taken together, our results demonstrate the therapeutic detoxification of CGA against AP-induced liver injury, and TLR3/4 and NFκB signaling pathway are involved in such process.

1. Introduction

Chlorogenic acid (CGA, 5-caffeoylquinic acid) is a well-known dietary polyphenolic compound, and it is widely distributed in plant materials such as coffee, tea, and a variety of plant foods (including apples, peach, carrot, blueberry, tomato, oilseeds, eggplant, prunes, cherries, etc.) [1]. Coffee is the major source of CGA intake in humans, and the amount of CGA in raw coffee is 4–12% [2,3]. In addition, CGA can be absorbed in the small intestine of humans with a relatively high absorption rate (33%) [4]. CGA has a variety of biological functions including antioxidant, anti-inflammatory, and antibacterial properties [5–7], and its consumption is also reported to be helpful for reducing blood pressure [8], regulating glucose and lipids metabolism [9], inhibiting cancer invasion and metastasis [10], and improving major depression [11].

Drug-induced liver injury (DILI) is a major clinical issue, and of which the hepatotoxicity induced by acetaminophen (AP) due to its overdose is especially common. AP, also named paracetamol, has been extensively used in the world as the antipyretic and analgesic drug since 1955, and it is available in various formulations (including tables, capsules, suspensions, etc.). As a common over-the-counter (OTC) drug, AP is easy to be obtained from market, so the phenomena of AP overdose often takes place, which will cause serious acute liver injury. A perspective on the epidemiology of AP exposure and intoxication in the United States showed that approximately 6% of adults are prescribed overdose AP and about 30,000 patients are hospitalized for AP-induced hepatotoxicity every year [12]. AP has been the leading worldwide cause of drug-induced acute liver failure (ALF), and liver transplantation should be offered to patients under some serious conditions,

* Corresponding author at: The Shanghai Key Laboratory of Complex Prescription, The MOE Key Laboratory for Standardization of Chinese Medicines, and The SATCM Key Laboratory for New Resources and Quality Evaluation of Chinese Medicines, Institute of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine, 1200 Cailun Road, Shanghai 201203, China. Tel.: +86 21 51322517; fax: +86 21 51322505.
E-mail address: lichenyue1307@126.com (L. Ji).

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otherwise AP-induced acute liver failure will cause patients to death [13].

In the previous studies, CGA has been reported to prevent liver injuries induced by tetrachlorobenzoquinone, griseofulvin, ischemia/reperfusion, and lipopolysaccharide [14–17]. In addition, our previous study has showed that CGA prevented AP-induced liver injury in mice when it was orally given for consecutive 7 days before AP intoxication [18]. In general, AP-induced ALF due to its overdose is unpredictable, so the therapeutic detoxification of AP-induced liver injury is more meaningful than preventive detoxification. The present study is designed to observe the therapeutic detoxification of CGA against AP-induced liver injury when it was orally given at 1 h after AP intoxication in mice. In addition, this study further investigated the mechanism engaged in the therapeutic detoxification of CGA against AP-induced liver injury.

2. Materials and methods

2.1. Chemical compounds and reagents

CGA (5-caffeoylquinic acid, CAS-327-97-9), a white powder with 98% purity, was purchased from Shanghai Hitsanns Co., Ltd (Shanghai, China). AP was purchased from Sigma Chemical Co. (St. Louis, MO). Kits for analysis of alanine/aspartate aminotransferase (ALT/AST) and myeloperoxidase (MPO) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). MPO antibody was obtained from Abcam (Cambridge, UK). The DAKO EnVision™ detection system was purchased from DAKO Corporation (Carpinteria, CA). NFκBp65, IκB, phospho-NFκBp65, phospho-IκB, β-actin, MyD88, TLR9 and Histone H3 antibodies were all purchased from Cell Signaling Technology (Danvers, MA). Antibodies for TLR2, TLR3, TLR4 and TLR6 were all purchased from Biobasic Inc (Shanghai, China). Peroxidase-conjugated goat anti-Rabbit IgG (H + L) and peroxidase-conjugated goat anti-Mouse IgG (H + L) were purchased from Jackson ImmunoResearch (West Grove, PA). NE-PER® nuclear and cytoplasmic extraction reagents, and Pierce® BCA Protein Assay Kit were purchased from ThermoFisher Scientific (Waltham, MA). Enzyme-linked immunosorbent assay (ELISA) kits were purchased from RapidBio (West Hills, CA). Trizol reagent was purchased from Life Technology (Carlsbad, CA). PrimeScript® RT Master Mix and SYBR® Premix Ex Taq™ were purchased from Takara (Shiga, Japan). All of the other reagents were purchased from Sigma (St. Louis, MO), unless otherwise indicated.

