609.2 → 301.1 for hesperidins (IS) respectively. The chromatographic analysis was achieved on a hypersil gold column (100 mm × 2.1 mm i.d., 1.9 μm particle size; Thermo, USA). The mobile phase consisted of 0.05% formic acid in water (A) and acetonitrile (B) at a flow rate of 0.4 mL/min. The UHPLC gradient system began with 15% B at 0–1 min, 15–60% B at 1–5 min, 60–100% B at 5–6 min. The column temperature was maintained at 40°C, while the sample-tray temperature was kept at 4°C.

The method had linear calibration curves over the concentrations of 1.95–1500.0 ng/mL for PD in rat plasma, 1.47–1500 ng/mL in HBSS buffer and 1.47–1500 ng/mL in inactivated fecal lysate solution. The extraction recoveries were 74.3–86.6%, 101.3–104.8% and 94.4–97.5% for PD in rat plasma, HBSS buffer and inactivated fecal lysate solution respectively. The lower limit of quantification for PD was 1.95 ng/mL in rat plasma, 1.47 ng/mL in HBSS buffer and 1.47 ng/mL in inactivated fecal lysate solution, respectively. The intra and inter-batch precision and accuracy were <20% for all quality control samples in rat plasma, transport buffer and fecal lysate solution.

Data analysis

The data in this paper were presented as mean tedt for each group, if not specified otherwise. Significance was assessed by one way analysis of variance and Student's *t*-test. A P value of < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Oral pharmacokinetics of platycodin D in rats

The plasma concentrations of PD in rats were determined after following oral administration of single PD at 20 mg/kg and equivalent dose of PRE. The results [Figure 2 and Table 1] showed that single PD was rapidly absorbed into the circulatory system and reached its peak concentration. PD was detected in rat plasma at 10 min after oral administration of single PD and PRE, and reached C_{max} at approximately 30 min and 75 min with C_{max} approximately 44.45 ng/mL and 17.94 ng/mL, respectively. The $AUC_{(0-\infty)}$ of PD in rats receiving the single PD was low (73.00 ± 24.17 ng h/mL), which was consistent with the previous results.[16,18] The $AUC_{(0-\infty)}$ of PD after an equivalent dose of PRE to rats was observed to 96.06 ± 48.51ng h /mL, which was significantly higher than that in rats receiving the single PD, indicating PD in PRE is more exposed to blood circulation than single PD. MRT describes the average time for all the drug molecules to reside in the body. $MRT_{(0-t)}$ of PD was also increased from 1.38 ± 0.20 h (single PD) to 6.10 ± 1.03 (PD in PRE), which showed PD in PRE duration *in vivo* prolonged, compared to single PD. Previously, Zhan *et al.*,[18] reported the pharmacokinetics of PD between in monomer PD and in PRE. There were some differences between the previous report and our study, which may be attributed to the different doses to rats. In Zhan's study, the oral dosage of single PD and PRE was 80mg/kg and 18.75 g/kg respectively, while the oral dosage was 20 mg/kg and 10 g/kg (corresponding equivalent PD) in our present study. The appearance indicated that the other ingredients contained in PRE, like saponins, may influence the absorption or metabolism of PD.

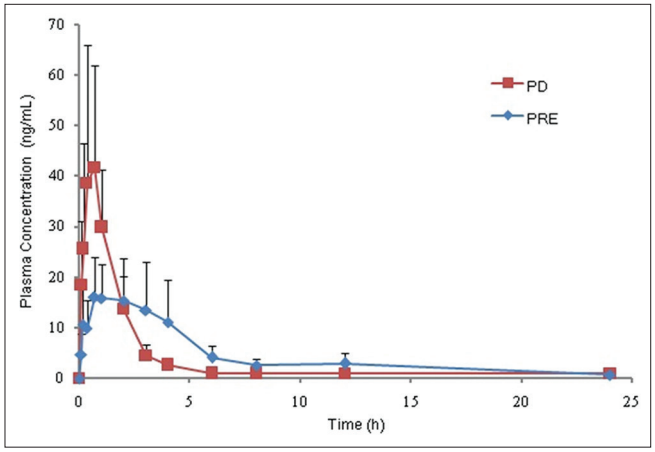

Figure 2: Mean concentration-time profiles of platycodin D in rat plasma after oral administration of *Platycodi radix* extract (10 g/kg) and single platycodin D (20 mg/kg) (mean ± standard deviation, $n = 6$)

Transcellular transport of platycodin D across Caco-2 cell monolayer

We determined the apparent permeability of PD both in PRE and in monomer PD in Caco-2 cell monolayers, a model employed to mimic human intestinal absorption characteristics.[21,22] Transport of 0.45 μg/mL PD and PRE (200 μg/mL, equivalent to 0.45 μg/mL PD) from apical side to basolateral side in Caco-2 cells were determined within 2 h to understand whether the coexisting components in PRE could influence the transport of PD.

