Available online at SciVarse ScienceDirect

Chinese Herbal Medicines (CHM)

ISSN 1674-6384

Journal homepage: www.tiprpress.com E-mail: chm@tiprpress.com

Original article

Anti-inflammatory and Anti-oxidant Effects of Licorice Flavonoids on Ulcerative Colitis in Mouse Model

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ARTICLE INFO

Article history

Received: March 11, 2017

Revised: July 27, 2017

Accepted: August 14, 2017

Available online:

October 10, 2017

DOI:

10.1016/S1674-6384(17)60116-3

ABSTRACT

Licorice (*Glycyrrhizae Radix* or *Liquiritiae Radix*) is traditionally used to treat various diseases including inflammation and gastric ulcers. Licorice is rich in flavonoid compounds and possesses anti-inflammatory activities. To investigate the protective effects of licorice flavonoids (LFs) in both acetic acid-induced and dextran sulphate sodium (DSS)-induced ulcerative colitis (UC) mouse model and its underlying mechanism. Acute UC was induced by intra-rectal acetic acid (4% v/v) after pretreatment with LFs (100, 200, and 400 mg/kg, p.o.), 0.9% saline (20 mL/kg, p.o.) or Sulfasalazine (SASP) (600 mg/kg, p.o.) for 10 d. Quantitative analysis of chemical components of LFs was also conducted by HPLC. Our results showed that pre-treatment with LFs significantly reduced the wet weight/length ratio of colon, percentage of affected area, macroscopic and histological damage scores in acid-induced UC mice. LFs also significantly decreased the oxidative stress and pro-inflammatory cytokines, upregulated nuclear factor erythroid 2-related factor 2 (Nrf2) pathway and downregulated nuclear transcription factor kappa B (NF-κB) pathway. At last, LFs also showed obvious antiulcer effect on the DSS-induced UC model. The major components of LFs were licochalcone A, glabrone, licoflavone, and licoflavone B. This study demonstrates that the protective effect of LFs may at least in part be due to its anti-oxidant activity through Nrf2 pathway and anti-inflammatory activity through NF-κB pathway.

Key words

licochalcone A; licorice flavonoids; ulcerative colitis; Nrf2; NF-κB

1. Introduction

Ulcerative colitis (UC), an inflammatory autoimmune disease which affects millions of people worldwide, is characterized by chronic uncontrolled inflammation of intestinal mucosa (Packey and Sartor, 2008). Chronic intestinal inflammation may pose an elevated risk of developing colorectal cancer, which is the third most common malignancy in humans (Saleh and Trinchieri, 2011). Even though the pathogenesis of UC remains largely unknown, it has been

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Fund: National Natural Science Foundation of China (81660654). Fast finding active chalcone resist to UC based on a simple active filter system and studying on their mode of action

considered as a result of the combination of environmental factors, genetic factors, intestinal microbiota modifications and immune responses (Ko and Auyeung, 2014; Cammarota et al, 2015). Over-expression of pro-inflammatory mediators such as reactive oxygen mediator, neutrophil infiltration and cytokines has been known to contribute to the inflammatory cascade in the pathological process of colitis (Neurath, 2014). Moreover, the nuclear transcription factor kappa B (NF-κB) is identified as one of the pivotal regulatory components in inflammatory diseases and inhibition of its activity may alleviate the severity of UC (Atreya et al, 2008). Furthermore, nuclear factor erythroid 2-related factor 2 (Nrf2) is a key transcription factor for different kinds of cells and tissues against oxidative stress, which is closely related to UC (Jena and Trivedi, 2014). The most current standard therapy for UC includes aminosalicylates (e.g., sulphasalazine and mesalamine), glucocorticosteroids (e.g., prednisolone and budesonide), immunosuppressant (e.g., azathioprine, 6-mercaptopurine and methotrexate) and biologic agents (e.g., infliximab and adalimumab) (Triantafillidis et al, 2011). Although these treatments are effective, they may cause severe side effects, including diarrhea, cramps, abdominal pain accompanied by fever, and high blood pressure (Rogler, 2010) in the context of continued administration. Therefore, it is crucial to develop a new and safe drug to prevent or treat UC.

