



## Original article

## Comparison of two approaches of intestinal absorption by puerarin

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## 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  $0.199 \times 10^{-5}$  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.

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## 1. Introduction

Although lots of innovations in the drug delivery system have come into being with the progress of pharmaceuticals, 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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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<sub>3</sub>, 5.3 mM NaH<sub>2</sub>PO<sub>4</sub>, 0.4 mM MgCl<sub>2</sub>, 6.7 mM CaCl<sub>2</sub>, 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<sub>2</sub>.

#### 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<sub>2</sub> : 5% CO<sub>2</sub> 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 empenage 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

abdomen was shaved and a longitudinal midline incision of 3–4 cm was carefully made to expose the jejunum and the mesenteric vein. Then the distal mesenteric vein was isolated by blunt dissection, and the 24 G catheter with heparinized saline was intubated into mesenteric vein and secured with 4-0 suture. Subsequently, the catheter was also connected by the silicone tube to the same peristaltic pump utilized for blood supply. The surgery operations for intestinal perfusion were the same as SPIP, and the selected intestinal segment about 20–30 cm length should be corresponding to the operating mesenteric vein. The experiment setup is illustrated in Fig. 1. At the conclusion of surgery operation, the proximal mesenteric vein was ligated with silk suture, and then the two catheters and pumps were switched on immediately. The perfusate was perfused at a flow rate of 0.2 mL/min and the blood was supplied at a flow rate of 0.5 mL/min. Mesenteric blood and luminal perfusate were discarded to waste between 0 and 30 min of the perfusion. During the perfusion, the perfusate from the outlet of the intestine was collected at 10 min intervals in a pre-weighed 5-mL centrifuge tube and the blood from mesenteric vein was simultaneously collected in heparinized centrifuge tubes at 10 min intervals. Following the termination of the experiment, the perfused intestinal segment was removed, and the length of the segment was measured without stretching.

### 2.3. Preparation of assay solution

#### 2.3.1. Preparation of the aqueous extract solution of *Puerariae Radix*

25 g of crude drugs *Puerariae Radix* was weighed and pulverized. An eightfold mass of water was added and then the mixture was boiled for 1 h for the first decocting, and a fourfold mass of water was boiled for 1 h for the second decocting. The two decoctions were mixed. The mixtures were filtered two times. The filtrates were combined and concentrated to 50 mL. Then 10 mL concentrated liquid was removed and diluted with Krebs–Ringer solution to 100 mL.

#### 2.3.2. Preparation perfusion solution of puerarin

The perfusion solution of 80 µg/mL (or 1200 µg/mL) puerarin was prepared by dissolving 40 mg (or 600 mg) puerarin in 500 mL Krebs–Ringer solution.

### 2.4. HPLC analysis

Puerarin was separated by a Phenomenex C18 column (250 mm × 4.6 mm, 5 µm) guarded with a precolumn. The mobile phase consists of acetonitrile and water containing 1.0% glacial acetic acid (14/86, v/v). The flow rate was 1.0 mL/min and the detection was achieved at 250 nm. The column temperature was set at 30 °C and the injection volume was 20 µL. The linearity was evaluated by linear regression analysis. With the linear concentration range (20.0–120.0 µg/mL), good linearity ( $r^2 > 0.9995$ ) was achieved.

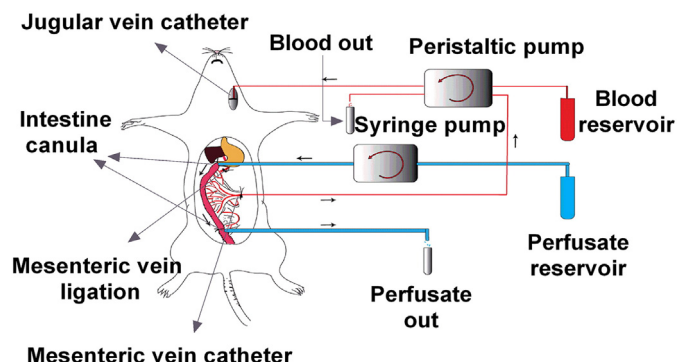


Fig. 1. Illustration of the experimental setup for IPVS.

