Absorption characteristic of paeoniflorin-6′-O-benzene sulfonate (CP-25) in in situ single-pass intestinal perfusion in rats

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Abstract

1. Paeoniflorin-6′-O-benzene sulfonate (CP-25) was synthesized to improve the poor oral absorption of paeoniflorin (Pae).
2. This study was performed to investigate the absorptive behavior and mechanism of CP-25 in in situ single-pass intestinal perfusion in rats, using Pae as a control.
3. The results showed that intestinal absorption of CP-25 was neither segmental nor sex dependent. However, the main segment of intestine that absorbed Pae was the duodenum. Furthermore, passive transport was confirmed to be the main absorption pattern of CP-25. More importantly, the absorption of CP-25 was much higher than Pae in the small intestine.
4. Among the ABC transporter inhibitors, the absorption rate of Pae increased in the presence of P-gp inhibitors verapamil and GF120918, which indicated that Pae was a substrate of P-glycoprotein (P-gp), however, such was not observed in the presence of breast cancer resistance protein and multidrug resistance-associated protein 2. Finally, the ABC transporter inhibitors did not have any significant impact on CP-25 as demonstrated in the parallel studies.
5. CP-25 could improve the poor absorption of Pae, which may be attributed to both the lipid solubility enhancement and its resistance to P-gp-mediated efflux.

Keywords

ABC transporters, drug transport, intestinal absorption, paeoniflorin, paeoniflorin-6′-O-benzene sulfonate, P-glycoprotein

Introduction

Total glucosides of peony (TGP) is an active compound isolated from the roots of Paeonia lactiflora Pall, a traditional Chinese medicine (TCM). TGP was approved by China Food and Drug Administration (CFDA) as an anti-inflammatory and immunomodulatory drug in 1998. It has since been used in the treatment of rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) (Chen et al., 2013; Wang et al., 2009; Zhang et al., 2011). Recent studies demonstrated that TGP also has potential therapeutic applications against chronic nephritis (Zhang et al., 2014a), atherosclerosis (Li et al., 2011), and Sjögren’s syndrome (Chang et al., 2013). Although TGP is effective in treating RA and some other autoimmune diseases without evident toxic or side effect, it has been shown to have a slow onset of action by oral administration in the clinics (Wang et al., 2012).

TGP mainly contains paeoniflorin (Pae), hydroxy-paeoniflorin, albiflorin, and benzoylpaeoniflorin (Kaneda et al., 1972). Among these compounds, Pae is the most abundant component (>40%) and it is the main biologically active ingredient of TGP (Su et al., 2010). Recent investigations of Pae exhibited many pharmacological activities such as anti-inflammatory (Chang et al., 2011; Wang et al., 2013), anti-neoplastic (Wu et al., 2013), anti-hyperglycemia (Yang et al., 2004), and neuroprotective effects (Liu et al., 2005a). According to the previous studies, Pae was not significantly absorbed across the gastrointestinal epithelium after oral administration, leading to a very low bioavailability (3–4%). This may account for the slow onset of action of TGP (Takeda et al., 1995). Recently, several studies have proposed some interpretations of the poor oral absorption. Since Pae is characterized as a water-soluble glucoside with a low lipophilicity, it is restrained from passing through the membranes of intestinal cells. In addition, P-glycoprotein (P-gp)-mediated transport may be a key factor to limit oral bioavailability of Pae. P-gp expressed on intestinal epithelial cells captures Pae when they pass through the cell membrane...
and releases them outside the cell, thus, affecting systemic drug concentration. When co-administrated with sinomenine, an inhibitor of P-gp, the pharmacokinetic parameters of Pae were markedly enhanced (Liu et al., 2005b). Moreover, two well-known P-gp inhibitors, verapamil and quinidine, could significantly elevate the absorption of Pae by 2.1- and 1.5-fold, respectively (Chan et al., 2006; Chen et al., 2012).

