Biomedicine & Pharmacotherapy 103 (2018) 140–146

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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

Qingchangligan formula alleviates acute liver injury by attenuating extracellular histone-associated inflammation

Lan Ding[a], Xiangying Zhang[a,b], Li Li[a], Chunyan Gou[a], Xiaolan Luo[a], Yang Yang[a], Tao Wen[c,*], Xiuhui Li[a,**]

[a] Beijing You An Hospital, Capital Medical University, Beijing 100069, China
[b] Beijing Institute of Hepatology, Beijing 100069, China
[c] Medical Research Center, Beijing Chao-Yang Hospital, Capital Medical University, Beijing 100020, China

ARTICLE INFO

Keywords:
Qingchangligan formula (QCLGF)
Acute-on-chronic liver failure (ACLF)
Extracellular histones
Systemic inflammation

ABSTRACT

Qingchangligan formula (QCLGF) is a traditional Chinese medicine that has exhibited remarkable clinical efficacy for patients with acute-on-chronic liver failure (ACLF). However, the hepatoprotective mechanisms of QCLGF are not completely understood. Extracellular histones were recently identified as the novel inflammatory mediators involved in the pathogenesis of acute liver injury. This study aimed to investigate whether QCLGF provides hepatoprotection by targeting extracellular histones. We showed that QCLGF significantly improved the survival rate of the ConA-treated mice, ameliorated hepatotoxicity, and markedly decreased the levels of extracellular histones and the associated cytokines. We further demonstrated that QCLGF attenuated systemic inflammation by inhibiting the mitogen-activated protein kinase (MAPK) signaling pathway. In addition, exogenous histones induced a significant HL-7702 cell damage, which could be prevented by administration of QCLGF. Lastly, we observed that extracellular histones and the associated cytokines were consistently lower in ACLF patients receiving conventional medical therapy plus QCLGF than in patients receiving only conventional medical therapy. Collectively, these results provide evidence that QCLGF has therapeutic potentials for treating ACLF, which may be due to its ability to interfere with extracellular histone-mediated cellular damage and systemic inflammation.

1. Introduction

Acute-on-chronic liver failure (ACLF) is the most common type of liver failure, which is defined as acute deterioration of liver function manifesting as jaundice and coagulopathy, complicated within 4 weeks by ascites and/or encephalopathy in patients with chronic liver diseases [1]. ACLF is associated with the development of multiple organ failure and a consequent poor prognosis. Generally, the onset of ACLF is involved with one or more precipitating events including bacterial infection, superimposed viral hepatitis or reactivation of hepatitis, alcohol, or hepatotoxic drug abuse. However, the underlying mechanisms are not fully understood [2]. Despite advances in current medical therapies, clinical management of ACLF remains limited and challenging. Liver transplantation is the only option in eligible patients when medical treatment fails [3].

In China, traditional Chinese medicine (TCM) has long been used as an important alternative and complementary therapy to treat ACLF. The mechanism of TCM in the treatment of ACLF is complex. From the perspective of TCM, three key factors including "toxins", "stasis", and "phlegm" define the pathogenesis of ACLF. Most of the TCM formulas show clinical efficacy against ACLF by targeting one or more of these factors. Of these, Qingchangligan formula (QCLGF) is comprised of five herbs, Rheum palmatum, dried Rehmannia root, Magnolia officinalis, Taraxacumofficinalae, and Fructusaurantii (Table1). QCLGF has been used in clinical practice for decades and has exhibited remarkable efficacy to improve ACLF [4]. The mechanisms of QCLGF may be involved with removal of inflammatory molecules and cytokines, and improvement in systemic and hepatic recovery. Our previous study also

Abbreviations: ACLF, acute-on-chronic liver failure; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ConA, concanavalin A; ERK, extracellular regulated protein kinase; H & E, hematoxylin and eosin; IFN-γ, interferon-γ; IL-1β, interleukin-1β; IL-6, interleukin-6; IL-10, interleukin-10; IL-18, interleukin-18; JNK, c-jun-N-terminal kinase; MAPK, mitogen-activated protein kinase; PAGE, polyacrylamide gel electrophoresis; PVDF, polyvinylidene difluoride; TBST, Tris-buffered saline with Tween-20; TNF-α, tumor necrosis factor-α

* Corresponding author.
** Corresponding author at: Department of Integrated TCM and Western Medicine, Beijing You An Hospital, Capital Medical University, Beijing 100069, China.
E-mail addresses: drdylan@126.com (L. Ding), zhangxiangying1984@163.com (X. Zhang), bjfhlc@126.com (L. Li), goucy@sina.vip.com (C. Gou), lxllfjlyl@163.com (X. Luo), 985166559@qq.com (Y. Yang), wentao5281@163.com (T. Wen), lixiuhui@sohu.com (X. Li).

https://doi.org/10.1016/j.biopha.2018.01.121
Received 22 November 2017; Received in revised form 15 January 2018; Accepted 24 January 2018

Table 1
Contents of Qingchangligan.

Chinese name	Botanical name	Common name	Genus	Family	Weight (g)	Part used
Da Huang	*Rheum palmatum* L.	*Rheum palmatum*	Rheum	Polygonaceae	15	Root and rhizome
Di Huang	*Rehmannia glutinosa* (Gaetn.) Libosch. ex Fisch. et	Dried rehmannia root	Rehmannia	Plantaginaceae	30	Root
Hou Po	*Magnolia officinalis* Rehder& E.H. Wilson	*Magnolia officinalis* Rehd et Wils	Magnolia	Magnoliaceae	15	Bark
Pu Gong Ying	Taraxacum abbreviatum Rail	*Taraxacum officinalae*	Taraxacum	Compositae	15	Flower, leaf and rhizome
Chi Qiao	*Citrus × aurantium* L.	Fructus aurantii	Citrus	Rutaceae	15	Fruit

demonstrated that QCLGF can protect the liver from acute injury by modulating autophagy [5]. Nevertheless, the protective mechanisms of QCLGF have not been well investigated.