### 2.5. Calculations and statistical analysis

$P_{app}$  obtained with the EGS method was calculated according to Eq. (1).

$$P_{app} = \frac{\Delta Q / \Delta t}{2\pi r L \cdot C_0} \quad (1)$$

where  $P_{app}$  (cm/s) is the apparent permeability coefficient,  $\Delta Q / \Delta t$  (µg/s) is the amount of compound permeated during 60 min,  $2\pi r L$  (cm<sup>2</sup>) is the surface area available for permeation with length,  $L$  (cm), and luminal radius,  $r$  (cm), respectively, and  $C_0$  (µg/mL) is the initial concentration on mucosal side.  $P_{eff}$  was calculated according to Eq. (2).

$$P_{eff} = \frac{-Q_{in} \cdot \ln(C_{out(corr)} / C_0)}{2\pi r L} \quad (2)$$

where  $P_{eff}$  (cm/s) is the effective permeability coefficient,  $Q_{in}$  is the flow rate of perfusate inlet ( $Q_{in} = 0.2$  mL/min),  $2\pi r L$  (cm<sup>2</sup>) is the surface area of intestinal segment with length,  $L$  (cm), and luminal radius,  $r$  (cm), respectively,  $C_0$  (µg/mL) is the initial concentration of perfusate, and  $C_{out(corr)}$  (µg/mL) is the outlet concentration of perfusate which is corrected for volume change in segment using the gravimetric method (Issa, Gupta, & Bansal, 2003).  $C_{out(corr)}$  was calculated according to Eqs. (3) and (4).

$$C_{out(corr)} = C_{out} \frac{Q_{out}}{Q_{in}} \quad (3)$$

$$Q_{out} = \frac{M_{out} / D_{out}}{t} \quad (4)$$

where  $Q_{out}$  is the measured flow (mL/min) of exiting intestinal perfusate using the actual intestinal perfusate density (g/mL),  $C_{out}$  (µg/mL) is measured outlet concentration of perfusate,  $M_{out}$  (g) is the mass of collected perfusate per interval,  $D_{out}$  (g/mL) is the density of the collected perfusate per interval,  $t$  is the interval time of sampling ( $t = 10$  min).  $P_{blood}$  obtained from IPVS was calculated according to Eq. (5).

$$P_{blood} = \frac{\Delta M_B / \Delta t}{2\pi r L \cdot \langle C \rangle} \quad (5)$$

where  $P_{blood}$  (cm/s) is the blood permeability coefficient,  $\Delta M_B / \Delta t$  (µg/mL) is the amount of compound appearance in mesenteric blood per interval,  $2\pi r L$  (cm<sup>2</sup>) is the surface area of intestinal segment with length,  $L$  (cm), and luminal radius,  $r$  (cm), respectively, and  $\langle C \rangle$  (µg/mL) is the logarithmic mean concentration of solute in the intestinal lumen.  $\langle C \rangle$  was calculated according to Eq. (6).

$$\langle C \rangle = \frac{C_{out(corr)} - C_0}{\ln(C_{out(corr)} / C_0)} \quad (6)$$

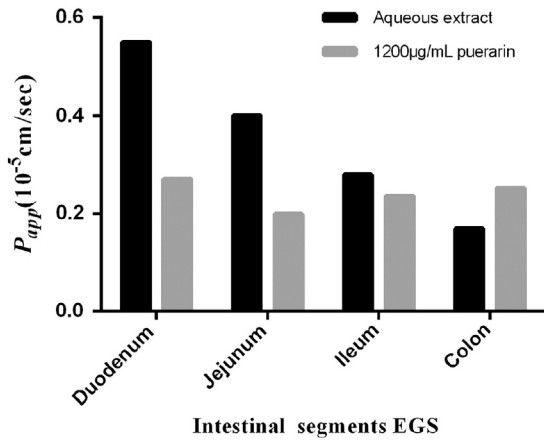
where  $C_{out(corr)}$  (µg/mL) is the corrected outlet concentration of perfusate, and  $C_0$  (µg/mL) is the initial concentration of perfusate.

