International Immunopharmacology 50 (2017) 95–106

Contents lists available at ScienceDirect

International Immunopharmacology

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

Wogonin attenuates inflammation by activating PPAR-γ in alcoholic liver disease

Hai-Di Li, Xin Chen, Yang Yang, Hui-Min Huang, Ling Zhang, Xin Zhang, Lei Zhang, Cheng Huang, Xiao-Ming Meng, Jun Li*

The Key Laboratory of Major Autoimmune Diseases, Anhui Province, Anhui Institute of Innovative Drugs, School of Pharmacy, Anhui Medical University, China
The Key Laboratory of Anti-inflammatory of Immune Medicines, Ministry of Education, China
Institute for Liver Diseases of Anhui Medical University, China

ARTICLE INFO

Keywords:
Wogonin
PPAR-γ
Inflammation
NF-κB
Alcoholic liver disease (ALD)

ABSTRACT

Alcoholic liver disease (ALD) is one of the predominant causes of liver-related morbidity and mortality worldwide. However, effective therapy for ALD is still lacking. Wogonin, a major flavonoid compound, is found in *Scutellaria baicalensis* Georgi. Accumulating studies have revealed that wogonin possesses anti-inflammatory and anti-tumour activities in various models. However, the hepatoprotective activity of wogonin in ALD is still obscure. In this study, we found that wogonin significantly attenuated inflammatory response in EtOH-fed mice, and reduced the expression of inflammatory cytokines such as TNF-α and IL-6 in EtOH-induced RAW264.7 cells. Furthermore, our findings showed that wogonin remarkably induced the expression of PPAR-γ *in vivo* and *in vitro*. Compared with the wogonin-treated group, blockade of PPAR-γ with inhibitor (T0070907) or PPAR-γ small interfering (si)-RNA were applied in RAW264.7 cells to evaluate the involvement of wogonin in alleviating EtOH-induced inflammation. Moreover, forced expression of PPAR-γ further suppressed the expression of TNF-α and IL-6 when treated with wogonin on EtOH-induced RAW264.7 cells. In addition, it was demonstrated that wogonin remarkably suppressed PPAR-γ-meditated phosphorylation and activation of NF-κB-P65. In conclusion, our results indicated that wogonin may serve as an effective modulator of PPAR-γ by down-regulating NF-κB pathway, thereby attenuated inflammatory response in ALD.

1. Introduction

Alcoholic liver disease (ALD), a class of liver injury disease, caused by long-term alcoholic consumption, along with high incidence and high mortality [1,2]. ALD can cause pathological progression including steatosis, liver fibrosis, cirrhosis, and even finally evolved into hepatic carcinoma [3,4]. As referenced, alcohol use and alcohol-use disorders greatly increase economic cost and global burden [5,6]. Despite extensive studies have revealed some pathogenetic factors of ALD, there are little treatments that could significantly reduce liver injury. Therefore, effective strategies for ALD patients are still required and need to be further explored [7,8]. In the initiation and exacerbation of ALD, inflammatory response plays a significant role [9], and liver macrophages are particularly critical in ALD [10,11].

Alcohol can induce the activation of macrophages, increase their membrane fluidity, and induce the generation of endotoxin which further activates macrophages, triggering a series of inflammatory reactions including the release of inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), and eventually leads to liver injury [12,13]. Moreover, alcohol can stimulate the activation of upstream NF-κB pathway in liver macrophages [14], which further induces the expression of TNF-α and IL-6. It appears to be useful to identify an inflammatory mediator in the treatment of liver inflammation in the progress of ALD.

Peroxisome proliferator-activated receptor-γ (PPAR-γ), a member of the nuclear hormone receptor family, has been prominently proved to limit inflammation in various macrophage models by inhibiting the expression of many pro-inflammation genes and enhancing the transcription of anti-inflammatory and anti-oxidant genes [15–17]. Moreover, PPAR-γ is a ligand-activated transcription factor that plays an important role in regulating nuclear factor-κB (NF-κB)-induced inflammation [18,19].

Traditional Chinese medicines (TCM) are widely concerned on the promising pharmacological effects and relatively low cytotoxicity. It has been reported that a lot of Chinese medicines have remarkably anti-inflammatory activity, some of which have been used for clinical

* Corresponding author at: School of Pharmacy, Anhui Medical University, Hefei, Anhui, China.
E-mail addresses: lj@ahmu.edu.cn, 1165796154@qq.com (J. Li).

http://dx.doi.org/10.1016/j.intimp.2017.06.013
Received 7 March 2017; Received in revised form 1 June 2017; Accepted 14 June 2017
Available online 21 June 2017

