International Immunopharmacology 44 (2017) 97–104

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Chlorogenic acid ameliorated concanavalin A-induced hepatitis by suppression of Toll-like receptor 4 signaling in mice

Yinglin Yuan [a,1], Xia Gong [b,1], Li Zhang [c], Rong Jiang [c], Junxia Yang [a], Bin Wang [d], Jingyuan Wan [a,*]

[a] Chongqing Key Laboratory of Biochemistry and Molecular Pharmacology, Chongqing Medical University, Chongqing 400016, China
[b] Department of Anatomy, Chongqing Medical University, Chongqing 400016, China
[c] Laboratory of Stem Cell and Tissue Engineering, Chongqing Medical University, Chongqing 400016, China
[d] Department of Anesthesiology, The First Affiliated Hospital of Chongqing Medical University, Chongqing 400016, China

ARTICLE INFO

Article history:
Received 19 June 2016
Received in revised form 25 December 2016
Accepted 10 January 2017
Available online 13 January 2017

Keywords:
Chlorogenic acid
Concanavalin A
Hepatitis
Adhesion molecules
Leukocytes
Toll-like receptor 4

ABSTRACT

Chlorogenic acid (CGA), one of the most abundant dietary polyphenolic compounds, has been reported to exhibit anti-inflammatory ability. However, the hepatoprotective effects and molecular mechanisms of CGA on concanavalin A (Con A)-induced hepatitis have not been explored. In the present study, we found that pretreatment with CGA dose-dependently inhibited the elevation of plasma aminotransferases and alleviated hepatic pathological damage as well as hepatocyte apoptosis in Con A-exposed mice. Additionally, CGA markedly suppressed the production of serum tumor necrosis factor (TNF)-α and interferon (IFN)-γ, alleviated the infiltration of hepatic macrophages, neutrophils, and activated $CD4^+$ T lymphocytes in Con A-primed mice. Moreover, CGA downregulated Con A-induced hepatic expression of adhesion molecules (ICAM-1, VCAM-1 and ELAM-1) mRNA and protein, and inhibited Con A-activated Toll-like receptor (TLR) 4 signal molecules including TLR4, p-IRAK1, p-IκB, and p-p38. Finally, our results also showed that CGA exhibited a therapeutic effect, which CGA posttreatment improved hepatic damage at 1, 3, and 6 h after Con A. Taken together, these data suggested that CGA could effectively prevent mice from Con A-induced hepatitis, which might be through suppressing the activation of TLR4 signaling, downregulating the expression of adhesion molecules, and alleviating the infiltration and activation of hepatic leukocytes and the production of pro-inflammatory cytokines.

1. Introduction

Acute hepatitis including viral hepatitis and autoimmune hepatitis represent major threats to human health worldwide. T cell-mediated immune responses play an important role in the development and progression of these diseases [1–3]. Concanavalin A (Con A)-induced acute liver injury and systemic immune activation in mice is a well characterized model of T cell-mediated hepatitis with pathological properties similar to immune-mediated hepatitis in humans [4,5]. This model has been widely used to study the pathogenesis and preclinical treatment of immunological hepatitis [6,7]. The hepatic infiltration and activation of leukocytes such as macrophages, neutrophils, and $CD4^+$ T cells, secrete amount of cytokines, such as tumor necrosis factor (TNF)-α, interferon (IFN)-γ, to mediate hepatocyte apoptosis and necrosis, leading to acute liver injury [8,9]. Previous studies indicated that toll-like receptor (TLR) 4 plays a critical role in the therapeutic target in many inflammation-related diseases [10,11]. Furthermore, several studies found that TLR4 signal pathway was involved in the pathogenesis of Con A-induced liver damage [12,13].

