Poor permeability and absorption affect the activity of four alkaloids from *Coptis*

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Abstract. Coptidis rhizoma *(Coptis)* and its alkaloids exert various pharmacological functions in cells and tissues; however, the oral absorption of these alkaloids requires further elucidation. The present study aimed to examine the mechanism underlying the poor absorption of alkaloids, including berberine (BER), coptisine (COP), palmatine (PAL) and jatrorrhizine (JAT). An ultra-performance liquid chromatography (UPLC) method was validated for the determination of BER, COP, PAL and JAT in the above experimental medium. In addition, the apparent oil-water partition coefficient ($P_{o/w}$); apparent permeability coefficient (Papp), determined using a parallel artificial membrane permeability assay (PAMPA) plate; membrane retention coefficient (R %); and effect of P-glycoprotein (P-gp) inhibitor on the Papp of the four alkaloids were investigated. The intestinal absorption rate constant (Ka) and absorption percentage (A %) of the four alkaloids were also determined. The results of the present study demonstrated that the $P_{o/w}$ of the four alkaloids in 0.1 mol·l^{-1} HCl medium was significantly higher (P<0.01), compared with those of the alkaloids in phosphate buffer (pH 7.4). The Papp of BER was 1.0-1.2x10^{-6} cm·s^{-1}, determined using a PAMPA plate, and the Papp of BER, COP, PAL and JAT decreased sequentially. The concentrations of the four alkaloids on the apical-to-basolateral (AP-BL) surface and the basolateral-to-apical (BL-AP) surface increased in a linear manner, with increasing concentrations between 10 and 100 μmol. In addition, the transportation of BER on the BL-AP surface was significantly faster (P<0.01), compared with that on the AP-BL surface and, following the addition of verpamil (a P-gp inhibitor), the Papp (AP-BL) of the four alkaloids increased, whereas the Papp (BL-AP) was significantly decreased (P<0.01). The rat intestinal perfusion experiment demonstrated that the four alkaloids were poorly absorbed; however, the Ka of BER was significantly higher, compared with the three other alkaloids. Furthermore, the A % and Ka provided evidence that the absorption of BER was increased in the jejunum, compared with in the ileum. In conclusion, the four alkaloids from *Coptis* appeared to be poorly absorbed, determined using a shake flask, pre-coated PAMPA plates, a Caco-2 cell monolayer model and intestinal perfusion; however, absorption was higher in the jejunum than in the ileum. Among the four alkaloids, the permeability of BER was markedly higher than the others, and P-gp efflux had a significant effect on the absorption of those alkaloids.

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Introduction

Coptidis rhizoma (*Coptis*), which is the rhizome of *Coptis chinensis* Franch from the Ranunculaceae family, is widely used in traditional Chinese medicine (TCM). *Coptis* has been used to treat various diseases, including dysentery, hypertension, inflammation, tumor and liver diseases (1-3). Modern pharmacological studies have demonstrated that *Coptis* exerts numerous activities, including antibacterial (4), antiviral (5), anti-inflammatory (6,7), antineoplastic (8,9), anti-diarrheal (10), antihypertensive (11), anti-oxidative (12), antidiabetic, anti-hypercholesterolemic and hepatoprotective effects (13-16). The bioactive compounds in *Coptis* remain to be fully identified; however, it is generally considered that the predominant bioactive components are protoberberine alkaloids, including berberine (BER), palmatine (PAL), jatrorrhizine (JAT) and coptisine (COP) (Fig. 1) (17,18). Previous studies have suggested that the protoberberine alkaloids have multiple pharmacological functions, including the ability to improve glycemic control and lipid profile, as well as antibiotic, anti-inflammatory, anti-diarrheal, antineoplastic, antiarrhythmic and immunosuppressive properties (19-23). Although the pharmacological effects of BER and other *Coptis* extracts have been widely reported, information regarding their oral absorption remains to be fully elucidated. Previous pharmacokinetic studies have demonstrated that the four protoberberine alkaloids have markedly low plasma concentrations and poor oral bioavailability in rats, beagle dogs and humans following oral administration (24-27). Furthermore, several studies have reported that BER is a P-glycoprotein (P-gp)

substrate, which is extensively metabolized by CYP2D6 and CYP1A2 in the liver (17,28-34). Accordingly, poor absorption, P-gp efflux and extensive metabolism may be responsible for the poor bioavailability and low plasma concentration of these alkaloids. The present study aimed to determine the oil/water partition coefficient ($P_{o/w}$) of these four alkaloids. In addition, the permeability of the four alkaloids was determined using an *in vitro* Caco-2 cell monolayer model and pre-coated parallel artificial membrane permeability assay (PAMPA) plates. Intestinal absorption was determined in various intestinal segments using an *in situ* rat gut circulation perfusion model.