Extensive efforts are being focused on resolving the issue of poor bioavailability of Pae. For example, it was reported that performed acetylation can improve the lipophilicity of Pae and augment its absorption in vitro (Yang et al., 2013). In an animal study, significant increases in bioavailability were observed in benzoylpaeoniflorin sulfonate compared with Pae and paeoniflorin sulfonate (Cheng et al., 2010). Therefore, the lipophilicity enhancement is considered to be an effective way to improve the oral bioavailability of Pae. With this in mind, we prepared CP-25, which is the product of paeoniflorin-6′-O-benzene sulfonate. We have previously confirmed that the oral bioavailability of CP-25 was better than Pae in normal rats, and its anti-inflammatory and immunoregulatory effects were significantly higher than Pae and TGP in collagen-induced arthritis mice (data not shown). However, the intestinal absorption trait of CP-25 was still not fully understood. In this study therefore, we characterized the transport of CP-25 in situ in single-pass intestinal perfusion (SPIP) model. This procedure is considered a robust tool for simulating real in vivo conditions following oral drug administration. Hence, the potential application of CP-25 for improving the absorption of Pae was investigated.

Materials and methods

Animals

Sprague-Dawley (SD) rats, weighting 220–250 g were purchased from the Animal Department of Anhui Medical University (Hefei, Anhui Province, China) and were allowed to acclimatize for 1 week. They were housed in cages (4 rats in each), kept at a standard laboratory conditions at a constant temperature of 24 °C ± 1 °C, and controlled lighting that provided a 12-h light/dark cycle. Water and a standard diet were available ad libitum. All rat manipulations were carried out in the morning to minimize the effects of circadian rhythms. The studies were approved by the Ethical Committee on Animal Research at the Institute of Clinical Pharmacology of Anhui Medical University.

Drugs and reagents

Pae (white powder, purity >95.0%) was provided by the Institute of Clinical Pharmacology of Anhui Medical University (Figure 1A). CP-25 (white powder, purity >98.0%) was synthesized from Pae via the esterification of benzene sulfonate, and the target compound was confirmed by 1H NMR, 13C NMR, and mass spectra (Figure 1B). Verapamil, GF120918, MK571, KO143, were from Sigma Chemical Co. (St. Louis, MO). Methanol was of chromatographic grade (JT Baker, Center Valley, PA). All other chemicals used in the present study were of reagent grade or better.

Stability tests

Prior to the experiment, stability tests of CP-25 and Pae were performed using established methods adapted from literature (Juan et al., 2010; Surampalli et al., 2015). Briefly, adult SD rats were fasted overnight for 12–16 h with free access to water. They were anesthetized using an intraperitoneal injection of chloral hydrate (350 mg/kg of body weight), and placed on a heated pad to keep normal body temperature. Upon verification of the loss of pain reflex, a midline longitudinal abdominal incision was made. The intestinal segment was placed inside the abdomen and the cannulae were connected to a perfusion system, which was equipped with a peristaltic pump and a heated bath that kept the temperature of the perfusion medium at 37 °C. Care was taken to handle the small intestine gently and to minimize the surgery in order to maintain an intact blood supply. The surgical area was covered with gauze soaked in saline solution.

Perfusion started by flushing 30 mL of 0.9% (wt:v) NaCl to remove the contents of the small intestine, followed by air pumping to drain residual fluid in the intestine. Krebs-Rings buffer, which is the perfusion solution, contained 7.8 g NaCl, 0.35 g KCl, 1.37 g NaHCO3, 0.02 g MgCl2, 0.22 g NaH2PO4, and 1.48 g glucose in 1000 mL of purified water. Perfusion buffer contained CP-25 (2, 5, 10, 20, 40 μg/mL) or Pae (5, 10, 20 μg/mL) was perfused with a flow rate of 0.2 mL/min. After allowing 30 min to reach steady state outlet concentrations, outlet perfusate samples were collected every 15 min till 120 min with tarred conical flask and were weighed after sample collection. At the end, the length and inner diameter of the segment were measured without stretching, and finally, the animals were euthanatized with an overdose of ether anesthesia. Samples were stored at −20 °C until analysis.
The SPIP experiment is based on reaching steady state with respect to the diffusion of the compound across the intestine. Gravimetric method with density correction was used to correct the volume change, which prevents the results from the interference by water absorption and secretion during the perfusion (Zhang et al., 2014b).

\[
V_{in} = \frac{m_{in}}{\rho_{out}} \\
V_{out} = \frac{m_{out}}{\rho_{out}}
\]

where \( m_{in} \), \( m_{out} \), \( V_{in} \), and \( V_{out} \) are the quality, density, volume of the drug perfusate; \( \rho_{in} \), \( \rho_{out} \), \( V_{in} \), and \( V_{out} \) are the quality, density, volume of the sample after correction.