Recently, many reports have focused on the use of natural products and dietary supplements derived from plants, which might offer alternative and effective anti-inflammatory therapies with low toxicity and minimal side effects (Sakthivel and Guruvayoorappan, 2013; Shigeshiro et al, 2013).

Licorice, the roots and rhizomes of *Glycyrrhiza* species, is one of the most frequently used herbs in ancient Egyptian, Greek and China (Sun and Pan, 2006). According to traditional Chinese medicine (TCM) theory, licorice primarily effective for digestive system, respiratory system and cardiovascular system (Wang et al, 2013). Licorice is almost the most widely prescribed crude drug in China, constituting about 60% of all TCM prescriptions (Wang and Yang, 2007). In clinic, licorice is effective to relieve the following conditions: phlegm, cough, dyspnea, spasms, pain, and toxicity, and cooperates with other medicines. Moreover, one of the well-known folk uses of licorice has been in the treatment of gastric ulcer (Aly et al, 2005). It is used to treat gastric ulcers when administered 20 to 30 min before meals through lining the stomach wall (Omar et al, 2012) and its antiulcer and mucosal protective actions have been confirmed by numerous clinical trials and animal experiments (Kassir, 1985; Aly et al, 2005). So far, about 400 compounds have been isolated from licorice (Yang et al, 2014), including more than 20 triterpenoids such as glycyrrhetic acid and approximately 300 flavonoids. Some of these flavonoids have been proved to have a remarkable anti-inflammatory and anti-oxidant activities (Furusawa et al, 2009; Simmler et al, 2013; Song et al, 2015). A recent study showed the healing effect of licorice extract in acetic acid-induced UC in rat model (Takhshid et al, 2011). Accordingly, to provide detailed experimental evidence about the therapeutic potentials of licorice flavonoids (LFs) on UC, we studied the antiulcer effect and the possible mechanism of LFs in both acetic acid- and dextran sulphate sodium (DSS)-induced UC mouse models, and analyzed the potential main active constituents of LFs.

2. Material and methods

2.1 Ethics statement

All animal experiment was approved by the Beijing Association for Science and Technology (approval ID SYXK (Beijing) 2007−0023) and complied with the guidelines of Beijing Laboratory Animal Welfare and Ethics of Beijing Administration Committee of Laboratory Animals.

2.2 Animals

Male ICR mice (20−22 g) and Balb/C mice (20−22 g) were purchased from Vital River Laboratory Animal Technology Co., Ltd. and housed for 3-day prior to experiments. Mice were kept in a 12 h light/dark cycle with controlled humidity (50%−70%) and temperature (20−24 °C). Food and water were available.

2.3 Reagents

Dextran sulphate sodium (DSS, MW 36 000−50 000) was obtained from MP Biomedicals, USA. Sulfasalazine (SASP) was purchased from Sanwei Pharmaceutical Co. Ltd. (Shanghai, China). The ELISA kits for mouse tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6) were purchased from R&D Systems (USA). The kits for biochemical analysis of myeloperoxidase (MPO), nitrogen monoxide (NO), superoxide dismutase (SOD), and reduced glutathione (GSH) were purchased from Jiancheng Bio-engineering Institute (Nanjing, China). Primary antibodies Nrf2, Kelch-like ECH-associated protein 1 (Keap 1), γ-glutamyl cysteine ligase (GCL), hemeoxygenase-1 (HO-1), IκB kinase α (IKKα), Lamin B and actin were purchased from Santa Cruz Biotechnology, Inc. (USA). Ploy clonal antibody against p65 NF-κB was purchased from Abcam, Inc. (USA). All other reagents used were of analytical grade.

2.4 Preparation of LFs

The raw materials of *Glycyrrhiza inflata* Bat. were collected from The Xinjiang Uygur Autonomous Region, China. The voucher specimen was identified by Prof. Xiao-guang Jia (Xinjiang Institute of Chinese Materia Medica and Ethical Materia Medica, China). The dried rhizomes of *G. inflata* (1.5 kg) were powered and extracted for three times with water at 100 °C (each for 3 h). The extracts were filtered and evaporated under reduced pressure for glycyrrhizic acid. Then the air-dried residues of G. *inflata* were refluxed twice (each for 1h) with 60% ethanol at 90 °C. The ethanol concentration of the extract was diluted to 35%−40% with water, then subjected to column (70 mm × 1800 mm) filled