Journal of Ethnopharmacology 162 (2015) 207–214

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

Research Paper

Anti-inflammatory effects of Huangqin tang extract in mice on ulcerative colitis

Peidong Chen*, Xi Zhou, Li Zhang, Mingqiu Shan, Beihua Bao, Yudan Cao, An Kang, Anwei Ding

Jiangsu Collaborative Innovation Center of Chinese Medicinal Resources Industrialization, Nanjing University of Chinese Medicine, Xianlin Road 138#, Nanjing 210046, China

ARTICLE INFO

Article history:
Received 3 April 2014
Received in revised form
30 October 2014
Accepted 22 December 2014
Available online 7 January 2015

Keywords:
Ulcerative colitis
Huangqin Tang extract
Cyclo-oxygenase-2
Myeloperoxidase
Tumor necrosis factor-α
Interleukin-1β

ABSTRACT

Ethnopharmacological relevance: HuangqinTang (HQT) is a traditional Chinese formula which is composed of *Scutellaria baicalensis* Georgi, *Paeonia lactiflora* Pall, *Glycyrrhiza uralensis* Fisch, and *Ziziphus jujube* Mill. HQT has been used in China for a wide range of disorders, especially in gastrointestinal inflammation with symptoms of nausea, vomiting, diarrhea, abdominal cramps and so on.

Aim of the study: To investigate the protective effects of HQT extract on 2, 4, 6-trinitrobenzenesulfonic acid (TNBS) induced colitis in mice.

Materials and methods: Different doses of HQT extract (1, 2 and 4 g/kg/day) and salicylazosulfapyridine (SASP, 500 mg/kg/day) were administered by gavage for 7 days after the induction of colitis with TNBS. The effects were studied by macroscopic score, histological analysis, immunohistochemical study of Cyclo-oxygenase-2 protein expression, as well as by determination of inflammation markers such as myeloperoxidase (MPO) and mRNA expression levels of pro-inflammatory cytokines, including TNF-α, IL-1β and IL-6.

Results: In TNBS induced group, mice body weight decreased gradually and did not recover at the end of the experiment, as compared with that of control group ($p < 0.01$). Edema and redness were also discovered in the colons profoundly and scores representing inflammation were all high in this group ($p < 0.01$). The level of colonic MPO activity and the tissue levels of TNF-α, IL-1β and IL-6 were markedly increased ($p < 0.01$). The mice treated with HQT extract and SASP recovered significantly compared with the TNBS group ($p < 0.01$).

Conclusion: Our results suggested that the efficacy of HQT extract, especially at the higher dose, was analogous to that of SASP, which implicated its potential application as a natural alternative medicine in colitis treatment.

1. Introduction

Ulcerative colitis (UC) is a relapsing non-transmural inflammatory disease restricted to the colon and is one of the idiopathic disease of inflammatory bowel disorders (IBD) (Baumgart and Sandborn, 2007). Although the precise cause of inflammatory bowel disease was unknown, the widely accepted hypothesis was that intestinal symbiotic bacteria triggered an inappropriate, overactive, and ongoing mucosal immune response which elicited intestinal tissue damage in genetically susceptible individuals (Duerr et al., 2006). It had been generally accepted that the pro-inflammatory cytokines such as tumor necrosis factor α (TNF-α), interleukins 1β, 6, 12 and 23(IL-1β, IL-6, IL-23) played a crucial role in UC development (Ebrahimi Daryani et al., 2014; Lee et al., 2012; Ordás et al., 2012). Anti-inflammatory drugs, such as aminosalicylates, corticosteroids and immunosuppressive agents, were frequently applied for the treatment of this disease. However, side effects were seriously and the recrudescence rates of IBD were rather high (Abdallah and Ismael, 2011; Liu and Wang, 2011). Therefore, new therapies should be developed and compounds from medicinal herbs were the drug candidates for treating this disease (Jia et al., 2014). Huangqin Tang (HQT) was a traditional Chinese formula which composed of 4 herbs: *Scutellaria baicalensis* Georgi, *Paeonia lactiflora* Pall, *Glycyrrhiza uralensis* Fisch and *Ziziphus jujube* Mill. This prescription had been used over a thousand years in China for a wide range of