2.2. Experimental animals

Specific pathogen free male ICR mice (16–20 g body weight) were purchased from Shanghai Laboratory Animal Center of Chinese Academy of Science (Shanghai, China). The animal room was maintained at a temperature of 22 ± 1 °C with a 12 h light–dark cycle (6:00–18:00) and 65 ± 5% humidity. The mice were fed with a standard laboratory diet and given free access to tap water. All mice were received humane care in compliance with the institutional animal care guidelines approved by the Experimental Animal Ethical Committee of Shanghai University of Traditional Chinese Medicine.

2.3. Treatment of animals

Mice were divided into 5 groups as follows: (1) vehicle control, (2) AP (400 mg/kg), (3) AP (400 mg/kg) + CGA (10 mg/kg), (4) AP (400 mg/kg) + CGA (20 mg/kg), (5) AP (400 mg/kg) + CGA (40 mg/kg). Each group contains 8 mice. Mice were administered orally with AP (400 mg/kg) or vehicle for once. Mice were separately given a single dose of CGA (10, 20 or 40 mg/kg, i.g.) at 1 h after AP administration. Mice were killed 4 h after AP administration, and plasma and livers were collected.

2.4. Serum biochemical analysis

The blood samples obtained were kept at room temperature for 2 h. Serum was then collected after centrifugation at 840*g* for 15 min. Serum ALT and AST were measured with kits according to the manufacturer's instructions.

2.5. Histological observation

Liver pieces were fixed in 10% PBS–formalin and embedded in paraffin for histological assessment of tissue damage. Samples were sectioned (5 μM), stained with haematoxylin–eosin (H&E), and observed under a light microscope (Olympus, Japan) for evaluating the liver damage.

2.6. MPO analysis

Liver MPO enzymatic activity was determined according to the manufacturer's instruction. Protein concentration was analyzed by BCA kit and MPO activity was expressed as units/g protein.

2.7. Immunohistochemical analysis

Paraffin-embedded liver sections (5 μm thickness) were firstly deparaffinized in xylene, and rehydrated in an ethanol gradient with distilled water. After endogenous peroxidase activity was quenched, liver sections were incubated with 5% bovine serum albumin to diminish nonspecific binding. After that, liver sections were incubated with MPO antibody at 4 °C overnight, and further detected using DAKO EnVision™ detection kits. All sections were counterstained with haematoxylin. The images were taken using an inverted microscope under the magnification of 200×. The MPO expression was determined by image information object definition (IOD) values analyzed by Image-Pro Plus 6.0 (Media Cybernetics, Silver Spring, MD).

2.8. Nuclear and cytoplasm protein extraction

Liver cytosolic and nuclear proteins were isolated as described in NE-PER® nuclear and cytoplasmic extraction kits. Protein concentration of cytosolic and nuclear samples was normalized to equal amount of protein of each sample.

2.9. Western-blot analysis

Liver sections were homogenized in ice-cold lysis buffer containing 50 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA, 20 mM NaF, 0.5% NP-40, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 10 μg/ml pepstatin A. Liver homogenates were centrifuged at 3000*g* for 10 min before the supernatant was transferred to new tubes. Protein concentrations in the supernatants were assayed by BCA Protein Assay Kit and every sample was normalized to the equal protein concentration. The samples were subjected to SDS–PAGE and then electrophoretically transferred onto a Immobilon®-P PVDF membranes (Millipore). The membranes were incubated with primary and secondary antibodies. Immunoblots were visualized using a chemiluminescent reagent. The gray densities of the protein bands were normalized using β-actin or Histone H3 density as an internal control, respectively.

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