Data were analyzed by ANOVA with Microsoft Excel program. Significant differences between the two groups were detected by using HSD test. A probability level of  $p < 0.05$  was set as the criterion of significance. Data was presented by mean ± S. E. M.

## 3. Results

### 3.1. Puerarin in aqueous extract of *Puerariae Radix* filtered into EGS

As expected, Puerarin was one of the ingredients in an aqueous extract solution of *Puerariae Radix*, which was measured by HPLC, and could be filtered into four intestinal segments in EGS (Fig. 2). We

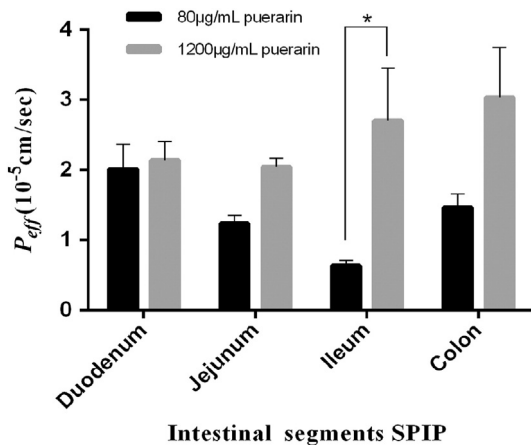


**Fig. 2.** No influence of intestinal segments on the  $P_{app}$  determined by four intestinal segments in EGS after incubation of 60 min in 1200 µg/mL puerarin (gray columns), but the  $P_{app}$  in the aqueous extract of the root of *Puerariae Radix* (black columns) decreases gradually from the proximate intestine to the distal intestine ( $n = 1$ ).

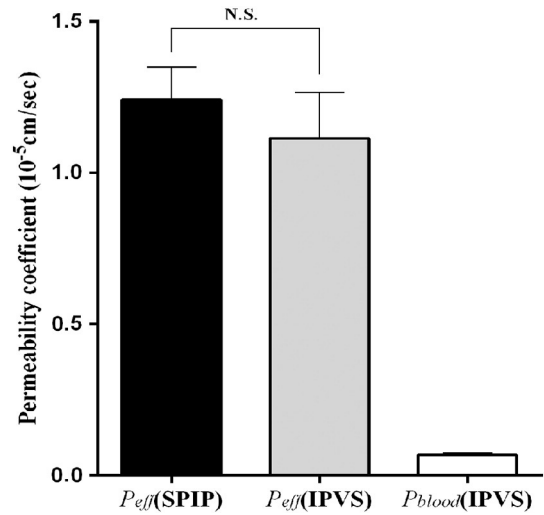
found that the  $P_{app}$  of puerarin in an aqueous extract solution of *Puerariae Radix* decreased gradually from the duodenum, jejunum, ileum, to the colon, but there was no similar behavior at 1200 µg/mL puerarin. The concentration of 1200 µg/mL puerarin was designed according to the calculation of the most usage in an aqueous extract solution of *Puerariae Radix*, to evaluate the permeability of puerarin.

**3.2. The influence of puerarin concentration and intestinal segments on the  $P_{eff}$  in SPIP experiments**

In the SPIP experiments, the low concentration of puerarin (80 µg/mL) and the high concentration of puerarin (1200 µg/mL) were designed in order to evaluate the concentration effect on  $P_{eff}$ . As the result showed (Fig. 3), the  $P_{eff}$  ( $2.705 \pm 0.752 \times 10^{-5} \text{ cm/s}$ ) of puerarin at high concentration was significantly greater than the  $P_{eff}$  ( $0.639 \pm 0.075 \times 10^{-5} \text{ cm/s}$ ) at low concentration in the ileum ( $p < 0.05$ ), but the influence of concentration on  $P_{eff}$  was not found in the other intestinal segments (two-way ANOVA). Furthermore, there was no intestinal position effect on  $P_{eff}$  within two concentration groups (one-way ANOVA).



**Fig. 3.** Influence of concentration on the  $P_{eff}$  of four intestinal segments in SPIP. Columns represent average ( $\pm$ S.E.M.;  $n = 3-4$ )  $P_{eff}$  at 80 µg/mL puerarin (black columns) and 1200 µg/mL puerarin (gray columns). \* indicates statistical significance at the  $p < 0.05$  level (ANOVA).



