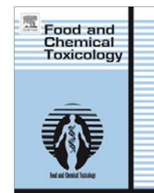
Food and Chemical Toxicology 49 (2011) 1329–1334

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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



Herbal formula CGX ameliorates LPS/D-galactosamine-induced hepatitis

Jang Woo Shin, Jing Hua Wang, Hye Jung Park, Min Kyeong Choi, Hyeong Geug Kim, Chang Gue Son *

Liver and Immunology Research Center, Institute of Traditional Medicine and Bioscience of Daejeon University, 22-5 Daeheung-dong, Jung-gu, Daejeon 301-724, Republic of Korea

ARTICLE INFO

Article history:
Received 3 January 2011
Accepted 10 March 2011
Available online 15 March 2011

Keywords:
Liver injury
LPS
D-Galactosamine
CGX
Traditional Korean medicine
Antioxidant

ABSTRACT

CGX, a traditional herbal drug, has been prescribed for patients suffering from various liver diseases, including hepatitis B, alcoholic liver disease, and fatty liver. We investigated whether CGX has hepatoprotective effects against lipopolysaccharide/D-galactosamine (LPS/D-GalN)-induced acute liver injury and its underlying mechanism(s). Mice were administered CGX orally for 7 days prior to an injection of LPS (5 μg/kg)/D-GalN (700 mg/kg). Complete blood count, serum diagnostic markers, antioxidant activities, caspase activity, and histopathological examinations were conducted 8 h after the injection. To evaluate the immunological mechanism of CGX, serum TNF-α and IL-10 were investigated 1.5 h after LPS/D-GalN injection. CGX pretreatment (100, 200, and 400 mg/kg) inhibited the elevation of serum AST and ALT levels as well as histopathological alterations. Moreover, CGX pretreatment inhibited activation of caspase-3/7. CGX attenuated LPS/D-GalN-induced lipid peroxidation with concomitant improvement in total antioxidant activities (superoxide dismutase, catalase, and glutathione peroxidase). CGX elevated the antioxidant capacity of the liver in both the pathological and normal conditions. Furthermore, LPS/D-GalN-induced alterations of neutrophil and lymphocyte populations were ameliorated and serum TNF-α was decreased significantly by CGX. From these data we conclude that CGX protects the liver from LPS/D-GalN-induced hepatitis through antioxidant mechanisms as well as immune modulation.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

Fulminant hepatic failure (FHF) is a life-threatening clinical syndrome characterized by rapid and massive hepatic dysfunction in the absence of previous histological evidence of liver disease (Bernuau et al., 1986). This syndrome results in encephalopathy, jaundice, severe coagulopathy, and high rates of mortality (Bernal et al., 2010). Pathogen infections and hepatotoxic drugs have been the main causes of FHF identified to date, although the origin of the disease still remains unknown in a large proportion of patients (Bernal et al., 2010). No therapy is currently available except liver transplantation, an expensive surgical procedure with an acute shortage of donors (Ng and Lo, 2009).

A mouse model of LPS/D-galactosamine (D-GalN)-induced liver injury has been effectively used to examine the efficiency of hepatoprotective agents, and can be clinically extrapolated to human liver dysfunction (Rahman and Hodgson, 2000). Although LPS stimulates inflammatory and Kupffer cells to produce proinflammatory cytokines, including TNF-α, IFN-γ, IL-6, and IL-12 (van Deuren et al., 1992), it is difficult to induce lethal effects using LPS alone, with an LD_{50} of 200 μg reported in CF-1 mice (Silverstein et al., 1989). Co-treatment with D-GalN dramatically enhanced the susceptibility of mice to the lethal effects of LPS, leading to FHF. D-GalN is a specific hepatotoxin that selectively depletes uridine nucleotides, consequently inhibiting macromolecule synthesis in hepatocytes (Decker and Keppler, 1974).

CGX is a traditional Korean medicine consisting of 13 herbal plants, which is generally prescribed for patients suffering from chronic liver diseases. Systemic toxicity tests in rats and dogs and clinical experience since 1991 have consistently confirmed the safety of this herbal drug combination. Previously, we reported hepatoprotective and anti-fibrotic effects of CGX in chemically induced liver injury models as well as in the clinic (Cho et al., 2000; Hu et al., 2008; Shin et al., 2006). In this study, we investigated the effect of CGX on LPS/D-GalN-induced hepatocellular injury, and further explored the underlying mechanisms, including antioxidant enzymes and cytokines.