The absorption rate constant (\( K_a \)) and effective permeability coefficient (\( P_{eff} \)) were calculated using the following equations:

\[
K_a = \left(1 - \frac{C_{out}}{C_{in}} V_{in} \right) \frac{Q_{in}}{\pi r^2 l}
\]

\[
P_{eff} = -\frac{Q_{in} \ln(C_{out}V_{out}/C_{in}V_{in})}{2\pi rl}
\]

where \( Q_{in} \) is the perfusion rate (0.2 mL/min); \( C_{in} \) and \( C_{out} \) are the concentrations of Pae or CP-25 in the solution entering and exiting the intestine. \( r \) is the inner radius of the intestine, and \( l \) is the length of the intestine, which are both measured at the end of the experiment.

**Intestinal absorption of CP-25 and Pae**

SPIP has been used to investigate the potential application of CP-25 for improving the absorption of Pae. Permeability was determined in six individual rats with three doses (5, 10, 20 \( \mu \)g/mL) of CP-25 and Pae, respectively. The whole small intestine was chosen as the study segment, which is from the beginning of duodenum to the end of ileum.

**Absorption of CP-25 with different concentrations**

In order to study whether the absorption of CP-25 has dose-dependent effects, perfusion solutions with different concentration (2, 5, 10, 20, 40 \( \mu \)g/mL) of CP-25 were introduced into the whole small intestine segment. Rats were randomly assigned to five groups with six rats each.

**Absorption of CP-25 and Pae at different intestinal segments**

To investigate whether the intestinal absorption of CP-25 and Pae was segment-dependent, SPIP was performed in four isolated intestinal segments. Rats were randomly assigned to eight groups with six rats each. Duodenum (2 cm away from pylorus), jejunum (15 cm away from pylorus), ileum (20 cm proximal to cecum), and colon (proximal to cecum) were perfused with the same concentration of CP-25 or Pae (10 \( \mu \)g/mL).

**Absorption of CP-25 in different genders**

To study the effect of gender on the absorption of CP-25, male and female rats were studied in SPIP with three doses (5, 10, 20 \( \mu \)g/mL) of CP-25. Rats were randomly assigned to six groups with six each.

**Study of P-gp, MRP2, and BCRP in SPIP studies**

To explore the underlying transport mechanism involved in transepithelial absorption, a total of 60 male SD rats, were divided randomly into 10 groups and perfused with CP-25 (10 \( \mu \)g/mL) and Pae (10 \( \mu \)g/mL) in the absence and presence of the efflux protein inhibitors. Verapamil (49.1 \( \mu \)g/mL) and GF120918 (5.6 \( \mu \)g/mL) were selected as P-gp blocker. MK571 (5.3 \( \mu \)g/mL) and KO143 (0.5 \( \mu \)g/mL) were chosen as multidrug resistance-associated protein 2 (MRP2) blocker and breast cancer resistance protein (BCRP) blocker, separately.

**Sample preparation**

Immediately after the experiments, outflow samples (1 mL) were added to 1 mL methanol (100% v:v), mixed on a vortex for 2 min, and centrifuged at 12 000 r\( \cdot \)min\(^{-1} \) for 15 min at 4 °C. At last, 20 \( \mu \)L of the supernatant was injected into high performance liquid chromatography (HPLC) for analysis.

**HPLC analysis**

The HPLC system (Agilent 1200 series HPLC, Agilent Technologies, Santa Clara, CA) was used in the analysis. The analytical column was a reversed-phase C18 column (Agilent TC-C18, 5 \( \mu \)m \( \times \) 250 mm; Agilent Technologies, Santa Clara, CA). The mobile phase for the chromatographic separation consisted of methanol-water (48:52, v:v) with filtering through a 0.45 \( \mu \)m millipore filter (Millipore Corporation, Billerica, MA) at a flow rate of 0.8 mL/min. The detection wavelength was 230 nm. All of the analyses were performed at room temperature. The injection volume was 20 \( \mu \)L and the analysis time was 20 min per sample. Figure 2 shows the HPLC chromatogram of blank perfusion solution and blank perfusion solution spiked with CP-25 and Pae. For the HPLC analysis of intestinal perfusion fluid for CP-25 and Pae, the retention times were about 18 and 6 min, respectively. No interfering peaks were observed within the time frame in which Pae or CP-25 were detected.

The calibration curve for CP-25 was prepared as \( Y = 11.916X + 0.0903, \ r = 0.9999 \) (n = 3) (Supplementary Figure 1). The recoveries were found to be accurate to 92.71%, 100.80%, and 90.27% in 5, 10, and 20 \( \mu \)g/mL respectively (Supplementary Table 3). The inter-daily and intra-daily variants for the CP-25 samples were lower than 5%, meeting the requirements of quantitative analysis (Supplementary Table 4).