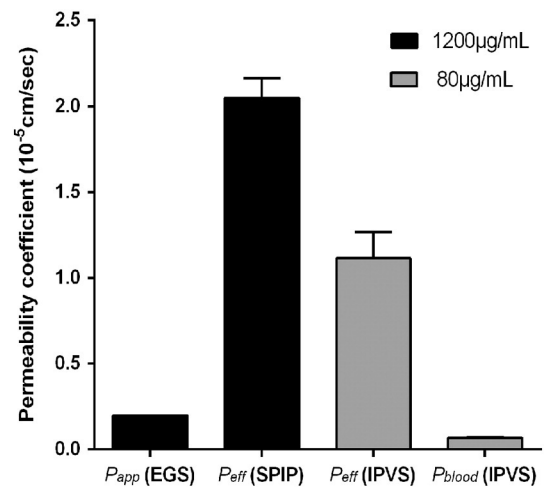
**Fig. 4.** Comparison of permeability coefficient of jejunum between SPIP and IPVS at 80 µg/mL puerarin. Columns represent average ( $\pm$ S.E.M.;  $n = 3-4$ )  $P_{eff}$  in SPIP (black columns),  $P_{eff}$  in IPVS (gray columns), and  $P_{blood}$  in IPVS (blank columns). N.S. indicates no statistical significance at the  $p < 0.05$  level (t test).

**3.3. Blood permeability coefficient of puerarin in the jejunum**

In order to quantify the puerarin absorbed into the blood via gut wall, we carried out the IPVS experiments in jejunum which is the longest region of rat small intestine. Through IPVS experiments, we gained two permeability coefficients of puerarin ( $P_{eff}$  and  $P_{blood}$ ), and we found the  $P_{blood}$  ( $0.068 \pm 0.002 \times 10^{-5} \text{ cm/s}$ ) was 16-fold lower than the  $P_{eff}$  ( $1.114 \pm 0.153 \times 10^{-5} \text{ cm/s}$ ). As expected, the  $P_{eff}$  of SPIP and IPVS were not statistically significant difference at 80 µg/mL puerarin (Fig. 4).

**3.4. The comparison of permeability coefficients of jejunum in EGS, SPIP and IPVS experiments**

In the present study, we used three models to determine the permeability of puerarin in jejunum, EGS, SPIP and IPVS, so that we observed three permeability coefficients,  $P_{app}$ ,  $P_{eff}$ , and  $P_{blood}$ . As the data showed (Fig. 5), at 1200 µg/mL puerarin,  $P_{app}$  ( $0.199 \times 10^{-5} \text{ cm/s}$ ) was 10-fold lower than  $P_{eff}$  ( $2.047 \pm 0.116 \times 10^{-5} \text{ cm/s}$ ), and the  $P_{blood}$



**Fig. 5.** Comparison of permeability coefficient of jejunum in EGS, SPIP and IPVS experiments. Columns represent average ( $\pm$ S.E.M.;  $n = 3$ ) of permeability coefficient at 1200 µg/mL puerarin (black columns) and at 80 µg/mL puerarin (gray columns).

( $0.068 \pm 0.002 \times 10^{-5}$  cm/s) was 16-fold lower than the  $P_{eff}$  ( $1.114 \pm 0.153 \times 10^{-5}$  cm/s) in IPVS experiments at 80  $\mu$ g/mL puerarin.

#### 4. Discussion

In the present paper, we focused on the determination of the permeability coefficient for puerarin using three rat models, and compared three permeability coefficients,  $P_{app}$ ,  $P_{eff}$ , and  $P_{blood}$ , to analyze puerarin intestinal absorption. Through this study, we practiced the combinational usage of three models for absorption study of CHM through the examination of puerarin, one of the bioactive ingredients of *Puerariae Radix*.