2. Materials and methods

2.1. Preparation and standardization of CGX

Briefly, 120 kg of the 13-herb mixture (Table 1) was boiled in 1200 L of distilled water (DW) for 4 h at 100 °C, and lyophilized after filtration using a 300-mesh filter (50 μm) and condensation. The final yield was 10.1% (w/w) from the original dried mixture. CGX was manufactured by Kyeungbang Pharmacy (Incheon, Korea).

* Corresponding author. Address: Daejeon Oriental Hospital of Daejeon University, Liver and Immunology Research Center, 22-5 Daehueng-dong, Jung-gu, Daejeon 301-724, Republic of Korea. Tel.: +82 42 257 6397; fax: +82 42 257 6398.
E-mail address: ckson@dju.ac.kr (C.G. Son).

0278-6915/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fct.2011.03.015

Table 1
Composition of CGX.

Herbal name	Scientific name	Voucher specimen number	Ratio
Artemisiae Capillaris Herba	*Artemisia capillaris*	AC-2007-01-He	5
Trionycis Carapax	*Trionyx sinensis*	TS-2007-01-Ca	5
Raphani Semen	*Raphanus sativus*	RS-2007-01-Se	5
Atractylodis Rhizoma Alba	*Atractylodes macrocephala*	AM-2007-01-Rh-Al	3
Poria	*Poria cocos*	PC-2007-01-Po	3
Alismatis Rhizoma	*Alisma orientalis*	AO-2007-01-Rh	3
Atractylodis Rhizoma	*Atractylodes chinensis*	AC-2007-02-Rh	3
Salviae Miltiorrhizae Radix	*Salvia miltiorrhiza*	SM-2007-01-Ra	3
Polyporus	*Polyporus umbellatus*	PU-2007-01-Po	2
Ponciri Fructus	*Poncirus trifoliata*	PT-2007-01-Fr	2
Amomi Fructus	*Amomum villosum*	AV-2007-01-Fr	2
Glycyrrhizae Radix	*Glycyrrhiza uralensis*	GU-2007-01-Ra	1
Aucklandiae Radix	*Aucklandia lappa*	AL-2007-01-Ra	1

All herbs used in the CGX formulation satisfied the Korean Pharmacopoeia criteria and CGX extract satisfied the criteria using a confirmation test for each herb, heavy metals, bacteria, fungi, specific pathogens, and a quantitative test (dimethylescuretin >0.15 mg/750 mg extract and glycirizin >1.1 mg/750 mg extract). Three-dimensional fingerprinting using a high-performance liquid chromatography-diode array detector-mass was performed as described previously (Shin et al., 2010b).

2.2. Animal and experimental design

For the experiments, 48 specific pathogen-free male BALB/c mice (6 weeks old) were obtained from a commercial animal breeder (Orientbio, Gyeonggido, Korea) and acclimated for l week before use. They were housed in an environmentally controlled room at 22 ± 2 °C under a 12/12 h light/dark cycle. They were fed commercial pellets (Cargill Agri Furina, Gyeonggido, Korea) with tap water *ad libitum*.

The mice were randomly divided into six groups ($n = 8$ each): normal, CGX 400 mg/kg, LPS/D-GalN and CGX (100, 200, or 400 mg/kg), and LPS/D-GalN. CGX or distilled water (DW) was administered orally once per day for 6 days, 1 h before LPS (Sigma, MO, USA). The mice received LPS/D-GalN (5 μg/kg and 700 mg/kg, respectively) intraperitoneally after 4 h fasting. All mice were sacrificed under deep ether anesthesia and blood was obtained from an abdominal vein after 8 h. A small portion of blood was used for the analysis of complete blood counts (CBC) and the remaining portion was allowed to clot at room temperature, centrifuged, serum separated, and then used for analysis of hepatic enzymes. The livers were stored separately at −80 °C for analysis of oxidative stress-related biomarkers and in phosphate-buffered formalin (10%) for histopathological evaluation.

For cytokine analyses, additional animal experiments were performed with a further 40 mice. The mice were divided into five groups (no CGX control group) and treated using the same protocol as above but were sacrificed at 1.5 h after the LPS/D-GalN challenge.

The animal experiments were conducted in accordance with the rules for Use of Laboratory Animals as adopted and maintained by the US National Institute of Health following approval of the Institutional Animal Care and Use Committee of Daejeon University (JUARB2009-014, 2010-037).

2.3. Biochemistry and histology

Serum levels of ALT and AST were determined using an Auto Chemistry Analyzer (AU400; Olympus, Tokyo, Japan). Formalin-fixed liver tissue was processed into paraplast-embeded blocks, sectioned, and slides stained with hematoxylin and eosin (H&E). Representative histopathological microphotographs were produced using a light microscope (Leica, Wetzlar, Germany) by an individual who was not aware of the animal experimental groups.