For the HPLC analysis of intestinal perfusion fluid for Pae, the retention time was about 6 min. The calibration curve for Pae was prepared as \( Y = 13.873X - 0.7602, \ r = 1.0000 \) (n = 3) (Supplementary Figure 1). The recoveries were found to be accurate at 92.41%, 99.62%, and 99.72% in 5, 10, and
20μg/mL respectively (Supplementary Table 3). The inter-daily and intra-daily variants for the CP-25 samples were lower than 5%, meeting the requirements of quantitative analysis (Supplementary Table 4).

**Figure 2.** HPLC chromatogram of CP-25 and Pae in the perfusion solution. (A) Blank perfusion solution. (B) Blank perfusion solution spiked with Pae. (C) Blank perfusion solution spiked with CP-25.

**Statistical analysis**

The data in this paper are presented as mean ± SD, unless otherwise specified. For multiple-group comparisons, one-way analysis of variance (ANOVA) followed by LSD test was
Table 1. Stability of CP-25 and Pae in the blank intestinal circulating solution ($\overline{X} \pm s, n = 4$).

| Time       | CP-25 (mAU-min) | Pae (mAU-min) |
|------------|-----------------|---------------|
| 0(h)       | 107.67 ± 0.78   | 146.40 ± 0.98 |
| 0.5(h) at 37°C | 106.97 ± 1.14   | 142.57 ± 0.88 |
| 1(h) at 37°C | 106.43 ± 0.87   | 140.60 ± 1.65 |
| 2(h) at 37°C | 103.90 ± 1.22   | 141.73 ± 1.26 |
| 3(h) at 37°C | 103.27 ± 0.78   | 140.80 ± 0.99 |
| RSD (%)    | 1.84%           | 1.66%         |

used. For a two-group comparison, a two-tailed Student’s $t$-test was employed. A $p$ value of less than 0.05 was considered as statistically significant.

Results
The stability of CP-25 and Pae
Before commencing the perfusion, drug stability studies were conducted. Our results showed that in blank perfusates, CP-25 and Pae degraded little in 3 h at 37°C (Table 1). Therefore, the stability of CP-25 and Pae was not affected by external factors. Thus, any loss of CP-25 and Pae from the perfusates was due to intestinal absorption.

Intestinal absorption characteristics of CP-25 and Pae
The intestinal absorption characteristics of CP-25 and Pae at different doses (low, medium, and high), are shown in Figure 3. $P_{eff}$ of Pae (5 µg/mL) was $6.92 ± 0.39 \times 10^{-4}$ cm/s, indicating that Pae was poorly permeable. However, a significant difference was observed from the experimental results between Pae and CP-25. For example, when compared with Pae (5 µg/mL), CP-25 gave a 1.9-fold and 1.8-fold $K_a$ and $P_{eff}$, respectively. Absorption characteristics of the other two doses of CP-25 were also significantly higher than that of Pae.

Effect of intestinal site on CP-25 intestinal permeability
Previous research has suggested that the absorption of drugs may vary conspicuously between different regions of the intestinal tract (Lindahl et al., 2004; Rice et al., 2005). In this investigation, the permeability values for each section are plotted in Figure 4. The absorption level of Pae was higher in duodenum than those in other intestinal regions ($p < 0.05$). However, there were no obvious differences of $K_a$ values for CP-25 between the four intestinal sites. This outcome was similar what was observed for the order of $P_{eff}$ ($p > 0.05$).

Effect of differential concentrations on CP-25 intestinal permeability
Table 2 shows the effects of different concentrations on the intestinal absorption of CP-25. Concentration of 40 µg/mL was set as the upper boundary because of the low aqueous solubility of CP-25. There were no significant differences in $K_a$ and $P_{eff}$ between different doses of CP-25, indicating that intestinal absorption of CP-25 may be passive transfer by diffusion across the lipid membranes.