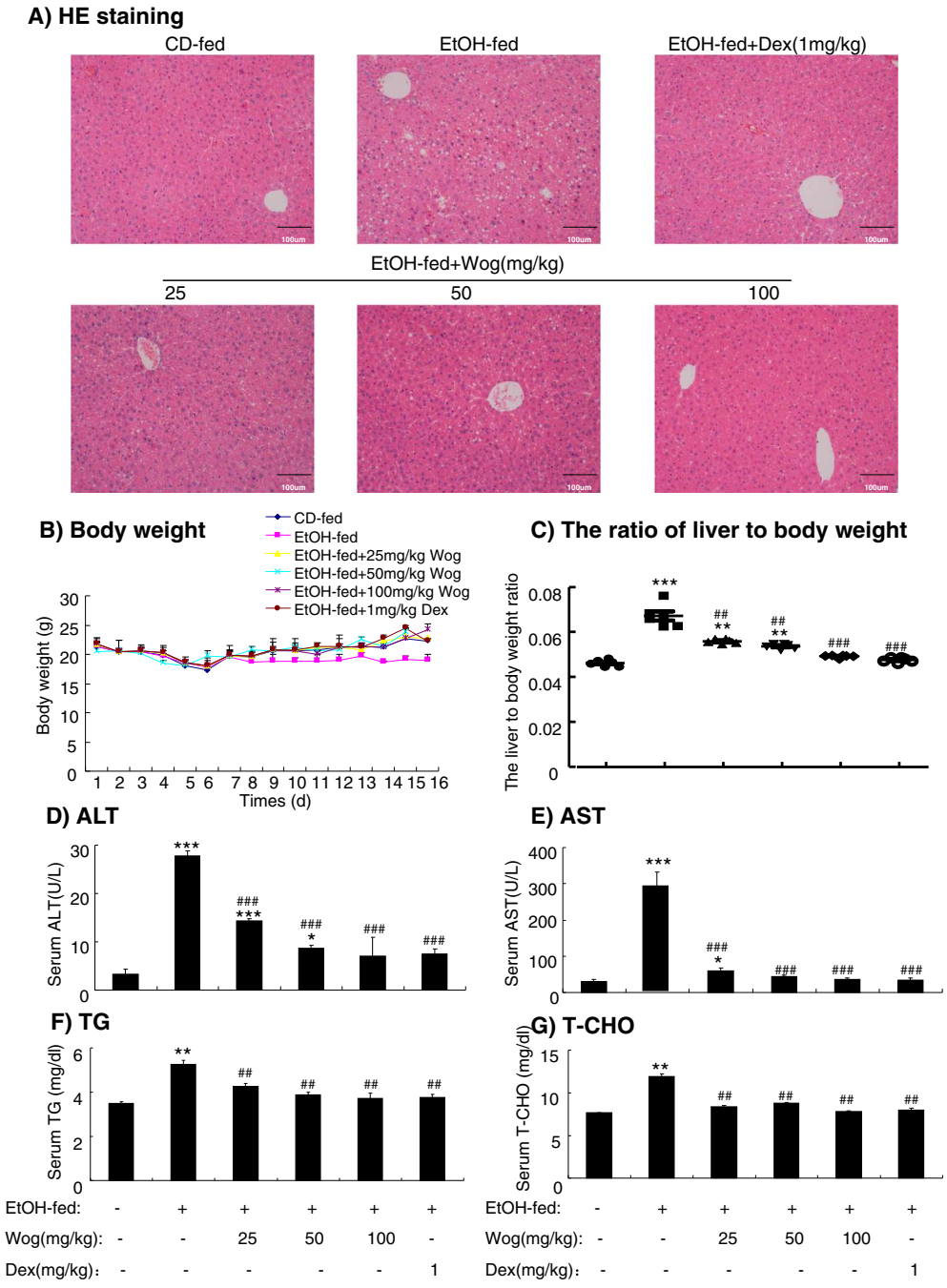

Fig. 1. Wogonin protects against liver injury and pathological characteristics of ALD in mice.
A: representative hematoxylin and eosin (H & E) staining of liver tissues in different groups, including CD-fed mice, EtOH-fed mice, 25 mg/kg, 50 mg/kg, 100 mg/kg wogonin-treated mice and positive (dexamethasone, 1 mg/kg)-treated mice. B and C: body weights and the liver to body weight ratio after ethanol feeding. D and E: serum ALT and AST levels. F and G: Hepatic triglyceride (TG) and total cholesterol (T-CHO) levels. The values represent means ± SD for at least 6–8 independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to CD-fed mice. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ compared to EtOH-fed mice. CD: control diet, Wog: wogonin, Dex: dexamethasone.

purposes. Wogonin, a major flavonoid of *Scutellaria baicalensis* Georgi, is well-known for its anti-inflammatory and anti-tumor effects [20]. Increasing evidence has also demonstrated the anti-inflammatory activity of wogonin in several animal models, including LPS-induced acute liver injury, acute lung liver, and kidney injury [21–23]. It is noteworthy that wogonin can activate the expression of PPAR-γ and then suppresses NF-κB pathway [21]. However, the anti-inflammatory effect of wogonin in ALD has not been studied. Therefore, our study examined the protective effect and mechanisms of wogonin in EtOH-induced ALD.

2. Materials and methods

2.1. Materials and reagents

Wogonin was provided from Meilune Biology Technology (MB6663, CAS 632-85-9, DaLian, China). Dexamethasone (Dex, purity > 99%) and T0070907 (T8703) was purchased from Sigma Chemical (St.Louis, MO, USA). The antibodies for β-actin, NF-κB-P65, NF-κB-p-P65 were purchased from Cell Signaling Technology (Danvers, MA). The polyclonal antibodies for TNF-α and IL-6 were purchased from abcam (Cambridge, UK). Enzyme Linked Immunosorbent Assay (ELISA) kit was purchased from Elabscience Biotechnology Co.Ltd (Wuhan, China). ALT (C009-2) assay kit, AST (C010-2) assay kit, TG (A110-1) and TCH (A111-1) assay kits were purchased from Jiancheng Biology Institution PeproTech (Nanjing, Jiangsu, China).

2.2. Animal treatment

C57BL/6 mice, male, 6–8 weeks old, weighing 18–22 g, were provided from the Laboratory Animal Center of Anhui Medical University. Animal experiments were approved by the Institutional Animal