The result of 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide assay showed that single

Table 1: Pharmacokinetic parameters of PD in rats following oral administration of PRE (10 g/kg) and single PD (20 mg/kg) (mean±SD, *n*=6)

Parameter	PD	PRE
C_{max} (ng/mL)	44.45±22.40	17.94±9.33*
$t_{1/2}$ (h)	1.32±0.64	2.86±1.07*
T_{max} (h)	0.44±0.17	1.22±0.62*
$AUC_{(0-t)}$ (ng h/mL)	71.26±24.17	88.97±45.42
$AUC_{(0-\infty)}$ (ng h/mL)	73.00±24.17	96.06±48.51*
$MRT_{(0-t)}$ (h)	1.38±0.20	6.10±1.03*

*P<0.05 compared with PD group. PD: Platycodin D; PRE: Platycodi radix extract; AUC: Area under the curve; MRT: Mean residence time; C_{max}: The peak concentration; T_{max}: The time to reach peak concentration; SD: Standard deviation; $t_{1/2}$: Half-life

PD and PRE at the working concentrations had no toxicity on Caco-2 cells within 2 h. The TEER values (>300 Ωcm^2) were not different significantly before and after the transport experiment. The results showed that the permeability of PD in monomer and in PRE was 3.52×10^{-7} cm/s and 2.11×10^{-7} cm/s respectively, corresponding to incomplete absorption in humans (1×10^{-6} cm/s),[21] which indicated that PD, like a lot of saponins, was poorly permeable.[23] PD in PRE also exhibited poor permeability but a little lower than that of in monomer PD, which implied that the coexisting components in PRE could affect the transport of PD. A lot of other platycodins in PRE may competitively inhibit the transport of PD from apical side to basolateral side. Transcellular transport across Caco-2 cell monolayer declared that the absorption did not result in the phenomenon that higher level of PD exposure in PRE than single PD.

Hydrolysis of platycodin D in fecal lysate

The result of *in vitro* hydrolysis study of PD by rat fecal lysate was presented in Figure 3. PD was hydrolyzed rapidly in fecal lysate, and decreased by 64% within 30 min. The intensity of hydrolyzation slightly diminished and the concentration of PD plateaued around 2 h, only about 20% PD presented at this time point. There are two unbranched sugar chains attached to the carbons C-3 and C-28 in the aglycones in PD, and each chain is composed of one (C-3) and four monosaccharide (C-28) residues. In intestinal bacteria, the major pathway of metabolism of PD is the hydrolysis of C-3 glycoside, followed by acetylation at the multi-site or, alternatively, further hydrolysis at the C-28 oligosaccharide and dehydroxylation of the alglycone part.[24,25] Furthermore, we are identifying the intestinal bacteria, such as β-glucosidases, potentially responsible for this metabolism to facilitate future metabolic studies of PD.

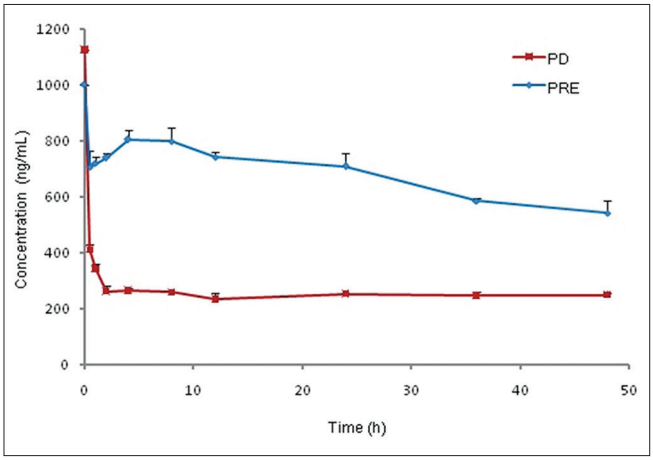

Figure 3: Metabolic profile of platycodin D from single platycodin D (1000 ng/mL) and *Platycodi radix* extract solution (0.5 mg/mL, corresponding equivalent platycodin D) in rat fecal lysate (mean ± standard deviation, $n = 3$)

Be similar to single PD, PD in PRE was hydrolyzed immediately in a remarkably short time (30 min), about 30% PD disappeared. However, the concentration of PD increased slightly within 1–4 h, and then the hydrolyzation eased step by step. The residue of PD in PRE was more about 1.6 fold than free PD in fecal lysate within 48 h. This pattern of distinctive hydrolysis in fecal lysate between single PD and PD in PRE, to a large extent accounted for their difference of pharmacokinetics. In PRE, there are many platycodins, like PD, such as PD 2, PD 3, and platycoside E. All these platycosides can be defined as bidesmosidic oleanane-type triterpenes with two sugar moieties: A glucose unit attached through an ether linkage at C-3 of a triterpene, and the other embracing arabinose, rhamnose, and xylose in sequence with attachment of a glycoside linkage between C-28 and arabinose.[5] These bidesmosidic saponins can be easily transformed into second platycodins by alkaline hydrolysis or intestinal microflora. So the other platycodins of PRE may be deglycosylated to PD under the microbial hydrolysis, which accounted for the concentration of PD in PRE increased slightly within 1–4 h. The biotransformation and metabolic profile of plentiful platycodins by intestinal microflora using high resolution mass spectrometry will be further investigated.

CONCLUSION

In summary, the pharmacokinetics, intestinal absorption and microbial metabolism of PD both in monomer and in PRE have been studied. The transmembrane behavior of PD was investigated by Caco-2 cells, showed it could not be absorbed well in intestine. It was demonstrated that the distinctive hydrolysis, not absorption, was the main reason for their difference of pharmacokinetics. Better understanding of the interactions between PD and the coexisting platycodins in the PRE will be investigated for further development of *Platycodi Radix* resources.

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