Materials and methods

Equipment. The ultra performance liquid chromatography (UPLC) H-class system (Singapore) was equipped with Empower 3 software (Waters Corporation, Milford, MA, USA), and an H-class Waters column (ACQUITY UPLC BEH C18; 2.1x100 mm; 1.7 μm; Waters Corporation) for UPLC separation. The RF-5301PC fluorescence spectrophotometer was purchased from Shimadzu Scientific Instruments (Columbia, MD, USA). The Bio-Tech Synergy 22100 microplate reader (cat. no. 168-1002XC) was obtained from Bio-Rad Laboratories, Inc. (Hercules, CA, USA), and the CF16RX-II centrifuge was purchased from Hitachi Koki Co., Ltd (Tokyo, Japan). A Nikon ECLIPSE Ti-U biological microscope (Nikon Corporation, Tokyo, Japan) was used to count the number of cells. Transwell culture plates were purchased from Corning Costar (Corning Incorporated, Corning, NY, USA) and the Pre-Coated PAMPA Plate system was obtained from BD Biosciences (Bedford, MA, USA).

Chemicals and reagents. Standards of berberine hydrochloride and palmatine hydrochloride were purchased from the National Institutes for Food and Drug Control of China (Beijing, China). Standards of coptisine hydrochloride and jatrorrhizine hydrochloride were purchased from Shanghai Tauto Biotech Co., Ltd. (Shanghai, China). Fluorescein sodium, propranolol, verapamil, furosemide, hydrochlorothiazide, caffeine and metoprolol were purchased from Sigma-Aldrich (St. Louis, MO, USA), and the purity of all of the chemicals was >98%. Methyl thiazolyl tetrazolium (MTT) was dissolved in trypsin, which was purchased from Amresco LLC (Solon, OH, USA). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco Life Technologies (Carlsbad, CA, USA). Acetonitrile and methanol were obtained from Thermo Fisher Scientific (Waltham, MA, USA). Ammonium acetate was purchased from Fluka (Sigma-Aldrich Trading Co., Ltd.) and formic acid was obtained from TEDIA Company (Fairfield, OH, USA). These were commercially available products of high performance liquid chromatography grade.

Cells and animals. The Caco-2 human colon carcinoma cells were purchased from the Cell Culture Center of the Chinese Academy of Medical Sciences (Beijing, China). Sprague-Dawley (SD) male rats were purchased from Beijing HFK Bioscience Co., Ltd. (license no. SCXK2009-0007; Beijing, China). Animal and cell experimental procedures were performed according to the guide for the Care and Use of Laboratory Animals (National Research Council Of USA, 1996), and the associated ethical regulations of the ethics committee of Guang'anmen Hospital (Beijing, China).

UPLC method validation. Simultaneous determination of the four alkaloids was achieved on the UPLC system. The mobile phase was composed of acetonitrile-water 30:70 (containing 2 mmol formic acid and 0.05% ammonium acetate at pH 3.20) at a flow rate of 0.3 ml·min^{-1}. The column temperature was 30˚C and the detector was a photo-diode array detector (345 nm). The injection volume was 5 μl.

For specificity, each standard was diluted with different solvents [mobile phase: 10% methanol containing phosphate-buffered saline (PBS), Hanks' solution or Krebs-Ringer solution with 20 μg·ml^{-1} phenol red] and then determined according to the UPLC method described above. For linearity, a series of concentrations of the standards was used, and linear regression was calculated as the peak area (Y) versus the concentration (x), in order to obtain the linear correlation coefficient. For precision, mixed standard solutions at three concentrations, from low to high, were assessed under UPLC conditions for five sequential days. The inter-assay and inner-assay precision of the four alkaloids was calculated as the relative standard deviation (RSD%) of the obtained peak area (n=6). For stability, the four alkaloids in each type of medium were assessed at 0, 1, 2, 4, 8 and 24 h, and the RSD% was used to evaluate the stability of the alkaloids. For recovery, appropriate quantities of the four reference alkaloids were added to the blank sample to produce a final concentration of 150 ng·ml^{-1} with six duplicates for each medium, and the recovery was calculated compared with each standard. The lower limit of detection (LOD) and the lower limit of quantification (LOQ) were determined using a final concentration, according to the ratio of signal to noise (S/N)>3 for LOD and S/N>10 for LOQ.

