Journal of Ethnopharmacology 124 (2009) 635–638

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

Pharmacokinetic properties of hydroxysafflor yellow A in healthy Chinese female volunteers

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ARTICLE INFO

Article history:
Received 14 March 2008
Received in revised form
20 November 2008
Accepted 16 February 2009
Available online 4 March 2009

Keywords:
Hydroxysafflor yellow A
Safflor yellow
Safflower
Pharmacokinetic

ABSTRACT

Ethnopharmacological relevance: Hydroxysafflor yellow A (HSYA) was isolated from the dried flower of *Carthamus tinctorius* L. which was extensively used in traditional Chinese medicine to treat diseases due to blood stasis. However, there have been few detailed pharmacokinetic studies about HSYA on human beings.

Aim of the study: The aim was to investigate the pharmacokinetic characteristics of HSYA in healthy Chinese female volunteers.

Materials and methods: The volunteers were given intravenous infusion of single doses of safflor yellow injection (containing HSYA 35, 70 and 140 mg) in separate trial periods with 1 week washout period. The concentration levels of HSYA in plasma were determined with HPLC. Various pharmacokinetic parameters were estimated from the plasma concentration versus time data using non-compartmental methods.

Results: The C_{max} values were 2.02 ± 0.18, 7.47 ± 0.67 and 14.48 ± 4.71 μg/mL after the administration of single doses of 35, 70, and 140 mg of HSYA, respectively. The corresponding values of AUC_{0-15h} were 6.57 ± 1.20, 25.90 ± 4.62 and 48.47 ± 12.11 μg/(mL h^{-1}), and the values of $t_{1/2}$ were 3.21 ± 1.26, 3.33 ± 0.68 and 2.98 ± 0.09 h. The Student–Newman–Keuls test results showed that C_{max} and AUC_{0-15h} were both linearly related to dose.

Conclusions: In this study, the pharmacokinetic properties of HSYA are based on first-order kinetics over the dose range tested.

1. Introduction

The dried flower of the safflower plant, *Carthamus tinctorius* L. has been used extensively in traditional Chinese medicine for its purported ability to improve blood flow and to treat coronary heart disease, hypertension, and cerebrovascular and gynecological disease (Lou and Liu, 1956; Liu et al., 1992; Kohei et al., 2000; Liang, 2004; The State Pharmacopoeia Commission of China, 2005). The compounds existing in safflower include chalcones and flavonoids. Phytochemical investigation shows that safflor yellow is the main constituent in water-soluble extract of safflower (Li and Chen, 1998; Liu et al., 2005). Safflor yellow consists of hydroxysafflor yellow A (HSYA), safflor yellow B, safflomin A, etc. and has a wide range of pharmacological activities, including coronary dilatation, antioxidation, myocardial and cerebral protection and immunosuppressive activity (Liu et al., 1992; Li and Chen, 1998; Yang et al., 2001; Jin et al., 2004). Hydroxysafflor yellow A [Fig. 1(A)], the main active component of safflor yellow, has been demonstrated to have the activities of antioxidation and myocardial and cerebral protective effect (Zhu et al., 2003; Wei et al., 2005; He et al., 2008). HSYA was chosen as an active marker component for controlling the quality of safflower in Chinese Pharmacopoeia (The State Pharmacopoeia Commission of China, 2005).

Safflor yellow for injection (containing HSYA 35 mg) was approved as a new drug by the State Food and Drug Administration for treating patients with ischemic cardio-cerebrovascular disease in 2005. Some pharmacokinetic studies of HSYA have been performed on animals in recent years (Chu et al., 2006; Li et al., 2007). However, there has been no report studying the pharmacokinetics properties of HSYA in humans by now. Therefore, it is important to investigate the pharmacokinetic characteristics of HSYA in humans for reasonable clinical application. The aim of our study was to assess the pharmacokinetic properties of a single intravenous infusion dose of HSYA in healthy Chinese female volunteers.

2. Materials and methods

2.1. Materials

Safflor yellow for injection (containing 35 mg HSYA per vial) was supplied by Yongning pharmaceutical Co., Ltd. (Zhejiang,

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0378-8741/$ – see front matter © 2009 Published by Elsevier Ireland Ltd.
doi:10.1016/j.jep.2009.02.026

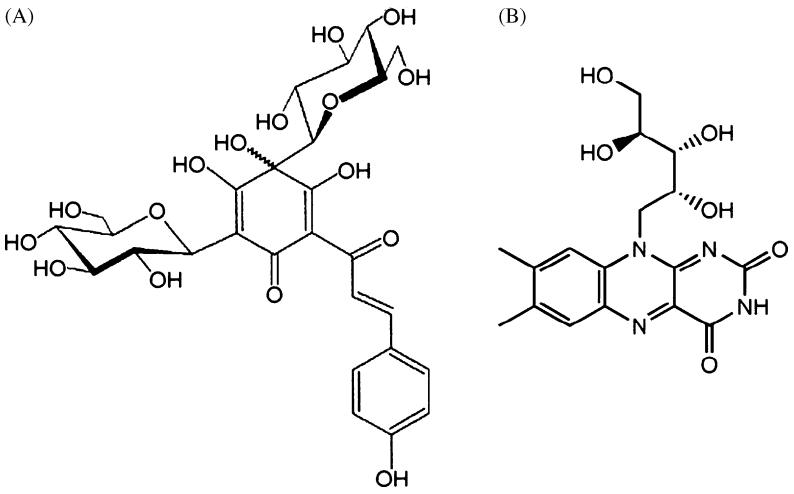

Fig. 1. The chemical structure of hydroxysafflor yellow A (A) and internal standard riboflavin (B).

