Fitoterapia 86 (2013) 202–207

Contents lists available at SciVerse ScienceDirect

Fitoterapia

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

Pharmacokinetics of puerarin in pregnant rats at different stages of gestation after oral administration

Li Cao [a,1], Jie Pu [a,1], Qing-Ri Cao [a], Bo-Wen Chen [b], Beom-Jin Lee [c], Jing-Hao Cui [a,*]

[a] College of Pharmaceutical Science, Soochow University, Suzhou, 215123, China
[b] Pharmaceutical preparation section, Suzhou Municipal Hospital, Suzhou, 215001, China
[c] College of Pharmacy, Ajou University, Suwon, 443-749, Republic of Korea

ARTICLE INFO

Article history:
Received 27 November 2012
Accepted in revised form 24 February 2013
Available online 14 March 2013

Keywords:
Puerarin
Pregnant rat
Pharmacokinetics

ABSTRACT

This study aims to observe the effects of gestational stage on the pharmacokinetics of puerarin after oral administration in rats. The pharmacokinetics of puerarin was studied in pregnant rats using a sensitive and reproducible high-performance liquid chromatography/ultraviolet method. The concentration–time curves in both normal and pregnant rats were fit into a two-compartment model. The results indicated that gestation influences the pharmacokinetics of puerarin at different levels, especially during the early stages of pregnancy. Furthermore, puerarin penetrates the placental barrier and maintains high concentrations in fetal rat plasma. Therefore, puerarin administration should be carefully considered in pregnant women.

1. Introduction

Puerarin (7,4′-dihydroxyisoflavone-8-β-glucopyranoside), with the chemical structure shown in Fig. 1, is the main active ingredient from the roots of *Pueraria lobata* (Willd.) Ohwi and *Pueraria thomsonii* Benth both of which have been used as traditional Chinese medicinal herbs for centuries. Various pharmacologic studies and clinical applications have indicated that puerarin has therapeutic effects on hypertension, diabetes, arteriosclerosis, and cerebral and myocardial ischemia [1–4]. Intravenous injection is the most common method for puerarin administration, although many researchers have attempted to improve its low bioavailability through oral administration using self-emulsifying microemulsions [5], solid lipid nanoparticles [6], phospholipid complexes [7], and hydroxypropyl–cyclodextrin complexes [8].

Pharmacokinetic studies on intravenously administered puerarin involving human volunteers showed a distribution half-life of 10.3 min, elimination half-life of 74 min, and mean residence time (MRT) of 1.28 h [9]. Therefore, the frequent intravenous injections administered in clinics could cause various side effects, especially in the treatment of chronic diseases. Side effects of puerarin injection have been detected in patients including fever, allergy, hemolysis, elevated transaminases, and acute renal failure [10,11].

Puerarin given alone or combined with other medicines for pregnancy-induced hypertension syndrome, gestational diabetes, and pre-eclampsia has been used and investigated [12–14]. Pharmacokinetic parameters such as the apparent volume of distribution (V_{app}), clearance, and elimination half-life ($T_{1/2,\beta}$), are affected by gestation because of changes in plasma volume and the protein binding ratio [15]. Animal and human pregnancy models exhibit variations in drug metabolism via the Cytochrome P450 and uridine diphosphate glucose full acid transferase pathways [16]. Furthermore, the insufficient information on drug permeability through the placental barrier and on fetal safety has attracted serious attention to drug administration among pregnant women. Therefore, the influence of gestation on the pharmacokinetics of traditional Chinese medicine and prescriptions should be investigated.

In this paper, the effect of gestational stage on the pharmacokinetics of oral puerarin administration was evaluated in rats. The plasma puerarin concentrations in fetal rats were also measured to determine drug permeability drug through

* Corresponding author. Tel./fax: +86 512 6588 2077.
E-mail address: jhcui@suda.edu.cn (J.-H. Cui).
[1] These authors contributed equally to this work.

0367-326X/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.fitote.2013.03.004

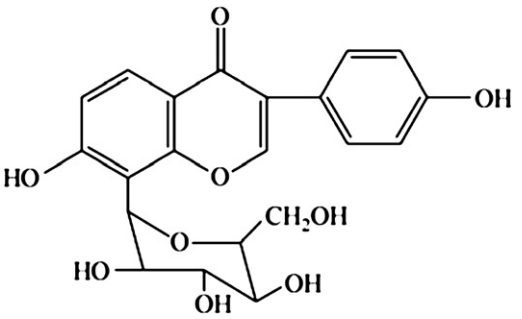

Fig. 1. Chemical structure of puerarin.

the placental barrier. The results of this study could provide beneficial preliminary information for the clinical administration of puerarin to pregnant women.