Discussion
In clinical practice, oral drug delivery is more essential compared with the intravenous route, because of better patient compliance, cheaper costs, greater convenience, and more simplicity for long-term treatment of chronic diseases (Guo et al., 2013). It is evident that absorption investigations are needed to understand the intestinal absorption processes from oral administration. Among all the methods for absorption studies, intestinal perfusion studies in rats emerges as the most reliable and cost effective option, as a high correlation was reported between human and rat small intestinal permeability ($r^2 = 0.8–0.95$) for drug intestinal permeability (Lozoya-Agullo et al., 2015). There are two kinds of techniques for the in situ intestinal perfusion method: recirculating perfusion model and single-pass perfusion model. But absorption in the recirculating perfusion model will be increased because the test drug has a long retention time for the solution in the intestine (Shao et al., 2015). Hence in this study, SPIP was chosen to investigate the absorptive behavior and mechanism as it is more likely to simulate human body environment. It has a slow rate (0.2 mL/min) which provides a greater relevance with intestinal peristalsis in humans (Luo et al., 2013). Instead of classic phenol red method, we used gravimetric method to determine the volume change of perfusate in these intestinal perfusion experiments. This decision was based on the report that phenol red was partly absorbed in small intestine and could interfere the transport measurement of some hydrophobic drugs (Nie et al., 2005).

Effect of gender differences on CP-25 intestinal permeability
As illustrated in Figure 5(A) and (B), there were no significant differences of $K_a$ and $P_{eff}$ values for CP-25 in female rats in comparison with male rats. These results imply that gender was not a critical influential factor of the CP-25 absorption behavior.

Effect of efflux protein inhibitors on Pae and CP-25 intestinal permeability
The effects of inhibitors, such as verapamil, GF120918 (P-gp inhibitors), MK571 (MRP2 inhibitor), and KO143 (BCRP inhibitor) on the intestinal absorption behavior of CP-25 and Pae are presented in Figure 6. $P_{eff}$ of Pae increased 2.2-fold by verapamil, whereas a 2.0-fold increase was exhibited by GF120918. However, as shown in Figure 6(C) and (D), the $P_{eff}$ and $K_a$ values of CP-25 were not significantly altered by verapamil and GF120918 treatment. Meanwhile, the $K_a$ and $P_{eff}$ values of both CP-25 and Pae were essentially unchanged by MK571 and KO143. It can therefore be speculated that Pae is a substrate of P-gp, but not MRP2 and BCRP. Hence, we can conclude that CP-25 may not be a substrate of P-gp, MRP2 and BCRP, or a low affinity to their receptors.
bioavailability of Pae. Therefore, the structure of Pae was modified via esterification to form a novel chemical structure, CP-25, which is a lipophilic compound. But there is no in situ experiment to explore the absorption of Pae lipophilicity analogs. Thus, in the present study, we have compared the absorption parameters of CP-25 with Pae in situ SPIP in rats. The results show that intestinal absorption parameters of CP-25 were significantly higher than that of Pae, which indicates

### Table 2. Absorption parameters of CP-25 at different concentrations ($\bar{X} \pm s, n = 6$).

| Concentration (µg/mL) | $K_a (\times 10^{-2} \text{ s}^{-1})$ | $P_{eff} (\times 10^{-3} \text{ cm s}^{-1})$ |
|-----------------------|-------------------------------------|----------------------------------|
| 2                     | 1.062 ± 0.028                       | 1.051 ± 0.025                    |
| 5                     | 1.115 ± 0.063                       | 1.244 ± 0.089                    |
| 10                    | 1.117 ± 0.095                       | 1.294 ± 0.220                    |
| 20                    | 1.098 ± 0.055                       | 1.112 ± 0.071                    |
| 40                    | 1.112 ± 0.037                       | 1.085 ± 0.054                    |

Figure 3. $K_a$ (A) and $P_{eff}$ (B) of CP-25 and Pae in three doses. The data were presented as mean ± SD ($n = 6$ in each group). **$p < 0.01$ versus Pae.

Figure 4. (A) $K_a$ of CP-25 in four different segments. (B) $P_{eff}$ of CP-25 in four different segments. (C) $K_a$ of Pae in four different segments. (D) $P_{eff}$ of Pae in four different segments. The data were presented as mean ± SD ($n = 6$ in each group). **$p < 0.01$ versus duodenum. **$p < 0.01$ versus duodenum.
that the absorption of CP-25 is much better than Pae in the small intestine. This enhancement of the CP-25 may be partly attributed to the lipophilic enhancement.

