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Biopharmaceutics classification of puerarin and comparison of perfusion approaches in rats

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ABSTRACT

The present study was conducted to characterize the biopharmaceutics classification system (BCS) category of puerarin in terms of intrinsic dissolution rate (IDR) and rat intestinal permeability and to investigate the poor intestinal absorption probably related to the drug metabolism in the gut wall of rats. Equilibrium solubility of puerarin was determined in various phosphate buffers and water, and IDR was estimated by measuring the dissolution of a non-disintegrating compact. Intestinal permeability (P_{eff} and P_{blood}) of puerarin was determined using the technology of *in situ* single-pass intestinal perfusion (SPIP) and intestinal perfusion with venous sampling (IPVS) in fasted rats. Metabolism of puerarin in intestinal tissue was tested by S9 incubation *in vitro*. The aqueous solubility of puerarin in phosphate buffers and water was good with a maximum solubility of 7.56 mg/mL at pH 7.4. Obtained IDR values of puerarin were in the range of 0.360–1.088 mg/min/cm^2, with maximum and minimum IDR value of pH 7.4 and pH 4.0, respectively. The P_{eff} was 1.252×10^{-5} cm/s determined by SPIP and the P_{blood} was 0.068×10^{-5} cm/s by IPVS in jejunum at puerarin 80 μg/mL. The metabolism rate of puerarin determined by the intestinal S9 fraction indicated that the gut wall metabolism of puerarin is one cause of poor absorption. According to the proposed classification of drugs and the results obtained from equilibrium solubility, IDR, P_{eff} and P_{blood}, it is concluded that puerarin could be categorized IV drug of the BCS based on its low solubility and low intestinal permeability values.

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1. Introduction

The biopharmaceutics classification system (BCS) has been accepted and increasing implementation since it was carried out by Gordon L. Amidon (Amidon et al., 1995). With the adoption of the BCS by FDA (FDA, 2000) and EMA (EMA, 2007), the scientific framework of the BCS and its concept are widely accepted by more scientists. The provisional biopharmaceutical classification of herbs used in Western medicine (Waldmann et al., 2012) and Chinese herbal medicine (Fong et al., 2013) have been predicted by software programs. However, only few experimental studies of the BCS have concentrated on the active ingredients in herbs (Smetanova et al., 2009; Zhang et al., 2012). Puerarin (7,4′-dihydroxyisoflavone-8-glucopyranoside) is a major active ingredient in the Chinese herbal medicine, *Puerariae radix*, which comes from the kudzu root. Total cumulative amounts of the barren and its metabolites were only 3.6% excreted in the urine after oral administration (Yasuda et al., 1995). And lower blood concentration after administering puerarin or *Pueraria lobata* extract orally is found in many reports (Jiang et al., 2013; Li et al., 2013, 2006; Luo et al., 2011; Quan et al., 2007; Wang et al., 2013). Although all the investigations above carried out in the last few years show the poor oral absorption of puerarin, the basic physicochemical data coming from solubility and intrinsic dissolution rate (IDR) were still limited from the published literatures. And no data about the permeability of puerarin has been compared with different methods of *in situ* intestinal perfusion of rat. The BCS with aqueous solubility and intestinal permeability can be a useful tool to demonstrate the problem of absorption of puerarin. Furthermore, the categorization of puerarin by the BCS can be used to facilitate the future formulation development. In the present study, aqueous solubility, IDR property and intestinal permeability of puerarin were investigated, and the intestinal metabolism was also studied by incubation of intestinal S9 fractions. The overall objective of this

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research is to establish the basic framework in understanding of the BCS of the herbal active component, puerarin. And the approaches of permeability test by *in situ* intestinal perfusion of rat are compared.

2. Materials and methods

2.1. Materials

Puerarin was purchased from the Xi'an Zhongxin Biotechnology Co., Ltd. Shanxi, China. HEPES were provided by Amresco, U.S.A. HPLC (high-performance liquid chromatography) grade acetonitrile was obtained from Fisher Scientific. All other reagents were of analytical grade and were purchased from Sinopharm Chemical Reagent Co., Ltd. Shanghai, China, unless otherwise mentioned. Krebs–Ringer solution(2 L): 267 mM NaCl, 9.4 mM KCl, 32.6 mM $NaHCO_3$, 5.3 mM NaH_2PO_4, 0.4 mM $MgCl_2$, 6.7 mM $CaCl_2$, 15.6 mM Glucose. HEPES buffer (1.15% KCl, 100 mL): 20 mM HEPES, 15.4 mM KCl. The HPLC analyses were done using a Waters 515 series HPLC system (Milford, MA, USA).

2.2. Aqueous solubility

The equilibrium solubilities of puerarin were determined in buffers over a pH value ranging from 1.0 to 7.4 (0.1 mol/L HCl to pH 1.0, phosphate buffers was adjusted by H_3PO_4 or NaOH to pH 4.5, pH 6.8, pH 7.0 and pH 7.4). Excess puerarin was added to various buffer solutions in a vial placed in a water bath, then shaken at 37 °C. Samples were filtered through 0.45 μm membrane filter prior to analysis. The filtrate was diluted with corresponding buffer and the concentration of puerarin was determined by the validated HPLC method.