Abbreviations: COX-2, cyclo-oxygenase-2; 5-FU, 5-fluorouracil; HE, hematoxylin and eosin; HQT, Huangqin Tang; HQTL, low dose of HQT extract with 1 g/kg; HQTM, medium dose of HQT extract with 2 g/kg; HQTH, high dose of HQT extract with 4 g/kg; IBD, inflammatory bowel disorders; IHC, immunohistochemical; IL-1β, interleukin 1β; IL-6, interleukin 6; MPO, Myeloperoxidase; SASP, salicylazosulfapyridine; TNBS, 2,4,6-trinitrobenzenesulfonic acid; TNF-α, tumor necrosis factor-α; UC, ulcerative colitis

* Corresponding author. Tel.: +86 25 85811512.
E-mail address: chenpeidong1970@163.com (P. Chen).

http://dx.doi.org/10.1016/j.jep.2014.12.039
0378-8741/

gastrointestinal ailments, including nausea, vomiting, diarrhea, and abdominal cramps (Zhang et al., 2010). HQT was demonstrated to have anti-diarrheal activity in irinotecan and 5-FU/leucovorin treated colorectal cancer patients and showed antitumor activity in pancreatic cancer (Saif et al., 2010). Flavonoids were the main constituents in *Scutellaria baicalensis* Georgi which were actively against inflammatory, virus and regulated immune system (Jeong et al., 2011; Yang et al., 2010;Yang et al., 2012a; Zeng et al., 2007). The total glucosides of paeony also exhibited both anti-inflammatory and immune-regulatory effects (Li et al., 2012; Zhou et al., 2012; Zhu et al., 2005). Flavonoids, triterpenoid saponins and isoflavonoid derivatives of glycyrrhiza exerted anti-inflammatory, anti-cancer and anti-oxidative effects (Hwang and Chun, 2012; Matsui et al., 2011; Seki et al., 2011). Triterpenoids and polysaccharides in *Ziziphus jujube* had anti-inflammatory and immunobiological activities (Lee et al., 2013; Li et al., 2013a). However, the anti-inflammatory effects of HQT extract, combination of these four herbs, on UC, had not been studied previously. In the present study, we assessed the protective effects of HQT extract on 2,4,6-trinitrobenzenesulfonic acid (TNBS) induced colitis by macroscopic score, histological analysis, immunohistochemical (IHC) study of Cyclo-oxygenase-2 (COX-2) protein expression, as well as by determination of inflammation markers such as myeloperoxidase (MPO) and the mRNA expressions of pro-inflammatory cytokines, such as TNF-α, IL-1β and IL-6.

2. Materials and methods

2.1. Plant materials and reagents

Component herbs of HQT: the root of *Scutellaria baicalensis* Georgi (Lamiaceae), *Paeonia lactiflora* Pall (Ranunculaceae); the root and rhizome of *Glycyrrhiza uralensis* Fisch (Leguminosae); and the fruit of *Ziziphus jujube* Mill. (Rhamnaceae) were collected in Neimenggu, Anhui, Shandong and Hebei province, the voucher specimens number were HQ-20121201, BS-20120809, ZGC20121106 and DZ20121126 respectively. All specimens were identified by Professor Dekang Wu, department of medicinal plants, Nanjing university of Chinese medicine and deposited at the laboratory for chemistry of Chinese medicine, Nanjing university of Chinese medicine. Baicalin, paeoniflorin, oleanolic acid, ursolic acid and glycyrrhetinic acid were purchased from China institute for control of pharmaceutical and biological products (Beijing, China). Oxypaeoniflora, albiflorin, betulinic acid, glycyrrhizic acid, licochalcone A, betulinic acid, liquiritigenin, isoacteoside, acteoside and chrysin were purchased from Chengdu Mansite Co. Ltd. (Chengdu china). Wogonin, oroxylin A and baicalein were isolated and purified from *Scutellaria baicalensis* Georgi in our laboratory. TNBS was obtained from Sigma Chemical Co. Ltd. (St. Louis, MO, USA). Salicylazosulfapyridine, commonly known as sulfasalaxine or SASP, was supplied by Sunve Pharmaceutical Co. Ltd. (Shanghai, China).

