Journal of Ethnopharmacology 154 (2014) 584–592

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

The effects of *Glycyrrhizae uralenis* and its major bioactive components on pharmacokinetics of daphnetin in *Cortex daphnes* in rats

Wen Zhang [a,b,c], Liu-qing Di [a,b,c,*], Jun-Song Li [a,b,c], Jin-jun Shan [d], An Kang [a], Shuai Qian [e], Le-tian Chen [a]

[a] School of Pharmacy, Nanjing University of Chinese Medicine, Nanjing 210023, PR China
[b] Jiangsu Engineering Research Center for Efficient Delivery System of TCM, PR China
[c] Nanjing Engineering Research Center for Industrialization of Chinese Medicine Pellets, PR China
[d] First Medicine School, Nanjing University of Chinese Medicine, Nanjing 210046, PR China
[e] School of Pharmacy, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, PR China

ARTICLE INFO

Article history:
Received 6 November 2013
Received in revised form
15 March 2014
Accepted 19 March 2014
Available online 3 April 2014

Keywords:
Comparative pharmacokinetics
Daphnetin
Glycyrrhizin
Liquiritin
Rheumatoid arthritis
UPLC–MS/MS

Chemical compounds studied in this article:
Daphnetin (Pubchem CID: 5280569)
Daphnin (Pubchem CID: 439499)
Glycyrrhizin (Pubchem CID: 14982)
Liquiritin (Pubchem CID: 503737)
Liquiritigenin (Pubchem CID: 114829)
Osthole (Pubchem CID: 10228)

ABSTRACT

Ethnopharmacological relevance: *Glycyrrhizae uralenis* (GU) is often prescribed together with *Cortex daphnes* (CD) in traditional Chinese medicinal practice to increase the efficacy of CD on the treatment of rheumatoid arthritis (RA), but the reasons were still unknown. In order to clarify the rationality of herbaceous compatibility between CD and GU, the comparative evaluations on pharmacokinetic behaviors of daphnetin (a predominantly active ingredient in CD) after intragastric administration of CD and CD–GU (combination of CD and GU) extract were studied. In addition, the effects of glycyrrhizin and liquiritin, active ingredients of *Glycyrrhiza* triterpenes and *Glycyrrhiza* flavones respectively, on the pharmacokinetics of daphnetin were also investigated.

Materials and methods: Five groups of rats were orally administered with CD extract, CD–GU extract, pure daphnetin, co-administration of daphnetin and glycyrrhizin as well as co-administration of daphnetin and liquiritin at the same single dose of daphnetin (20 mg/kg). The rat plasma concentrations of daphnetin were determined by our developed UPLC–MS/MS method. The pharmacokinetics of daphnetin in above groups were investigated and compared.

Results: Comparing with oral administration of CD extract, *AUC* and T_{max} of daphnetin significantly increased after giving CD–GU ($p < 0.05$). In addition, in comparison to daphnetin alone, co-administration of daphnetin with liquiritin significantly increased the *AUC* and C_{max} of daphnetin for ~1.5-fold, while co-administered with glycyrrhizin showed limited impact on the pharmacokinetics of daphnetin.

Conclusions: In this study, it was found that liquiritin, one of the major components of GU, significantly enhanced the bioavailability of the main component daphnetin in CD. In addition, the bioavailability of daphnetin in the CD–GU prescription was also significantly higher than that in CD alone, which could be due to liquiritin. Such results explained the mechanism of the increased efficacy in treating RA with the combined use of CD and GU.

1. Introduction

Traditional Chinese herbal medicine (TCHM) has been widely used for the treatment and prevention of various diseases for over thousands of years. In clinical practice, TCHM is prepared in decoctions with specific combination of different herbs as a formula to maximize synergistic effects and minimize adverse reactions.