China). HSYA standard was purchased from Shandong Lvye Natural Medicine Research and Development Center (Shandong, China) with 98.0% purity. The internal standard [Fig. 1(B)] was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). 0.9% normal saline was obtained from Shijiazhuang No.4 Pharmaceutical Co. Ltd. (Hebei, China). Acetonitrile of HPLC-grade was purchased from Fisher Scientific (Pittsburgh, PA, USA). Water was distilled and purified using a Milli-Q water purification system (Millipore, Bedford, MA, USA).The Potassium dihydrogen phosphate (KH_2PO_4), *ortho*-phosphoric acid and perchloric acid were all of analytical grade. Drug-free human plasma from healthy donors was kindly provided by the Blood Center of Xijing Hospital (Shaanxi, China).

2.2. Study protocol and volunteers

This single-dose, randomized, parallel, open-label, three-way crossover study was conducted at phase I clinical trial center of Xijing Hospital, the Fourth Military Medical University, Shaanxi, China. The study protocol was approved by the Ethics Committee of Xijing Hospital and written informed consent was obtained from each volunteer after full explanation of the objectives and design of the study. The study was conducted in accordance with the guidelines on Good Clinical Practice (European Agency for the Evaluation of Medicinal Products, 2005) and the ethical standards for human experimentation established by the Declaration of Helsinki and its amendments (World Medical Association Declaration of Helsinki, 2005).

Healthy Chinese female volunteers (aged 25–39 years) were recruited. The volunteers were free from significant cardiac, hepatic, renal, pulmonary, neurologic, gastrointestinal and hematologic disease, as determined by medical history, physical examination and routine laboratory tests (hematology, blood biochemistry and urinalysis). The volunteers were instructed to abstain from using any drug for 2 weeks before and during the study period and to abstain from smoking, alcohol, caffeine-containing food and beverages for at least 48 h before the study period.

2.3. Study drug administration

According to the randomized, parallel, open-label, and three-way crossover trial rule, a computer generated randomization schedule, and volunteers were allocated to receive single doses of safflor yellow injection (containing HSYA 35, 70 and 140 mg, respectively) intravenous infusion administered in separate trial periods with 1 week washout between study periods. After one overnight fast, the volunteers were administered a single dose of safflor yellow injection diluted with 250 mL 0.9% normal saline; no food was allowed until 4 h after administration and water intake was allowed 2 h after drug administration. Water, standardized meals were given to all volunteers according to a time schedule. Volunteers were under direct medical supervision at the study site and were ambulatory, but strenuous activity was prohibited.

2.4. Sample analysis

2.4.1. Collection

For HSYA assay, 4 mL blood samples were drawn into heparinized tubes through an indwelling cannula immediately before (0 h) and 0.5, 1, 1.25, 1.5, 2.0, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12 and 15 h after administration. Immediately after collection, blood samples were centrifuged (model TDZ4-ES, Xiangyi centrifuge instrument Co., Ltd. Hunan, China) at 1500 × *g* for 10 min at room temperature, and plasma was separated and stored at −20 °C in coded polypropylene tubes (Haimen Company, Jiangsu, China).

2.4.2. Preparation of standard and quality-control samples

The calibration curve in human plasma was prepared at 0.04, 0.08, 0.2, 0.4, 1, 2, 4, 8 and 20 μg/mL HSYA; the concentration of internal standard in plasma was 20 μg/mL. Quality-control samples of HSYA were freshly prepared in bulk at three concentrations of 0.4, 2 and 20 μg/mL, respectively.

2.5. Sample preparation

200 μL aliquot of plasma sample was added with 40 μL riboflavin (20 μg/mL) and 40 μL 50% methanol in a 1.5 mL tube. The mixture was vortexed for 30 s. 120 μL of 6% perchloric acid was added, vortexed for 2 min. The mixture was centrifuged at 12,000 × *g* for 10 min. 50 μL aliquot of the supernatant was injected into the HPLC system.

2.6. Apparatus and chromatographic conditions

Analyses were performed on a Shimadzu Scientific Instruments (Kyoto, Japan) liquid chromatographic system which was composed of a LC-10Avp binary pump, a SPD-10Avp variable wavelength detector and a computer system for data acquisition (LC-Solution). The analytical column employed was a Shim-pack VP-ODS C_{18} column (150 mm × 4.6 mm I.D., 5 μm particle size) protected with a