2. Materials and methods

2.1. Chemicals and reagents

Puerarin (HPLC purity > 98%) was provided by Xi'an Aiwo Biological Co., Ltd. (Xi'an, China). The puerarin reference standard was purchased from the National Chemicals and Biological Products Institute (Beijing, China). *p*-Hydroxybenzoic acid (internal standard, IS), citric acid, and sodium carboxymethylcellulose were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Methanol (high-performance liquid chromatography [HPLC] grade) was purchased from Honeywell Burdick and Jackson ACS (Shanghai, China). All other chemicals and solvents were of analytical grade and were used without further purification.

2.2. Standard and working solutions

Puerarin stock solution was prepared by dissolving 10.0 mg of puerarin in 50 mL of methanol. Working solutions were prepared from the stock solution by dilution in methanol. The *p*-hydroxybenzoic acid IS solution was diluted to a final concentration of 100 $\mu g \cdot mL^{-1}$ using methanol. Calibration standards at puerarin concentrations ranging from 1.0 $\mu g \cdot mL^{-1}$ to 100.0 $\mu g \cdot mL^{-1}$ were prepared by spiking the corresponding standard working solution with 100 μL of blank rat plasma.

2.3. Sample preparations

A 200 μL aliquot of the plasma sample was mixed with 20 μL of IS (100 $\mu g \cdot mL^{-1}$) in methanol, followed by 1 mL of acetonitrile. The mixture was vortexed for 5 min and centrifuged for 10 min at 15,000 rpm. The supernatant layer was transferred into an Eppendorf tube and evaporated to dryness at 40 °C under nitrogen. The residue was reconstituted with 100 μL of the mobile phase, and 20 μL of the sample solution was injected into the HPLC system for analysis.

2.4. HPLC system

Chromatographic analysis was conducted in a Shimadzu HPLC 15C system with a dual Essentia Pump, a spectral power distribution UV–Vis detector set to 252 nm, a 20 μL-injection loop, and a liquid chromatography solution workstation (Shimadzu, Suzhou, China). Chromatographic separation was achieved using a COSMOSIL 5 C18-MS-II reversed phase column (4.6 mm × 250 mm, 5 μm particle size) protected by an RP-18 guard column. The mobile phase consisted of 0.1% citric acid–methanol (26.5:73.5, v/v) filtered through a 0.45 μm Millipore filter and degassed prior to use. The flow rate was set to 1.0 $mL \cdot min^{-1}$ and column temperature was set to 40 °C.

2.5. Method validation

2.5.1. Linearity and sensitivity

The calibration curve was established through linear regression of the peak area ratio of puerarin to IS and the normal standard concentrations (0.1, 0.2, 0.5, 1.0, 2.5, 5.0, and 10.0 $\mu g \cdot mL^{-1}$) for puerarin with $1/x^2$ weighting factor, described as $Y = a + bX$.

The lowest limit of quantification (LLOQ) was defined as the amount that could be detected at a signal-to-noise ratio of 3. The LLOQ was determined in three replicates with a precision rate of less than 20% using the relative standard deviation (RSD) and an accuracy rate between 80% and 120% of the spiked concentration.

2.5.2. Accuracy and precision

The intraday and interday accuracies and precision rates were analyzed by determining quality control (QC) samples at three different concentrations (0.2, 1.0, and 5.0 $\mu g \cdot mL^{-1}$) over five consecutive days. The QC samples of the three different concentrations were tested using five replicates and calculated with calibration curves obtained daily. Accuracy is expressed as a percentage (% accuracy = [detected concentration / nominal concentration] × 100%). Precision was estimated as the percentage RSD. Precision was needed to be less than 15% and the accuracy needed to range from 85% to 115%.

2.5.3. Extraction recovery

Plasma samples were spiked with three different puerarin concentrations (0.2, 1.0, and 5 $\mu g \cdot mL^{-1}$) containing the IS. After the samples were processed according to the aforementioned method, the resulting peak areas were compared with those of a puerarin standard carried in the mobile phase to provide the recovery values.

2.5.4. Stability

The QC samples prepared at the three levels above were used to determine the short-term, long-term, and freeze–thaw stability. The concentrations of the stability samples were compared with corresponding spiked analyte concentrations. Short-term temperature stability was assessed by keeping the samples at room temperature for 4 h before sample preparation. Long-term stability was evaluated by keeping the samples at −40 °C for up to 2 weeks. Freeze–thaw stability was determined by freezing the samples at −40 °C and then thawing them at room temperature for 24 h through three cycles.

2.6. Pharmacokinetics in pregnant rats

Female specific pathogen-free Sprague–Dawley rats weighing 230 g to 270 g were obtained from the Shanghai