Cortex daphnes (CD, *Zushima* in Chinese) is the stem cortex and root bark of *Daphne giraldii* Nitsche, *D. tangutica* Maxim and *D. retusa* Hemsl (Li et al., 2002). Clinically, CD has been used as a TCHM for treatment of ache, traumatic injury and rheumatoid arthritis (RA) in China for very long period (Gao et al., 2008), and also as a folk medicine for lumbago treatment and fever reduction in Turkey (Süntar et al., 2012). Daphnetin (Fig. 1), as a major component of CD, has many biological activities, such as anti-inflammatory (Witaicenis et al., 2013), antioxidant (Thuong et al., 2010),

Abbreviations: CD, *Cortex daphnes*; GU, *Glycyrrhizae uralenis*; CD–GU, combination of CD and GU; TCHM, traditional Chinese herbal medicine; CYP, cytochrome; UGT, UDP-glucuronosyltransferase; C_{max}, peak plasma concentration; T_{max}, time to C_{max}; AUC_{0-t}, area under the curve from 0 to time; $AUC_{0-\infty}$, area under the curve from 0 to infinity; $T_{1/2z}$, terminal elimination half-life; *MRT*, mean residence time

* Correspondence to: Nanjing University of Chinese Medicine, 138 Xianlin Road, Nanjing 210046, PR China. Tel.: +86 25 85811071; fax: +86 25 85811067.
E-mail address: diliuqing928@163.com (L.-q. Di).

http://dx.doi.org/10.1016/j.jep.2014.03.047
0378-8741/

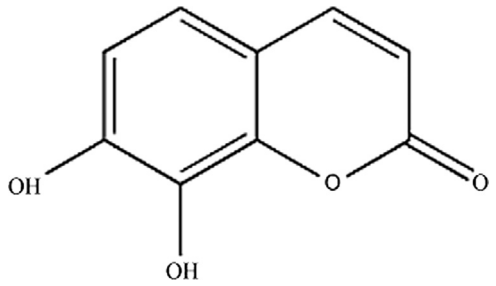

Fig. 1. Chemical structure of daphnetin.

antimicrobial (Cottiglia et al., 2001), and antimalarial properties (Huang et al., 2006) as well as protein kinase inhibition (Finn et al., 2004).

Glycyrrhizae uralenis (GU, Gancao in Chinese), derived from the root and rhizome of *G. uralenis* Fisch., is prescribed in many Chinese traditional formulas for its medical potential in the treatment of anti-inflammation (Fu et al., 2013), immunoloregulatin (Lee et al., 2009), and anti-allergic effects (Shin et al., 2007). Phytochemical investigations revealed that revealed triterpenes and flavones are regarded as the principal components responsible for the main pharmacological activities of GU (Tu et al., 2010; Wang et al., 2013). Glycyrrhizin and liquiritin are the major active component of these triterpenes and flavones, respectively (Zhang and Ye, 2009).

The combined use of CD and GU in treating RA has been reported in various literatures, e.g. *Handbook of Ningxia Chinese Herbal Medicine* (Editorial Board of Handbook of Ningxia Chinese Herbal Medicine, 1971) and *Journal of Shaanxi Traditional Chinese Medicine* (China Academy of Chinese Medical Sciences, 1962), which are famous in the field of TCHM. CD is often clinically used in combination with GU to produce better efficacy in curing RA (Gansu Institute for Drug Control, 1978; Meng et al., 2012). Our preliminary pharmacodynamic studies demonstrated that the effect of CD in curing adjuvant-induced arthritis was increased when combining with GU in rat model. Meanwhile, CD–GU prescription possesses the optimal curative effect in treating RA at a ratio of three to two (3:2) of CD and GU in weight (unpublished). However, the mechanism of such enhancement was not clear and needed to be discovered.

Pharmacokinetic study is a useful tool to explain and predict various events related to the efficacy of drugs and thus are valuable to evaluate the rationality and compatibility of herbs or prescriptions (Wu et al., 2009). Recently, several reports focus on how some components in the formulas interact with other ingredients (Xu et al., 2013). For example, the bioavailability of osthole was significantly increased when orally administered with Bushen Yizhi decoction compared to pure osthole (Zhang et al., 2014). The ingredients from Baihe might promote the bioavailability of timosaponin B-II and timosaponin A-III after administration of Zhimu–Baihe herb pair compared with Zhimu extract (Liu et al., 2013). However, it was still unclear how the synergistic effect is generated in biopharmaceutics when CD and GU are used together in treating RA.

