Journal of Pharmacological and Toxicological Methods 70 (2014) 6–11

Contents lists available at ScienceDirect

Journal of Pharmacological and Toxicological Methods

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

Original article

Comparison of two approaches of intestinal absorption by puerarin

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ARTICLE INFO

Article history:
Received 15 December 2013
Accepted 13 March 2014
Available online 25 March 2014

Keywords:
Everted gut sac
In situ single-pass intestinal perfusion
In situ intestinal perfusion with venous sampling
Methods
Puerarin

ABSTRACT

Introduction: Everted gut sac (EGS) and in situ single-pass intestinal perfusion (SPIP) have been widely used in the study of drug absorption and biopharmaceutical classification systems (BCS). Furthermore, they could also be applied in the research of drug intestinal first-pass metabolism. Since most of Chinese herbal medicines (CHMs) are orally administrated, it is necessary to study the permeability of active ingredients of CHMs. Thus, we attempted to apply the EGS and SPIP models to study the permeability of puerarin, one of the active marker compounds (AMCs) of *Puerariae Radix*. **Methods:** In the present study, three rat models of ex vivo and in situ, EGS, SPIP, and in situ intestinal perfusion with venous sampling (IPVS), were established to determine the permeability coefficient of puerarin. The apparent permeability coefficient (P_{app}) was obtained by EGS. The SPIP model was used to determine the effective permeability coefficient (P_{eff}) in different intestinal segments. The blood permeability coefficient (P_{blood}) was determined by IPVS. Puerarin concentration of perfusion and blood samples were measured by HPLC. **Results:** Puerarin could filter into EGS incubated in aqueous extract of *Puerariae Radix* or puerarin solution. In the SPIP experiment, the concentration effect on P_{eff} was observed in the ileum, but not in the other three intestinal segments. The P_{blood} was $0.068 \pm 0.002 \times 10^{-5}$ cm/s and was 16-fold lower than the P_{eff} ($1.114 \pm 0.153 \times 10^{-5}$ cm/s) in the IPVS experiment at 80 μg/mL puerarin. As expected, the P_{eff} ($1.24 \pm 0.11 \times 10^{-5}$ cm/s) in SPIP did not differ from the P_{eff} in IPVS. The P_{app} was 0.199×10^{-5} cm/s at 1200 μg/mL puerarin, 10-fold lower than P_{eff} ($2.047 \pm 0.116 \times 10^{-5}$ cm/s) in SPIP. **Discussion:** Three models for permeability were successfully practiced in the study of puerarin absorption and our research strategy will be useful for herbal constituent absorption in the future.

1. Introduction

Although lots of innovations in the drug delivery system have came into being with the progress of pharmaceutics, oral administration still remains as the preferred route of administration by virtue of its convenience, low cost, and high patient compliance compared to alternative routes. However, compounds intended for oral administration must have adequate aqueous solubility and intestinal permeability in order to reach an appropriate bioavailability. In 1995, (Amidon, Lennernas, Shah, & Crison, 1995) first introduced the BCS on the basis of aqueous solubility and intestinal permeability, by which compounds were categorized into one of four biopharmaceutical classes (Class 1–Class 4). It is the core of BCS to approximately predict the absorption of compounds in the human gastrointestinal tract. In August 2000, U.S. FDA guidance (CDER, 2000) stated that the BCS approach could be used to justify biowaivers for highly soluble and highly permeable drug substances (i.e., Class 1). In this guidance, EGS and SPIP are two proposed models to determine the permeability of drugs. These models show good correlation to the in vivo intestinal permeability and the fraction of dosage absorbed in humans (Fagerholm, Johansson, & Lennernas, 1996; Lennernas, 1997). Yang Liu and other researchers (Balimane, Chong, & Morrison, 2000; Luo et al., 2013; Volpe, 2010) have well reviewed the methods for drug permeability. Just as those reviews, human Loc-I-Gut protocol is the golden criteria, cell monolayers and artificial membranes are allowed for high-throughput screening of passive permeability, and EGS and SPIP are widely used for investigating the complex absorption process. Additionally, some papers (Kanazu, Okamura, Yamaguchi, Baba, & Koike, 2005; Masaki, Hashimoto, & Imai, 2007; Suzuki & Sugiyama, 2000; Zhang & Benet, 2001) using the EGS or SPIP for studying drug absorption had reported that the low permeability of drugs was partly attributed to the interaction of drugs with intestinal CYP and P-gp as intestine disposition. Since most of CHMs are orally administrated, despite other routes of administration such as skin and

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http://dx.doi.org/10.1016/j.vascn.2014.03.170
1056-8719/

intravascular administration, what natural substance are absorbed into blood and how they are disposed of by the intestines must be clarified to ensure a safe and scientific medication of CHM. So far, it is a great challenge for the advancement of CHM.