In recent years, not only P-gp, but also MRP2 and BCRP, which are mainly membrane proteins in ABC transporter family, were mostly investigated as membrane protein transporters in the intestine. Generally, orally administered compounds have to cross the intestinal enterocytes to reach the blood circulation and tissue. In this process, these transporters act as selective barriers which have the function of secreting
drugs (substrates) into the intestinal lumen. Consequently, the absorption of drugs administered orally can be inhibited by ABC transporters (Kis et al., 2013; Rong et al., 2013). Lately, researchers have discovered that structural modification can influence the interactions of drugs with P-gp. For example, by modifying specific moieties on the paclitaxel, new taxanes might be identified with reduced binding or recognition by P-gp and potentially enhanced their permeability properties (Rice et al., 2005; Turunen et al., 2008). And so the effects of ABC transporters on CP-25 and Pae were investigated with four inhibitors. The results demonstrated that CP-25 was not a substrate for P-gp, BCRP and MRP2, as permeabilities in the small intestine were not increased by co-perfusion of verapamil and GF120918, MK571 and KO143, respectively. However, Pae permeability obtained by the co-administration of verapamil and GF120918 increased ($p<0.01$). This suggests that Pae could be substantially transported by P-gp, but not MRP2 or BCRP in the gut wall. The results matched with the previous research that Pae was a substrate of P-gp, and that its absorption could be substantially improved by inhibiting P-gp-mediated efflux (Chan et al., 2006; Chen et al., 2012; Liu et al., 2005b). It can therefore be assumed that structural changes of Pae blocks the substrate site relevant for P-gp activity, so CP-25 could not be highly recognized or bound by P-gp and pumped out.

P-gp has a wide group of substrates including compounds of very different molecular weights, and compounds composed of different chemical groups. It is thus difficult to describe what chemical features a compound must exhibit in order to be a P-gp substrate. Based on the structure of these two compounds, we found CP-25 differs from Pae by the addition of a benzene sulfonate unit at C-6-OH of the skeleton. Thus, the existence of the hydroxyl group at C-6 is the key factor for the transport with or without efflux, because the only difference we observed between CP-25 and Pae is C-6-OH. Not only Pae, but also some compounds which have a glycoside without substituted group were proven to be a P-gp substrates, such as ginsenoside Rh2 (Xie et al., 2005) and icariin (Chen et al., 2008). Therefrom, a glycoside with the unbound state of 6-OH determines the involvement of the efflux mediated by P-gp. Taken together, the lipophilicity of CP-25 was higher than Pae, and CP-25 was not recognized as a substrate by P-gp. CP-25 showed 1.9-fold and 1.8-fold higher $K_a$ and $P_{eff}$ values, respectively, when compared with Pae. This $P_{eff}$ value of CP-25 was almost the same as, that of Pae in the presence of P-gp inhibitors, however, the value increased 2.0-fold higher when compared with that of Pae in the absence of P-gp inhibitors. Consequently, we expect the bioavailability of CP-25 to be around 6–8%, which may translate to 2-fold higher than that of Pae.

In the present study, the intestinal absorptive behavior of CP-25 was investigated to elucidate the absorption mechanism in the intestinal tract, which has significant implications for further investigation on pharmaceutics of CP-25. There are three main mechanistic pathways for drug transport: passive transport, carrier-mediated transport, and membrane mobile transport. Comparison of the $P_{eff}$ calculated from various concentrations exhibited no difference, indicating that CP-25 worked via a passive transport. Moreover, it is very necessary to understand the oral absorption processes in different regions of the intestinal tract. Further, comparing $P_{eff}$ and $K_a$ values obtained from the different segments revealed that CP-25 could be absorbed in the whole intestine with no segmental-dependent changes. However, the main segment of intestine that absorbed Pae was the duodenum ($p<0.05$). The regional permeability is determined by the interplay between the physicochemical properties of the drug and the intestinal environment (Lindahl et al., 2004). The physicochemical properties include solubility, degree of dissociation, lipotropy, PH, molecular size, and so on. Moreover, different segments of the intestine involve physiological differences, including mucosal blood flow, unstirred water layer, membrane proteins, enteroenzyme, and so on (Jiang et al., 2014). In this experiment, the results suggested that low-level expression of P-gp in duodenum may be the reason that Pae has a better absorption in duodenum, as P-gp was proven to be in increase expression from proximal to distal intestinal parts (Caroline et al., 2008). Drug permeability to intestine of different gender rat was also measured to find out if intestinal absorption of CP-25 exhibits gender-dependent changes. The results indicated that gender could not significantly affect the absorption behavior of CP-25.