Chlorogenic acid (CGA, Fig. 1A) is one of the most abundant polyphenol compounds found in many natural foods including coffee, fruits and vegetables [14]. Previous studies have shown that CGA exhibits a broad range of pharmacological effects such as anti-inflammatory [15], anti-oxidant [16], anti-bacterial [17] and anti-carcinogenic [18] activities. Furthermore, CGA has been proven to be effective in the protection of many liver diseases in various models, such as carbon tetrachloride (CCl_4)-induced liver fibrosis [16], acetaminophen-induced liver injury [15], and D-galactose-induced liver injury [19]. In addition, studies found that CGA could inhibit the production of pro-inflammatory cytokines (such as TNF-α and IFN-γ) [20], downregulate expression of various cell adhesion molecules [21], and prevent against apoptosis [22]. Shan et al. demonstrated that CGA inhibited lipopolysaccharide (LPS)-induced inflammatory response via attenuating the activation of NFκB and JNK/AP-1 signaling pathways [23]. But as far as we know, it is not clear that whether CGA ameliorated Con A-induced hepatitis in mice.

In this study, we explored the effects of CGA on Con A-induced hepatitis model and investigated the potential mechanisms. Our results indicated that CGA ameliorated liver damage, attenuated hepatocytes

* Corresponding author.
E-mail address: jywan@cqmu.edu.cn (J. Wan).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.intimp.2017.01.017
1567-5769/

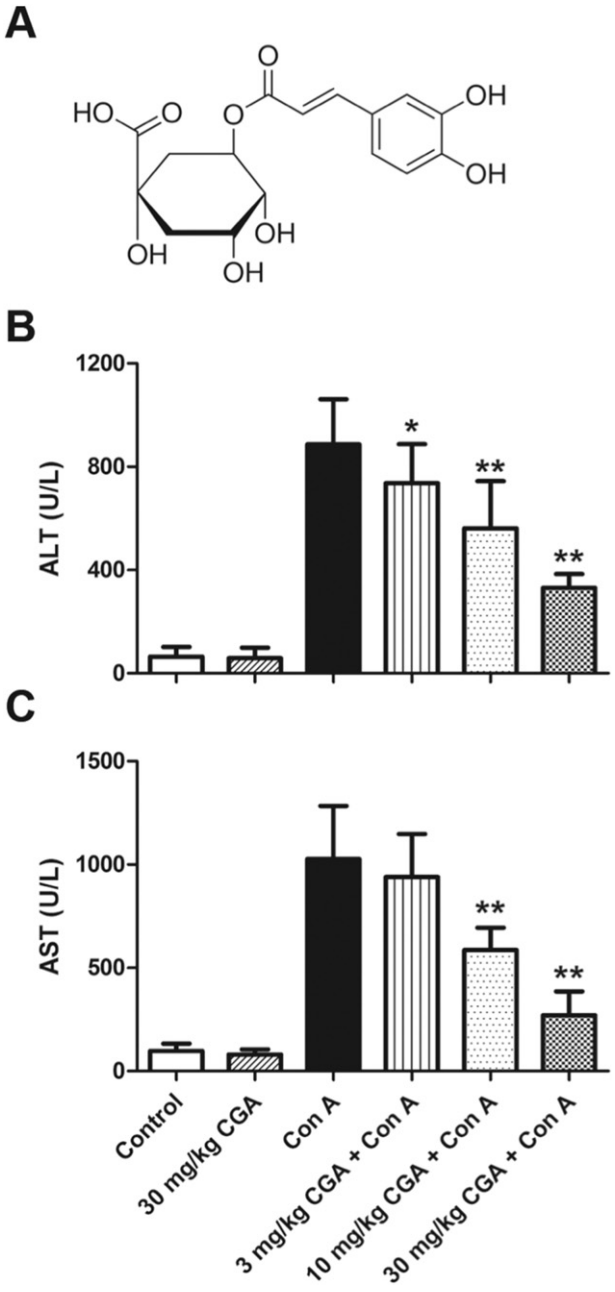

Fig. 1. Chlorogenic acid pretreatment ameliorated Con A-induced liver injury in mice. Mice were given oral gavage of CGA (3, 10, 30 mg/kg, respectively) every 8 h one time for three times within 24 h before Con A (20 mg/kg) injection. Blood samples were harvested at 12 h after Con A exposure to evaluate liver enzymes. (A) Chemical structure of CGA. (B) Alanine aminotransferase (ALT) activity. (C) Aspartate aminotransferase (AST) activity. Data were expressed as mean ± SD, $n = 6$. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the Con A group.

apoptosis, and improved liver function in Con A-challenged mice. Notably, CGA could effectively prevent from Con A-induced T cell-mediated hepatitis, which might be through suppressing TLR4 signaling pathways, downregulating the expressions of adhesion molecules, inhibiting the infiltration and activation of hepatic leukocytes, and decreasing the production of inflammatory cytokines.