2.4. Determination of caspase activity

Caspase-3/7 activity in liver tissues was detected using the SensoLyte Homogeneous AMC Caspase-3/7 Assay Kit (Anaspec, CA, USA) according to the manufacturer's protocol. Liver was homogenized in RIPA buffer, and 150 μL mixed with 50 μL working solution and incubated for 30 min. Fluorescence was determined with Victor 3 (PerkinElmer, MA, USA).

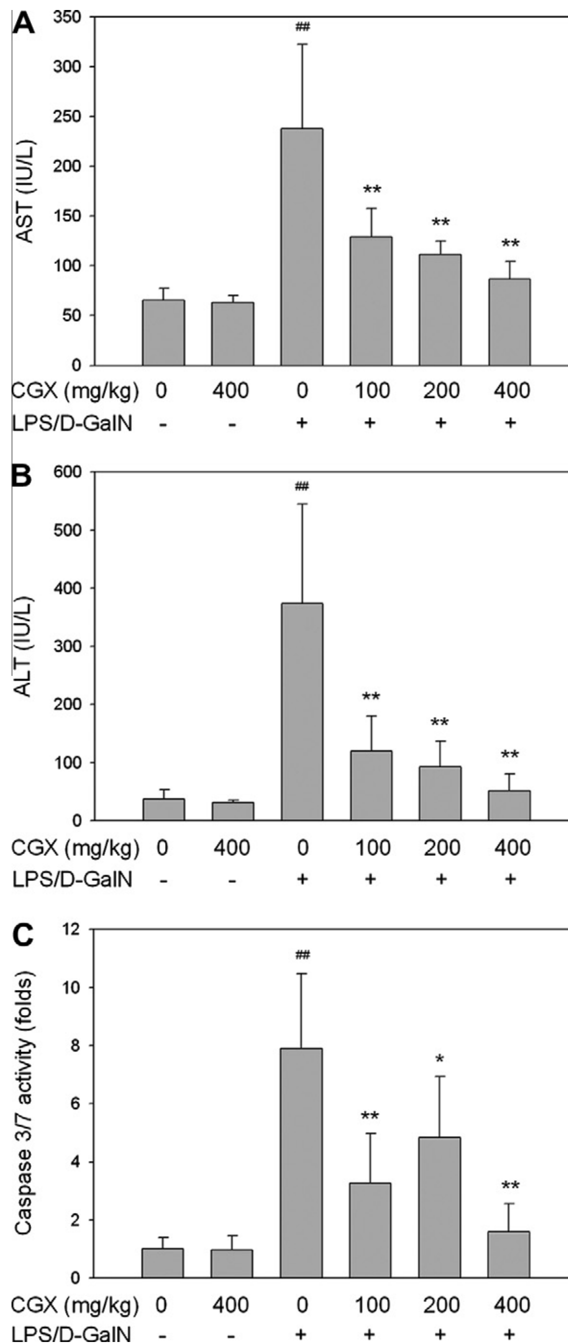


Fig. 1. Serum aminotransferase and caspase activity in liver tissues. Mice were pretreated with CGX (100, 200, and 400 mg/kg) or DW once per day for 6 days and 1 h before the LPS/D-GalN injection. After 8 h, blood was collected and serum was prepared. Serum AST and ALT were determined. Livers were removed and homogenized and caspase-3/7 activity in liver homogenate was determined. Data were expressed as mean ± SD ($n = 8$). $^{\#\#}P < 0.01$, compared with the normal group; $^{*}P < 0.05$ and $^{**}P < 0.01$, compared with LPS/D-GalN group.

2.5. Malondialdehyde (MDA) analysis in the liver tissue

Lipid peroxidation in liver tissues was examined using the method of thiobarbituric acid-reactive substances (TBARS) as described previously (Uchiyama and Mihara, 1978). The concentration of TBARS was expressed as μM of MDA/gram of tissue using 1,1,3,3-tetraethoxypropane (TEP) as a standard. Briefly, 0.15 g of liver tissue was homogenized in ice-cold KCl (1.5 mL, 11.5 g/L) using Fast Prep (MP Biomedicals, OH, USA), 0.13 mL of the homogenate was mixed with phosphoric acid (0.08 mL, 10 g/L), and thiobarbituric acid (0.26 mL, 0.67%). The resulting solution was heated for 45 min at 100 °C, followed by the addition of 1.03 mL *n*-butanol. This

Download English Version:

https://daneshyari.com/en/article/5853437

Download Persian Version:

https://daneshyari.com/article/5853437

Daneshyari.com