2.3. IDR measurement

A quantity of drug was prepared by compression of 200 mg of puerarin powder with a force of 8 Mpa for 1 min to make non-disintegrating compacts by using die and punch with a diameter of 8.0 mm. The surface area of the compacts was 0.5024 cm^2. After compression, compacts were placed in a molten beeswax-mold to ensure that the only one surface was exposed to the dissolution medium. Dissolution study was conducted using USP II dissolution apparatus with 900 mL dissolution media over the pH range 1.0–7.4 at a temperature of 37 °C with paddle rotate at 100 rpm. Samples were withdrawn with time intervals of 30 min through 0.45 μm syringe filters. The samples were analyzed by the validated HPLC method.

2.4. Animals and treatment

All animal experiments were performed at Beijing University of Chinese Medicine (BUCM) and conducted using protocols approved by the University Committee on Ethics in the Care and Use of Laboratory Animals, and the animals were housed and handled according to the Laboratory Animal Medicine guidelines of BUCM. Normal male Sprague-Dawley rats weighing 300–350 g were purchased from Vital River Laboratory Animal Technology Co., Ltd. China. Animals were kept under artificial light on a 12 h light/dark cycle and housed in rooms controlled between 23 ± 1 °C and 55 ± 5% relative humidity at the Laboratory Animal Center of BUCM. Rats were acclimated for at least 7 days with free access to animal chow and water before the study. Thenceforth were placed in individual cages with wide mesh floors and fasted overnight (water *ad libitum*) prior to the date of the experiment.

Rats were anesthetized with pentobarbital sodium (50 mg/kg) by intraperitoneal injection, then they were placed on a warming blanket and under a heating lamp to maintain body temperature during surgery and throughout the experiment. To sustain the anesthetic condition during the course, one third of the initial dose of pentobarbital sodium was administered throughout the remainder of the experiment.

2.5 *In situ* single-pass intestinal perfusion (SPIP)

SPIP studies were performed using established methods adapted from the literature (Ochsenfahrt and Winne, 1969). The abdomen of an anesthetized animal was shaved and a longitudinal midline incision of 3–4 cm was carefully made to expose the intestinal segments. The duodenum, jejunum and ileum were located respectively. The identification of the main parts of intestinal is: the duodenum from the pylorus to the ligament of Treitz, jejunum and ileum from the ligament of Treitz to ileocecal junction. As the difficulty to make a distinction between jejunum and ileum, the ileum segment was used about 10 cm long upwards ileocecal junction, the jejunum segment was selected about 10 cm in the middle of the ligament of Treitz to the site of ileum used. Careful handling to avoid disturbance of the intact blood supplying, the segment for surgery was located and the both ends were incised with a surgical scissors for cannula. Two silicone tubes were inserted through the small slits and sured. The segment was then rinsed with warm isotonic saline until the effluent was clear. As a means of expelling the remaining isotonic saline from the intestine, air was pumped slowly through the segment from a 50 mL airtight syringe. The exposed intestinal segment was kept moist by covering with a piece of sterilized gauze wetted with saline solution, and during the experiment, warm isotonic saline was also sprinkled on the gauze for many times by a syringe. After that, the inlet tubing was connected to a syringe pump (LSP02-1B Longer Pump, China). At the start of the study, the perfusion solution containing the drug was incubated in a 37 °C water bath to maintain the temperature. In order to assure a steady state, the perfusate was pumped at a flow rate of 0.2 mL/min for 30 min firstly. After reaching steady state, the perfused samples from the intestinal segment were collected at 15 min intervals up to 120 min. Following the termination of the experiment, the perfused intestinal segment was cut out, the length and radius of it were accurately measured. At last, animals were sacrificed by performing a bilateral thoracotomy.

2.6 *In situ* intestinal perfusion with venous sampling (IPVS)

The surgical procedures used to prepare the perfused rat jejunum with venous sampling are similar to those described methods adapted from the literature, with some modifications (Holmstock et al., 2012; Ochsenfahrt and Winne, 1969). Before perfusion surgical operation, the other five to seven rats were selected for donor blood per experiment, a total of 50–70 mL blood was obtained from the abdominal aorta with a heparinized syringe. The anticoagulation of blood collected from these donor rats was 1000 U/mL heparin solution. The blood incubated in a 37 °C water bath to maintain temperature was used to supply the blood loss via the mesenteric vein. The procedure of surgery preparation was the same as SPIP. Briefly, the left jugular vein of the anesthetized rat was exposed, isolated by blunt dissection, a cannula filled with heparinized saline (100 U/mL) was inserted approximately 1–2 cm into the vein and secured for the blood supply during the IPVS. Then the canal was connected to a peristaltic pump (BT 100-1F Longer Pump, China) which placed between the donor blood reservoir and the canal by a silicone tube filled with blood, and the other end of the silicone tube was immersed in the donor blood. The abdomen was shaved and a longitudinal midline incision of 3–4 cm was carefully made to expose the jejunum. The intestines were then placed to the rat's right, so that

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