Determination of $P_{o/w}$. The $P_{o/w}$ was determined using a shake flask method with solutions of octanol with 0.1 M HCl, and octanol with pH 7.4 buffer solution (1.36 g KH_2PO_4, 79 ml 0.1 M NaOH, diluted with H_2O to 200 ml). A total of 100 ml of the two solutions were placed separately into a separation funnel, and shaken three times prior to the natural separation of the solution into two layers at room temperature. Subsequently, 10 ml of the upper oil phase layer and 10 ml of the lower aqueous phase layer was transferred into a flask. The standard solution of the four alkaloids (20 μg/ml) was prepared using the octanol with HCl solution, and the octanol with buffer solution, respectively. The flasks were placed into a thermostatic water bath at 37˚C with agitation for 24 h, prior to being centrifuged (13,800 x g; 4˚C; 10 min). Subsequently, 1.0 ml of the solution was evaporated using nitrogen, and the residual solution was dissolved in the mobile phase and transferred into a glass vial. The samples were then analyzed in triplicate using the UPLC method, and the concentrations of each alkaloid in the oil phase (C_{oil}) and aqueous phase (C_{Water}) were obtained. The $P_{o/w}$was calculated using the following formula: $P_{o/w} = C_{oil} / C_{Water}$.

Permeability assay using the PAMPA plate. Drugs with poor passive absorption (furosemide and hydrochlorothiazide)

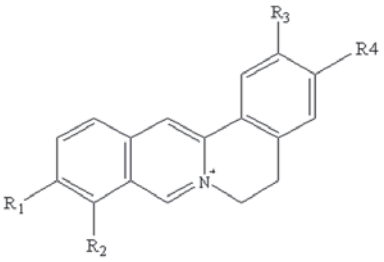
and drugs with good absorption (caffeine and metoprolol) were selected as a cocktail tool to investigate the permeability and absorption of the alkaloids, according to the BD Biosciences PAMPA plate permeability instructions (35). The test standards were prepared by dissolving BER, PAL, JAT and COP in methanol, which was then diluted in phosphate buffer solution (PBS), containing 8.0 g NaCl; 0.2 g KCl; 1.6 g $Na_2HPO_4 \cdot 12H_2O$ and 0.2 g KH_2PO_4 per liter in H_2O (adjusted to pH 7.40 using 0.1 mol NaOH), to obtain a concentration of 75 $\mu g \cdot ml^{-1}$ for each standard.

The PAMPA plate was pre-cooled at -20˚C prior to incubation at ambient temperature for 30 min. A total of 200 μl PBS was added to the upper layer of the plate and 300 μl of the BER, COP, JAT and JAT solutions (25, 50 and 75 $\mu g \cdot ml^{-1}$, respectively) were added to the lower layer. The layers were then placed together, ensuring no bubbles were present, and incubated at 25˚C for 5 h (n=6). Subsequently, 100 μl of the upper and lower layer solutions were transferred to a tube and centrifuged at 13,800 x g for 10 min. The supernatant was then transferred into a glass vial for analysis, according to the validated UPLC method. Drug-free PBS samples were used as a control group. The Papp and membrane retention coefficient (R%) were calculated as follows: Papp = -ln [1 - C A / Ce] / A x (1 / VD + 1 / VA) x t (cm/s); Ce = [CD (t) x V D + CAt x VA)] / (VD + VA); R (%) = [1 - (CDt x VD + CA t x VA)] / C0 x VD x 100%. t, incubation time (18,000 sec); C0, initial compound concentration in the donor well (mM); CDt, compound concentration in the donor well at t in mM; CAt, compound concentration in the acceptor well at t in mM; Ce, compound at equilibrium concentration; VD, donor well volume (0.3 ml); VA, acceptor well volume (0.2 ml); A, filter area (0.3 cm^2).

Bidirectional transportation of the alkaloids and the effect of the P-gp transporter. The present study aimed to verify whether the P-gp transporter affected the uptake of the four alkaloids. According to the literature, the Caco-2 cell monolayer model is an advantageous artificial tool for drug bidirectional transportation and P-gp efflux investigation (36-42). Verification of the active Caco-2 cell monolayer model is a procedure used to select a Caco-2 cell monolayer model with good absorption and transportation of propranolol, but weak absorption and transportation of fluorescent yellow (purity >98%; Sigma-Aldrich), which may then be used as a tool for drug evaluation. An MTT assay was used to evaluate the effects of the four alkaloids on Caco-2 cell activity (43).