**Conclusion**

In conclusion, the absorption of CP-25 is much better than Pae in the small intestine. The improving intestinal absorption effect of CP-25 is not only because of its enhanced lipophilicity, but also, possibly, because it will not be secreted back into the intestinal lumen by transporters as a substrate of P-gp. The intestinal absorption CP-25 was neither segmental-dependent nor gender biased, and passive transport was the main absorption pattern of CP-25. Therefore, CP-25 could be used orally to overcome the low bioavailability and absorption of Pae. However, further studies particularly in vitro is still needed to support these results.

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**Declaration of interest**

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**References**

Caroline M, Ulla M, Andreas R, Gert F. (2008). Closing the gaps: a full scan of the intestinal expression of P-Glycoprotein, breast cancer resistance protein, and multidrug resistance-associated protein 2 in male and female rats. Drug Metab Dispos 36:1249–54.

Chan K, Liu ZQ, Jiang ZH, et al. (2006). The effects of sinomenine on intestinal absorption of paconiflorin by the everted rat gut sac model. J Ethnopharmacol 103:425–32.
Chang BC, Chen WD, Zhang Y, et al. (2013). Effects of total glucosides of paeony for delaying onset of Sjogren’s syndrome: an animal study. J Craniomaxillofac Surg 41:610–15.

Chang Y, Zhang L, Wang C, et al. (2011). Paeoniflorin inhibits function of synoviocytes pretreated by rIL-1z and regulates EP4 receptor expression. J Ethnopharmacol 137:1275–82.

Chen Y, Wang J, Wang L, et al. (2012). Absorption and interaction of the main constituents from the traditional Chinese drug pair Shaoyao-Gancao via a Caco-2 cell monolayer model. Molecules 17:14908–17.

Chen Y, Zhao YH, Jia XB, Hu M. (2008). Intestinal absorption mechanisms of prenylated flavonoids present in the heat-processed Epimedium koreanum Nakai (Yin Yanghuo). Pharm Res 25:2190–9.

Chen Z, Li XP, Li ZJ, et al. (2013). Reduced hepatotoxicity by total glucosides of paeony in combination treatment with leflunomide and methotrexate for patients with active rheumatoid arthritis. Int Immunopharmacol 15:474–7.

Cheng Y, Feng C, Wen F, Zhang H. (2010). Pharmacokinetic comparisons of typical constituents in white peony root and sulfur fumigated white peony root after oral administration to mice. J Ethnopharmacol 129:167–73.

Guo M, Rong WT, Hou J, et al. (2013). Mechanisms of chitosan-coated poly(lactic-co-glycolic acid) nanoparticles for improving oral absorption of 7-ethyl-10-hydroxycamptothecin. Nanotechnology 24:245101.

Jiang CP, He X, Yang XL, et al. (2014). Intestinal absorptive transport of Genkwanin from Fls genkwa using a single-pass intestinal perfusion rat model. Am J Chin Med 42:349–59.

Juan ME, Gonzalez-Pons E, Planas JM. (2010). Multidrug resistance proteins restrain the intestinal absorption of trans-resveratrol in rats. J Nutr 140:489–95.

Kaneda M, Itaka Y, Shibata S. (1972). Chemical studies on the oriental plant drugs—XXXIII: the absolute structures of paeoniflorin, albiflorin, oxyacetyleniflorin and benzoylpaeciflorin isolated from Chinese peony root. Tetrahedron 28:4309–17.

Kis O, Zastre JA, Hoque MT, et al. (2013). Role of drug efflux and uptake transporters in atazanavir intestinal permeability and drug--drug interactions. Pharm Res 30:1050–64.

Li J, Chen CX, Shen YH. (2011). Effects of total glucosides of paeony (Paeonia lactiflora Pall) roots on experimental atherosclerosis in rats. J Ethnopharmacol 135:469–75.

Lindahl A, Sjögberg A, Bredberg U, et al. (2004). Regional intestinal absorption and biliary excretion of fluvalastin in the rat: possible involvement of mpr2. Mol Pharm 1:347–56.

Liu DZ, Xie QK, Ji XQ, et al. (2005a). Neuroprotective effect of paeoniflorin on cerebral ischemic rat by activating adenosine A1 receptor in a manner different from its classical agonists. Br J Pharmacol 146:604–11.

Liu ZQ, Jiang ZH, Chan K, et al. (2005b). Pharmacokinetic interaction of paeoniflorin and sinomenine: pharmacokinetic parameters and tissue distribution characteristics in rats and protein binding ability in vitro. J Pharmacol Sci 99:381–91.