2.2. Preparations of HQT extractions

According to the original prescription, the formula was made from *Scutellaria baicalensis* Georgi, *Paeonia lactiflora* Pall, *Glycyrrhiza uralensis* Fisch, *Ziziphus jujube* Mill., and the ratio was 3:2:2:3. *Scutellaria baicalensis* Georgi (300 g) was extracted twice with 50% ethanol (each 2500 mL for 2 h) and then transferred to D101 macroporous resin column (150 cm × 8 cm) chromatography eluted with 80% ethanol to provide a fraction with 11.87% yield (35.6 g). *Paeonia lactiflora* Pall (200 g) and *Glycyrrhiza uralensis* Fisch (200 g) were extracted twice with 50% and 70% ethanol respectively under reflux (each 1800 mL for 2 h), and then transferred to AB-8 macroporous resin column chromatography eluted with 80% ethanol to afford the fraction of total glycoside and flavonoids (9.96%, 11.5 g and 15.47%, 30.9 g, respectively). *Ziziphus jujube* Mill. (300 g) was extracted twice with 90% ethanol under reflux (each 2500 mL for 2 h) to provide the a fraction with 7.62% yield (22.9 g), the residue extracted with water three times (each 2500 mL for 1 h), the solution obtained was concentrated and added 10 times amount of 95% ethanol, stood for a night and concentrated to get polysaccharide with 22.70% yield (67.3 g). The HQT extracts were obtained after mixing all of the fractions mentioned above. The fraction was concentrated under reduced pressure to dryness and then suspended in 0.5% CMC-Na solution for pharmacological evaluation afterwards.

2.3. HPLC analysis

Chromatographic analysis was performed on a Waters2695 series HPLC system equipped with a Waters2489 UV detector. The chromatographic separation was performed on a Megres C18 (4.6 mm × 150 mm, 5 μm) with the column temperature at 25 °C. The flow rate was set at 1 mL/min and the injection volume was 10 μL. Isocratic analysis was applied for baicalin determination by using methanol – water–phosphoric acid (47:53:0.2) system and acetonitrile −0.1% phosphate (14:86) for paeoniflorin. Gradient analysis for glycyrrhizic acid determination was applied by using acetonitrile (A) and 0.1% phosphate in water (B). The detailed gradient conditions were listed below: 0–8 min,19%A; 8–35 min,50%A; 35–36 min,100%A; 36–40,19%A.

2.4. HPLC–MS analysis

The HPLC/MS/MS system was an Agilent 1290 infinity LC system consisted of a binary pump with integrated degasser, and an Agilent 6460 Triple Quadrupole LC/MS system with Agilent Jet Stream Technology. Agilent MassHunter workstation software (Version B.03.00) was used for instrument control and data acquisition. Analytical was performed on a Kromasil C18 column (250 mm × 6 mm, 5 μm). Column temperature was set at 30 °C and auto-sampler temperature was set at 4 °C, and the flow rate was 0.6 mL/min. The mobile phase consisted of 0.05% formic acid in water (A) and acetonitrile (B): 0–10 min, 85→80% A; 10–35 min, 80→75% A; 35–55 min, 75→70% A; 55–65 min, 70→60% A; 65–90 min, 60→35% A; 90–92.5 min, 35→5% A; 92.5–102.5 min, 5% A; 102.5–110 min, 5→85% A; 110–115 min, 85% A. Injection volume was set as 3 μL and wavelength was set as 254 nm. Mass spectrometer was operated using Agilent Jet Stream electrospray ionization (ESI) source in the negative ion mode for detection. Detailed MS parameters were as follows: drying gas (nitrogen) temperature, 350 °C, drying gas flow, 10 L/min, sheath gas (nitrogen) temperature, 320 °C, sheath gas flow, 11 L/min, nebulization gas pressure, 40 psi, capillary voltage, 3500 V.

2.5. Quantification of baicalin, paeoniflorin, glycyrrhizic acid and polysaccharide in individual herb of HQT extract

The contents of baicalin, paeoniflorin and glycyrrhizic acid in HQT extract were quantitatively analyzed. The extract residue was centrifuged at 15,000 rpm for 10 min, and then the supernatant was analyzed by HPLC. The contents of baicalin, paeoniflorin, glycyrrhizic acid in each extract were 19.87%, 12.04% and 12.33% respectively. Polysaccharide in *Ziziphus jujube* Mill was determined on an instrument of Perkin-Elmer Lambda 35, and the wavelength was set at 490 nm. The content of polysaccharide was 49.98%.

2.6. Animals

Male balb/c mice (20–24 g) were obtained from animal multiplication center of Qinglong Mountain (Nanjing, China). The mice were housed in an air-conditioned room at 22–24 °C with a 12 h