2. Materials and methods

2.1. Mice

Male Balb/c mice weighing between 20 and 25 g (6–8 weeks old) were purchased from the Experimental Animal Center of Chongqing Medical University (Chongqing, China). The animals were kept in an environmentally controlled room (20–25 °C, 50 ± 5% humidity) under a 12-h dark/light cycle. All animals were fed with a standard laboratory diet and water ad libitum. Mice were acclimatized for at least l week before use. All the experiments were performed in accordance with the guidelines from Chongqing Medical University Institutional Animal Care and Use Committee.

2.2. Reagents

CGA and Con A were purchased from Sigma (St. Louis, MO, USA). The alanine aminotransferase (ALT) and aspartate aminotransferase (AST) assay kits were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Enzyme linked immunosorbent assay (ELISA) kits for TNF-α and IFN-γ were purchased from Bender MedSystems (Vienna, Austria). In Situ Cell Death Detection Kit was from Roche Applied Science (Basel, Switzerland). FITC rat anti-mouse F4/80 and CD4, PE rat anti-mouse Ly6G, and PE hamster anti-mouse CD69 were purchased from BD Biosciences (Heidelberg, Germany). Actin Green 488 and Actin Red 555 were from Invitrogen (Carlsbad, CA, USA). Caspase 3 colorimetric assay kit was purchased from Beyotime Institute of Biotechnology (Jiangsu, China). Intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, endothelial leukocyte adhesion molecule (ELAM)-1, rabbit anti-TLR4 antibodies were purchased from Abcam (Cambridge, UK). Rabbit anti-GAPDH antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The rabbit anti-mouse phospho-IRAK1, phospho-IκB and phospho-p38 mitogen-activated protein kinase (MAPK) antibodies were purchased from Cell Signaling Technology (Boston, USA).

2.3. Animal treatment

Hepatitis was induced in mice by a single intravenous injection of 20 mg/kg of Con A. To evaluate the hepatoprotective action, vehicle or various doses of CGA (3 mg/kg, 10 mg/kg, 30 mg/kg, dissolved in 0.5% carboxymethyl cellulose sodium salt in 0.9%normal saline) were orally treated every 8 h one time for three times within 24 h before Con A administration. The serum and liver samples were harvested from mice at 12 h after Con A injection.

2.4. Analysis of serum enzyme activity and cytokine production

The serum was obtained 12 h after Con A administration. The enzyme activities of ALT and AST were assessed using the commercially available kits, the levels of TNF-α and IFN-γ were detected by using ELISA kits according to the manufacturer's instructions.

2.5. Histological analysis

Liver tissues of mice were partially fixed with 4% paraformaldehyde and subsequently embedded in paraffin. Tissue sections (thickness, 5 μm) were stained with hematoxylin & eosin (H&E) for histopathological evaluation under light microscope (Nikon, Tokyo, Japan).

Fig. 2. Chlorogenic acid pretreatment attenuated Con A-induced hepatic necrosis and apoptosis in mice. Mice were orally treated with vehicle or CGA (30 mg/kg) before Con A (20 mg/kg) or saline administration. Livers were collected for histological examination. (A) H&E staining (200×). (B) The apoptotic cells were determined by TUNEL assay and the TUNEL-positive cells showed a dark-brown nucleus (400×). (C) The activities of caspase 3 were determined. Data were expressed as mean ± SD, $n = 6$. $^{**}P < 0.01$ compared with the Con A group.