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Synergistic effects of rhubarb-gardenia herb pair in cholestatic rats at pharmacodynamic and pharmacokinetic levels

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Rhein (PubChem CID: 10168)
Aloe emodin (PubChem CID: 10207)
Emodin (PubChem CID: 3220)
Chrysophanol (PubChem CID: 10208)
1,8-dihydroxyanthraquinone (PubChem CID: 2950)

ABSTRACT

Ethnopharmacological relevance: Herb pair serves as the basic building block of a traditional Chinese medicine (TCM) formula. The rhubarb–gardenia herb pair (RGHP), composed of rhubarb and gardenia, has meaningful clinical effects to cure cholestasis diseases. This study was designed to confirm the expected synergistic effects of RGHP at pharmacodynamic and pharmacokinetic levels.
Materials and methods: Thirty male Sprague-Dawley rats were divided into control, model and drug-treated groups. After intragastrically administrated with α-naphthylisothiocyanate (ANIT) to induce cholestasis, rats were treated with rhubarb, gardenia or RGHP. For pharmacodynamic study, biochemical and histopathological tests were performed to assess the hepatoprotective effects. While for pharmacokinetic study, a LC–MS method was developed for determination of five main chemical markers, namely genipin, rhein, aloe emodin, emodin and chrysophanol in rat plasma.
Results: The biochemical and histopathological tests suggested that RGHP exerted enhanced hepatoprotective effects against the ANIT-induced cholestasis compared with single herbs. The pharmacokinetic study indicated RGHP could significantly elevate systemic exposure level and prolong retention time of five markers in comparison with rhubarb or gardenia alone.
Conclusions: The present study demonstrated the synergistic effects of RGHP in ANIT-induced cholestatic rats at pharmacodynamic and pharmacokinetic levels, and has significant enlightenments for the rational use of the related TCM formulas containing RGHP.

1. Introduction

Unveiling active components and the underlying mechanism of traditional Chinese medicine (TCM) formula is a key issue to its modernization and globalization, while the research has faced many obstacles due to the unimaginable complexity of multi-herb formula. Especially for those ones comprising dozens of herbs, to entirely identify their active components could be fairly difficult. For simplification purposes, the study of herb pair may act as a pointcut in the investigation of TCM formula (Wang et al., 2012a). Herb pair (Chinese name: yaodui), referring to a unique combination consisting of two or three herbal drugs, has special clinical significance in TCM (Deng et al., 2008; Ung et al., 2007). Guided by TCM theories, herb pair is typically formed by combining herbs with similar or opposite nature and action so as to exert enhanced or depressed pharmacological effects. In a typical formula, herb pair usually serves as the basic building block. Taking Liuwei-Dihuang Pills, the most highly prescribed pharmaceutical preparation in China as an example, it consists of six ingredients forming two set of herbal combinations: the tonifying combination (*Rehmannia glutinosa* root, *Cornus officinalis* fruit, and *Dioscorea opposite* rhizome) and the eliminating combination (*Alismatis orientalis* rhizome, *Poria*, and *Paeonia suffrutcosa* cork). The expected synergistic effects between the tonifying and eliminating combinations for treating inflammation, diabetic, hypertension, amnesia have been proved by a wealth of modern pharmacological studies (Hsu et al., 2014; Liu et al., 2013; Perry et al., 2014; Wang et al., 2012b).

The rhubarb-gardenia herb pair (RGHP) is composed of two commonly used herbs: the rhubarb is described in Chinese Pharmacopoeia as the dried root and rhizome of *Rheum palmatum* L., *Rheum tanguticum* Maxim. ex Balf., or *Rheum officinale* Bail. (Family Polygonaceae), the gardenia is officially documented as the dried ripe fruit of *Gardenia jasminoides* Ellis (Family Rubiaceae). According to TCM theories, both rhubarb and gardenia belong to “cold and bitter” herb, and have the similar functions of reducing heat in blood and counteracting toxicity, eliminating damp-heat

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and removing heat-toxicity from blood (Chinese Pharmacopoeia Committee, 2010). The former is mainly prescribed as a purgative for the treatment of fever with constipation, retention of feces and abdominal pain, jaundice caused by damp-heat; the latter is commonly used in conditions such as febrile diseases with restlessness, jaundice with dark urine and hematuria with difficult painful urination. Therefore, many classical formulas including Yinchenhao Decoction (consisting of *Artemisia capillaris* herb and RGHP), Zhizi-Dahuang Decoction (consisting of *Citrus aurantium* fruit, fermented soybean and RGHP) and Dahuang-Xiaoshi Decoction (consisting of *Phellodendron chinense* cortex, saltpeter and RGHP) are formed for the treatment of cholestatic liver diseases. Although the pharmacodynamic and pharmacokinetic researches of Yinchenhao Decoction and Zhizi-Dahuang Decoction have been conducted extensively (Cao et al., 2009; Lv et al., 2012; Zhang et al., 2011), the synergistic effects between rhubarb and gardenia in cholestatic conditions has so far not been experimentally supported.