Puerarin (7, 4′-dihydroxyisoflavone-8-glucopyranoside) is a major active ingredient in CHM, *Puerariae Radix*, which comes from the kudzu root. It is believed that the major isoflavone puerarin is responsible for the pharmacological actions of kudzu root on the cardiovascular systems, cerebrovascular systems, and anti-diabetic effects (Wong, Li, Li, Razmovski-Naumovski, & Chan, 2011). But literature on puerarin absorption in intestine is still absent. Therefore, we investigated the permeability of puerarin using the ex vivo and in situ models of rat with analysis of puerarin in both perfusate and mesenteric blood by HPLC.

2. Materials and methods

2.1. Chemicals

Puerarin (purity 98%) 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 unless otherwise mentioned were of analytical grade and were purchased from Sinopharm Chemical Reagent Co., Ltd. Shanghai, China. 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. Animals and surgical procedures

2.2.1. Animals care and anesthesia

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 250–300 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 the rats 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 peritoneal injection, and 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. At the end of the experiment, all animals were sacrificed by CO_2.

2.2.2. Everted gut sac (EGS) preparation

EGS studies were performed using established methods adapted from the literature (Barthe, Bessouet, Woodley, & Houin, 1998; Wilson & Wiseman, 1954). The abdomen of anesthetized animal was shaved and a longitudinal midline incision of 3–4 cm was carefully made to expose the intestinal segments. The duodenum, jejunum, ileum and colon of the rat intestines (approximately 10 cm each) were quickly excised, and the underlying mesenterium was removed. The intestinal segments were rinsed with normal saline solution using a syringe equipped with blunt end to remove the contents at ambient temperature. After that the intestinal segments were carefully placed in Krebs–Ringer solution which was continuously oxygenated. One end of the segment was ligated using silk braided sutures, and the segment was carefully everted using a stainless steel rod to make an empty gut sac. The everted gut sac was filled on the serosal side (inside) with 1 mL Krebs–Ringer solution, and then the other end of the gut segment was tightly ligated. Each sac was placed in a 50 mL Erlenmeyer flask containing 30 mL of test solution that was bubbled with 95% O_2 : 5% CO_2 and incubated in a 37 °C water bath to maintain temperature. After 60 min, the sacs were taken out, washed, blotted dry with a piece of filter paper, after that, all samples were taken out from the serosal side. The length and radius of gut sacs were accurately measured. The samples were filtered through 0.45 μm syringe filters and analyzed by the validated HPLC method.

2.2.3. Surgical procedures of in situ single-pass intestinal perfusion (SPIP)

SPIP studies were performed using established methods adapted from the literature (Dahan, West, & Amidon, 2009). Briefly, the abdomen of an anesthetized rat was shaved and a longitudinal midline incision of 3–4 cm was carefully made to expose the intestinal segments. The duodenum, jejunum ileum and colon were located respectively. The identification of the main parts of intestinal is: the duodenum from the pylorus to the ligament of Treitz, the colon from the cecum empennage to the top of the rectum, 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 selected 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. One polytetrafluoroethylene-(PTFE) cannula (1/16″ ID, 1/8″ OD) was inserted at each end (proximal and distal) and secured by 4-0 suture. The segment was then rinsed with warm isotonic saline until the effluent was clear. To expel the remaining isotonic saline from the intestine, the air was pumped slowly through the intestinal 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, 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 temperature. In order to assure steady state, the perfusate was pumped at a flow rate of 0.2 mL/min for 30 min firstly. After reaching steady state, the perfusate samples out of the intestinal segment were collected at 10 min intervals up to 120 min. Following the termination of the experiment, the perfused intestinal segments were cut out, and the length and radius of it were accurately measured.

2.2.4. Surgical procedures of in situ intestinal perfusion with venous sampling (IPVS)

The surgical procedures used to prepare the perfused rat jejunum with venous sampling are similar to previously described methods adapted from the literature (Castle et al., 1985; Cummins, Salphati, Reid, & Benet, 2003; Mudra & Borchardt, 2010; Singhal, Ho, & Anderson, 1998) with some modifications. Briefly, a total of 50–70 mL blood drawn from five or seven donor rats via the abdominal aorta was anticoagulated by 700 U heparin, and the blood incubated in a 37 °C water bath was prepared to be transfused into the recipient rat through the jugular vein for balancing the blood loss via the mesenteric vein. The left jugular vein of the anesthetized rat was exposed, isolated by blunt dissection, a 24 G BD Intima II catheter (Becton Dickinson Medical Devices CO., Ltd. China) filled with heparinized saline (100 U/mL) was inserted approximately 1–2 cm into the vein and secured by 4-0 suture. Then the catheter was connected by a silicone tube filled with blood to a peristaltic pump (BT 100-1F Longer Pump, China), which is placed between the donor blood reservoir and the catheter, and the other end of the silicone tube was immersed in the donor blood. The