Recently, extracellular histones have been identified as a novel class of inflammatory mediators implicated in various inflammatory diseases, including sepsis, peritonitis/appendicitis, acute lung injury, acute kidney injury, glomerulonephritis, and autoimmune diseases [6–9]. It has been suggested that extracellular histones possess various toxic effects, including direct cytotoxicity, induction of vascular permeability, coagulation activation, platelet aggregation, and cytokines production [10]. Moreover, histone-targeted therapy is effective for the treatment of various inflammatory injuries, thereby confirming a causal role for extracellular histones [11]. We have previously confirmed that extracellular histones play a pathologic and targetable role in patients with ACLF [12]. In the present study, we further intend to investigate whether QCLGF exerts its hepatoprotective effects by attenuating extracellular histone-associated inflammation.

2. Methods and materials

2.1. Drug preparation

The QCLGF granules, manufactured by Kangren Tang Pharmaceutical (Beijing, China), are prepared through a hospital prescription agreement. All the ingredients included in the QCLGF granules are listed in Table 1. Prior to usage, 1 g of dry herbal mixture was resuspended in 1 ml of distilled water.

2.2. Animals and treatment

Male C57BL/6 mice (8–12 weeks old) were purchased from Capital Medical University (Beijing, China), and they were maintained in the animal facility under sterile conditions. For a murine model of immune-mediated liver injury, ConA (15 mg/kg, Sigma, St Luis, MO, USA) was administered *via* tail vein.

To study the effects of QCLGF on the ConA-treated mice, QCLGF (50 mg/kg) was administered once a day for 3 days *via* gavage prior to ConA injection. The mice were sacrificed at 9 h after ConA injection, and blood and liver samples were collected for subsequent analyses. All animal studies were approved by the Ethics Committee of the Capital Medical University (approval ID: AEEI-2016-106).

2.3. Assessment of liver damage

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using a multiparameteric analyzer (AU 5400, Olympus, Japan). Liver tissues were fixed in formalin solution, washed with phosphate-buffered saline (PBS), embedded in paraffin wax, cut into 5-μm sections, and stained with hematoxylin and eosin (H&E) using a standard protocol. The sections were analyzed using light microscopy.

2.4. Human subjects

Plasma samples were collected from 23 patients with ACLF at Beijing Youan Hospital, Capital Medical University (Beijing, China), between March 2015 and August 2016. All patients with ACLF had been either positive for HBsAg or HBV DNA (by PCR assay) for more than 6 months before enrollment. The diagnostic standard of ACLF fulfilled the criteria set by the Asian Pacific Association for the Study of the Liver (serum bilirubin ≥ 5 mg/dL, international normalized ratio ≥ 1.5 or prothrombin activity $< 40\%$) [13]. In addition, according to the Guideline Principle for the Diagnosis and Treatment of Viral Hepatitis by the China Association of Chinese Medicine of Liver Diseases, ACLF was defined as the Damp-heat Obstruction Pattern of Chinese Medicine [14]. Patients with other forms of viral hepatitis, acute liver failure, acute decompensation of cirrhosis with prior history decompensation, Wilson's disease, autoimmune hepatitis, alcoholic liver disease, sclerosing cholangitis, biliary obstruction, or malignancies were excluded.

All patients underwent conventional therapeutic intervention, which included absolute bed rest, antiviral therapy, rectification fluid and electrolyte and acid-base abnormalities, and plasma expansion with albumin or crystalloids. In the QCLGF plus conventional therapy group, QCLGF (0.1 g/kg) was administrated once a day for 4 weeks *via* retention-enema. Plasma samples were collected before therapy and 4 weeks after therapy for further analyses. All human samples involved were approved by the Institutional Review Board at Beijing Youan Hospital, Capital Medical University, Beijing, P.R. China (approval ID: [2014]33).

2.5. Measurement of extracellular histones

The level of extracellular histones was measured using a cell death detection ELISA kit (Roche Applied Science, USA), which contains anti-histone antibodies that react with histones of various species, including humans and mice [15].

2.6. Measurement of plasma cytokines

Cytokines in plasma samples were quantified using the ProcartaPlex™Multiplex Immunoassay from eBioscience (San Diego, CA, USA), according to the manufacturer's instructions.

2.7. Western blotting

Proteins from frozen liver of mice were extracted in RIPA buffer, containing protease/phosphatase inhibitors. After protein quantification (Pierce™ BCA Protein Assay Kit, Thermo), the same amount of proteins were separated on 12% SDS polyacrylamide gel electrophoresis (SDS-PAGE) and transferred on to polyvinylidene difluoride (PVDF) membranes (Bio-Rad, Hercules, CA, USA). Membranes were probed with p-extracellular regulated protein kinase (p-ERK), p-c-jun-N-terminal kinase (p-JNK), p-p38, total ERK, total JNK, total p38 and β-actin antibodies (Cell Signaling Technology, Danvers, MA) and HRP-coupled secondary antibodies (Cell Signaling Technology, Danvers, MA). Immunoblots were visualized using the ECL chemiluminescence kit (Thermo Fisher Scientific, Rockford, IL, USA).

2.8. Cell culture and treatment

Normal human hepatocyte cell line (HL-7702) was purchased from the Shanghai Cell Bank (Shanghai, China), supplemented with