Previously, we reported the pharmacokinetics of pure daphnetin in rat plasma after oral administration (Shan et al., 2009). Given the complexity of chemicals in CD–GU decoction, some herbal ingredients in GU may affect the pharmacokinetic profile of daphnetin in CD. So it is important to investigate the pharmacokinetic behaviors of daphnetin following intragastric administration of CD and CD–GU extract. Additionally, glycyrrhizin and liquiritin, which are also the main components in CD–GU prescription, are used as phytochemical markers for the quality control of GU in Chinese Pharmacopoeia Commission (2010). Thus, it is necessary to compare the pharmacokinetics of daphnetin in the plasma to explore whether glycyrrhizin or liquiritin could affect the pharmacokinetic behavior of daphnetin, which have not been reported previously. It is expected that the results of this study would be helpful for proving the rationality of CD–GU prescription from the perspective of biopharmaceutics and promoting further pharmacological studies of daphnetin.

2. Experimental

2.1. Materials and reagents

Daphnetin (batch number 110900-201006, purity 98%), osthole (IS, internal standard, batch number 110822-200305, purity 98%) were supplied by National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Daphnetin BPC (batch number 007-121129), glycyrrhizin (batch number 004-121213) and liquiritin (batch number 009-121124) were obtained from Chengdu Ruifinsi, Biotechnology Co., Ltd. Their purities, determined by HPLC, were higher than 95.0%. HPLC grade of formic acid and acetonitrile were purchased from Merck kGaA (Darmstadt, Germany). Distilled deionized water was freshly generated by a MilliQ Ultra Pure Water System (Millipore, Billerica, USA). All other reagents were of analytical grade.

CD (batch number 20120812) was purchased from the Shaanxi Pharmaceutical Company (Shaanxi, China). GU (batch number 20120901) was purchased from Anhui Wan Sheng Chinese Medicine YinPian Co., Ltd. (Anhui, China). These plant materials were authenticated by Dr. Jianwei Chen (Department of pharmacy, Nanjing University of Chinese Medicine, Nanjing, China). The voucher specimens were deposited in Department of pharmacy, Nanjing University of Chinese Medicine.

2.2. Preparation of CD and CD–GU extract

CD and GU were cut into slices before using. After being crushed to pieces, the CD and CD–GU (3:2, *w*/*w*) prescriptions were each decocted twice with boiling water by refluxing for 2 h and 1 h. The ratio of the total weight of the herbs and the volume of water for the first and second repetition were 1:10 and 1:5, respectively. The combined aqueous solution were obtained by filtration and concentrated to contain about 2 mg/mL daphnetin under reduced pressure at 50 °C. The CD and CD–GU extract were kept in 4 °C for further investigations.

2.3. Contents of daphnetin, glycyrrhizin and liquiritin in CD and CD–GU extracts

The analyses were performed on a Waters 2695-2489 HPLC system (Waters Corp., Milford, MA, USA) consisting of a quaternary pump solvent management system, a UV detector, an autosampler and an on-line degasser.

To calculate the administration dose, the contents of daphnetin in CD extract as well as daphnetin, glycyrrhizin and liquiritin in CD–GU extract were quantified by our validated HPLC method. About 100 μL of aqueous extract were diluted with methanol for 20 times, followed by centrifugation (15,000 rpm for 10 min) and filtration (0.2 μm microporous membrane). An aliquot of 10 μL obtained filtrate was injected into HPLC-UV system for analysis. The column and autosampler temperature were set at 40 °C and 4 °C, respectively. Liquid chromatographic separations were achieved using a Hedera ODS-2 C_{18} column (4.6 × 250 mm, 5 μm) with acetonitrile (A) and water (0.05% H_3PO_4, B) as mobile phase at a rate of 1 mL/min. UV detection wavelength of daphnetin, glycyrrhizin and liquiritin were set at 327, 237 and 237 nm respectively (Chinese Pharmacopoeia Commission, 2010). The gradient program was as follow: 0–19 min, 83% B;