The co-research of pharmacodynamic and pharmacokinetic is helpful to deeply understand the mechanism of TCM. In the present study, the possible synergistic effects were investigated by pharmacodynamic and pharmacokinetic comparisons between RGHP and the two individual herbs (rhubarb or gardenia) alone. For pharmacodynamic study, six biochemical indicators, *viz.* total bilirubin (TBIL), direct bilirubin (DBIL), total bile acid (DBA), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), along with the liver histopathology in α-naphthylisothiocyanate-induced (ANIT) cholestatic rats were measured and evaluated. While for pharmacokinetic study, a LC–MS method was developed and validated for determination of five main chemical markers, namely genipin, rhein, aloe emodin, emodin and chrysophanol in rat plasma, and the pharmacokinetic parameters including AUC, MRT, $t_{1/2z}$, T_{max}, CL/F and C_{max} were compared.

2. Materials and methods

2.1. Materials, reagents and animals

The decoction pieces of rhubarb and gardenia were purchased from Bozhou traditional Chinese medicine market (Anhui Province, China). These two herbal drugs were unambiguously authenticated to be the rhizome of *Rheum palmatum* L. and the fruit of *Gardenia jasminoides* Ellis respectively, using the macroscopic and microscopic identification methods along with TLC analysis documented in Chinese Pharmacopoeia (Chinese Pharmacopoeia Committee, 2010) by Prof. Hui-Jun Li, China Pharmaceutical University. The representative specimens (No. R201403 for rhubarb and No. G201403 for gardenia) were deposited in State Key Laboratory of Natural Medicines. ANIT (purity > 95%) and sulfatase (type H-1, 10,000 units/g, from *Helix pomatia*, containing 300,000 units/g of β-glucuronidase) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The standards (purity > 98%) of genipin, geniposide, rhein, aloe emodin, emodin, chrysophanol and 1,8-dihydroxyanthraquinone (IS) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Their structures are shown in Fig. 1. Methanol and formic acid were of HPLC grade and obtained from Tedia (Fairfield, USA). Ultrapure water (18 MΩ) was purified with a Milli-Q system (Millipore, Milford, MA, USA). The other solvents and chemicals were of analytical grade or better.

Male Sprague-Dawley (SD) rats, weighing 240–280 g, were provided by Sino-British Sippr/BK Lab Animal Ltd. (Shanghai, China). The animal studies were conducted in accordance with the Provision and General Recommendation of Chinese Experimental Animals Administration Legislation and were approved by Department of Science and Technology of Jiangsu Province (license number: SYXK(Su) 2012–0005). These animals were housed under controlled conditions (temperature 22 ± 2 °C, relative humidity 50 ± 10%) with a natural light-dark cycle for one week before the experiment was carried out.

2.2. Instrumentation and conditions

The quality control (QC) samples and the real plasma samples were analyzed by a LC–MS system consisted of an Agilent 1200 series HPLC system (Agilent Technologies, Waldbronn, Germany) coupled to a SL G1946D quadruple mass spectrometer (Agilent Technologies, USA) equipped with an electrospray ionization source. Data were acquired and analyzed by Agilent ChemStation Software Version A.01.00 (Agilent Technologies, USA). Chromatographic separation was achieved on an Inertsil ODS-SP column (5 μm, 150 × 4.6 mm). The mobile phase consisted of 5 mM ammonium acetate (A) and methanol (B) at 0.8 mL/min with a gradient as follows: 0.00–2.00 min, 52% B; 2.01–2.50 min, 52–80% B; 2.51–12.00 min, 80% B; 12.01–12.50 min, 80–95% B; 12.51–18.50 min, 95% B. The MS was operated as follows: drying gas (N_2) flow rate, 11 L/min; drying gas temperature, 350 °C; nebulizing gas (N_2) pressure, 35 psi; capillary voltage, 3500 V; quad temperature, 100 °C. The analytes were monitored using negative selected ion monitoring (SIM) at m/z 225.0 for genipin $[M\text{-H}]^-$, m/z 282.9 for

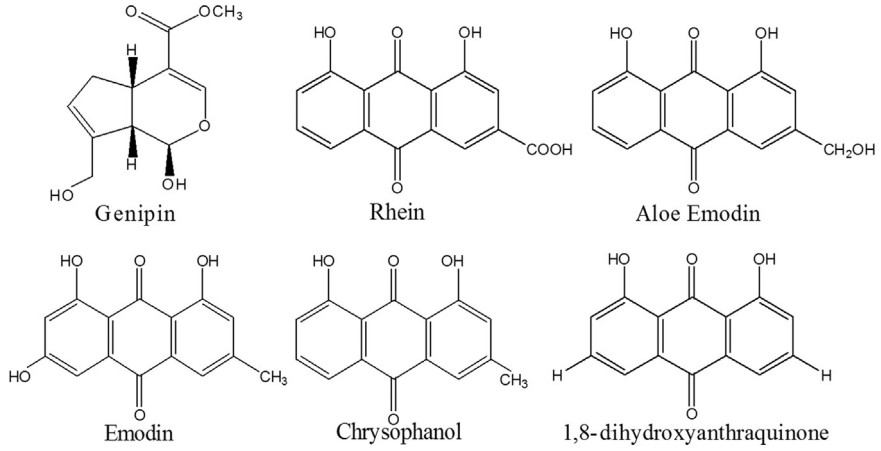

Fig. 1. Chemical structures of genipin, rhein, aloe emodin, emodin, chrysophanol and 1,8-dihydroxyanthraquinone (IS).