Lozoya-Agullo I, Zur M, Wolk O, et al. (2015). In-situ intestinal rat perfusions for human F abs prediction and BCS permeability class determination: investigation of the single-pass vs. the Doluisio experimental approaches. Int J Pharm 480:1–7.

Luo Z, Liu Y, Zhao B, et al. (2013). Ex vivo and in situ approaches used to study intestinal absorption. J Pharmacol Toxicol Methods 68:208–16.

Nie SF, Pan WS, Yang XG, et al. (2005). Evaluation of gravimetry in the rat single-pass intestinal perfusion technique. Chin New Drugs J 14:1176–9.

Rice A, Liu Y, Michaelis ML, et al. (2005). Chemical modification of paclitaxel (Taxol) reduces P-glycoprotein interactions and increases permeation across the blood-brain barrier in vitro and in situ. J Pharmacol Chem 48:832–8.

Rong Z, Xu Y, Zhang C, et al. (2013). Evaluation of intestinal absorption of amitolitin guayl in rats: breast cancer resistant protein as a primary barrier of oral bioavailability. Life Sci 92:245–51.

Shao B, Cui C, Ji H, et al. (2015). Enhanced oral bioavailability of pipeline by self-emuulsifying drug delivery systems: in vitro, in vivo and in situ intestinal permeability studies. Drug Deliv 22:740–7.

Su J, Zhang P, Zhang JJ, et al. (2010). Effects of total glucosides of paeony on oxidative stress in the kidney from diabetic rats. Phytomedicine 17:254–60.

Surampalli G, K Nanjwade B, Patil PA. (2015). Corroboration of naringin effects on the intestinal absorption and pharmacokinetic behavior of candesartan cilexetil solid dispersions using in-situ rat models. Drug Dev Ind Pharm 41:1057–65.

Takeda S, Isono T, Wakui Y, et al. (1995). Absorption and excretion of paeoniflorin in rats. J Pharm Pharmacol 47:1036–40.

Turunen BJ, Ge H, Oyetunji J, et al. (2008). Paclitaxel succinate analogs: anionic and amide introduction as a strategy to impart blood-brain barrier permeability. Bioorg Med Chem Lett 18:5971–4.

Wang C, Yuan J, Wu HX, et al. (2013). Paeoniflorin inhibits inflammatory responses in mice with allergic contact dermatitis by regulating the balance between inflammatory and anti-inflammatory cytokines. Inflamm Res 62:1035–44.

Wang C, Yuan J, Yang ZY, et al. (2012). Pharmacokinetics of paeoniflorin microemulsion after repeated dosing in rats with adjuvant arthritis. Pharmazie 67:997–1001.

Wang YN, Zhang Y, Wang Y, et al. (2009). The beneficial effect of total glucosides of paeony on psoriatic arthritis links to circulating Tregs and Th1 Cell function. Phytoster Other Res 28:372–81.

Wu JJ, Sun WY, Hu SS, et al. (2013). A standardized extract from Paeonia lactiflora and Astragalus membranaceus induces apoptosis and inhibits the proliferation, migration and invasion of human hepatoma cell lines. Int J Oncol 43:1643–51.

Xie HT, Wang GJ, Chen M, et al. (2005). Uptake and metabolism of ginsenoside Rh2 and its aglycon protopanaxadiol by Caco-2 cells. Biol Pharm Bull 28:383–6.

Yang HO, Ko WK, Kim JY, Ro HS. (2004). Paeoniflorin: an antihyperlipidemic agent from Paeonia lactiflora. Fitoterapia 75:55–69.

Yang XW, Guo J, Xu W. (2013). Absorption and transport characteristic of paeoniflorin and its derivatives in model of Caco-2 cell monolayers. Chin Tradition Herb Drugs 44:2097–104.

Zhang HF, Xiao WG, Hou P. (2011). Clinical study of total glucosides of paeony in patients with systemic lupus erythematosus. Zhongguo Zhong Xi Yi Jie He Za Zhi 31:476–9.

Zhang W, Zhao L, Su SQ, et al. (2014a). Total glucosides of paeony attenuate renal tubulointerstitial injury in STZ-induced diabetic rats: role of Toll-like receptor 2. J Pharmacol Sci 125:59–67.

Zhang Z, Chen Y, Deng J, et al. (2014b). Solid dispersion of berberine-phospholipid complex/TPGS 1000/SiO2: preparation, characterization and in vivo studies. Int J Pharm 465:306–16.

**Supplementary material available online**