The EGS model was used to determine the permeability coefficient of  $P_{app}$ , and was suitable to study regional intestinal absorption. As the results showed, puerarin in aqueous extract of *Puerariae Radix* could have higher permeability in the small intestine than in the puerarin solution, which was made manifest in the high  $P_{app}$  of puerarin in the aqueous extract of *Puerariae Radix* in duodenum, jejunum and ileum. For the decoctions of classical CHM dosage form, the EGS model could be also used to screen the components absorbed via the intestines and preliminarily characterize their permeability by virtue of its relative quickness, low cost, simple operation, and good correlation with human intestinal absorption (Alam, Al-Jenoobi, & Al-Mohizea, 2012). For example, this model has been used to study phytochemical components from herbal extracts for the evaluation of extracts from *Stevia rebaudiana* (Koyama et al., 2003), *Astragalus* (Xu et al., 2006) and herbal combinations (Kelber et al., 2006).

The effective permeability coefficient of  $P_{eff}$  determined by SPIP was widely applied in the drug absorption study (Dahan & Amidon, 2009; Kim et al., 2006; Lennernas, 1997; Lennernas & Abrahamsson, 2005; Volpe, 2010) to predict the extent of intestinal absorption of drugs in humans, and it was one of the primary parameters for oral drug developments. In the present study, the  $P_{eff}$  of puerarin was not affected by intestinal segments, indicating that puerarin could be uniformly absorbed in the four intestinal segments without a specific absorption window. Concentration dependent permeability was only found in the ileum, and it suggested that the high concentration of puerarin could improve the permeability of the ileum through unknown mechanisms. The  $P_{eff}$  of puerarin in rat jejunum was  $1.24 \pm 0.11 \times 10^{-5}$  cm/s and lower than that of metoprolol (Incecayir, Tsume, & Amidon, 2013),  $3.3 \pm 0.064 \times 10^{-5}$  cm/s, as a U.S. FDA reference drug for the low/high permeability class boundary (CDER, 2000). Thus, puerarin would belong to the low permeability substance in abstracto. Recently, herbal BCS classification (Fong et al., 2013; Waldmann et al., 2012) has been framed on the solubility and permeability of the AMCs predicted in silico, so that the permeability of AMCs will be more widely studied by means of classical SPIP models in the future.

The IPVS model was modified from SPIP for simultaneous profiling drug intestinal absorption and presystemic metabolism, and its suitability was verified by the lack of a significant difference in  $P_{eff}$  between SPIP and IPVS. Using the IPVS model, we found the intestinal first-pass metabolism of puerarin in jejunum by comparison between the  $P_{eff}$  and the  $P_{blood}$ , which revealed that the fraction metabolized (Singhal et al., 1998) of puerarin was 93.9% by calculation of fraction metabolized =  $1 - P_{blood} / P_{eff}$ . However, the mechanisms of intestinal metabolism for puerarin still need to be investigated with no answers in the present study. But, there were lots of papers that reported that the IPVS model of rat had been successfully used to study intestinal first-pass metabolism for AMCs or herbal extracts, e.g. for *Radix Scutellariae* (Li et al., 2012), for Tanshinone IIA (Yu et al., 2007), for Glabridin (Cao et al., 2007), and for Baicalein (Zhang, Lin, Chang, & Zuo, 2005), that strongly demonstrated that this model was the best applicable model for studying intestinal metabolism. Thus, we presented three permeability coefficients for puerarin,  $P_{app}$ ,  $P_{eff}$ , and  $P_{blood}$ , observed by methods of EGS, SPIP, and IPVS respectively. As the results showed (Fig. 5), there were large differences between them, such as between EGS and SPIP resulting

from an intrinsic property of the models which has been well reviewed previously (Balimane et al., 2000; Luo et al., 2013; Volpe, 2010), while the difference between SPIP and IPVS was a result of intestinal first-pass metabolism inferred from experimental data. In fact, all of models will be useful for any research if we are able to understand them comprehensively.

#### 5. Conclusion

During the study, we firstly identified that puerarin was an absorbable component in aqueous extract of *Puerariae Radix* using EGS, and determined the effective permeability coefficient of puerarin by SPIP. Then, we obtained the blood permeability coefficient from IPVS for examining intestinal first-pass metabolism. The results showed that puerarin was a low permeability compound and there was first-pass metabolism in the intestine. This research strategy was successfully practiced in the study of puerarin intestinal permeability and it will be useful information for herbal constituent absorption studies in the future.

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