Hanks' solution was prepared, and test solutions of BER, COP, JAT and PAL were prepared using Hanks' solution, in order to obtain a stock solution with a concentration of 100 $\mu mol \cdot l^{-1}$ of each alkaloid. The solutions were then diluted to 10, 20, 60 and 100 $\mu mol \cdot l^{-1}$ concentrations, respectively. Tool drugs were prepared at 30 $\mu g \cdot ml^{-1}$ for propranolol, 20 $\mu g \cdot ml^{-1}$ for fluorescent yellow and 100 $\mu mol \cdot l^{-1}$ for verapamil dissolved (containing 30 $\mu mol \cdot l^{-1}$ of BER) in Hanks' solution.

The Caco-2 cells were cultured in high glucose DMEM supplemented with 10% FBS, 1% non-essential amino acids (Sigma-Aldrich) and 1% green-streptomycin (Sigma-Aldrich) at 37˚C, in an incubator of 5% CO_2 under 90% relative humidity. The cells ($5x10^4/cm^2$) were then seeded into a Transwell 12-well plate, and the medium was replaced every other day;



Compound	Molecular formula	Chemical
BER	$[C_{20}H_{18}NO_4]^+$	R_1=R_2= OCH_3, R_3-R_4= -OCH_2O-
COP	$[C_{19}H_{14}NO_4]^+$	R_1=R_2= OCH_3, R_3-R_4= -OCH_2O-
JAT	$[C_{20}H_{20}NO_4]^+$	R_1=R_2= OCH_3, R_3-R_4= -OCH_2O-
PAL	$[C_{21}H_{22}NO_4]^+$	R1=R2= OCH3, R_3-R_4= -OCH_2O-

Figure 1. Chemical structure and molecular formula of the four alkaloids from *Coptis*. PAL, palmatine; COP, coptisine; JAT, jatrorrhizine.

the cells were continuously cultured for 21 days. Subsequently, a Caco-2 cell monolayer with good absorption and transportation of propranolol, but weak absorption and transportation of fluorescent yellow, was selected. Briefly, 0.5 ml propranolol (30 $\mu g \cdot ml^{-1}$) or fluorescent yellow (20 $\mu g \cdot ml^{-1}$) was added to the apical (AP) side and 1.5 ml blank Hanks' solution was added to the basolateral (BL) side. The cells were incubated at 37˚C in a water bath for 60 min. The concentrations of propranolol and fluorescent yellow in the BL side were used to evaluate transport ability.

An MTT assay was performed as follows: 100 μl cell suspension ($1x10^6$/ml) was added to a 96-well plate, and 200 μl culture medium was added to each well prior to plate incubation for 24 h. The medium in each well was then aspirated and 200 μl test solution (BER, COP, JAT and PAL) was added and incubated for 48 h. Subsequently, 20 μl MTT (5 $mg \cdot ml^{-1}$ in PBS) was added to each well and incubated for 4 h prior to termination with 150 μl/well dimethyl sulfoxide. The experimental group, the control group (no cells or drugs), and the negative group (cell culture medium only) were assessed in six duplicates. The absorbance value was measured at 570 nm using a microplate reader. Survival rates were calculated and compared between the experimental group and the untreated negative group using the following formula: Survival rate (%) = $(A_{experiment} - A_{blank}) / (A_{negative} - A_{blank})$.

Bidirectional transportation of the four alkaloids in the Caco-2 cell monolayer was determined, as previously described (29-31,44-46). Caco-2 cells cultured in Transwell plates ($1x10^6$ cells/ml) for 21 days with a resistance value >350 Ω were selected using an Epithelial Volt-Ohm meter (Millicell® ERS-2; Millipore Corporation, Billerica, MA, USA) for the bidirectional transportation experiments. The cells were washed twice with Hanks' solution at 37˚C and then incubated for 30 min at 37˚C. The culture medium was then discarded, and equal volumes of BER, COP, JAT and PAL at various concentrations were added to the AP side or to the BL side. Blank Hanks' solution was used as a control. The Transwell plates were then incubated at 37˚C in an oscillating water bath agitated at 50 $r \cdot min^{-1}$. Subsequently, 0.1 ml of each sample was collected at 30, 60, 90 and 120 min from the AP or BL side, with an equal volume of blank Hanks' collected

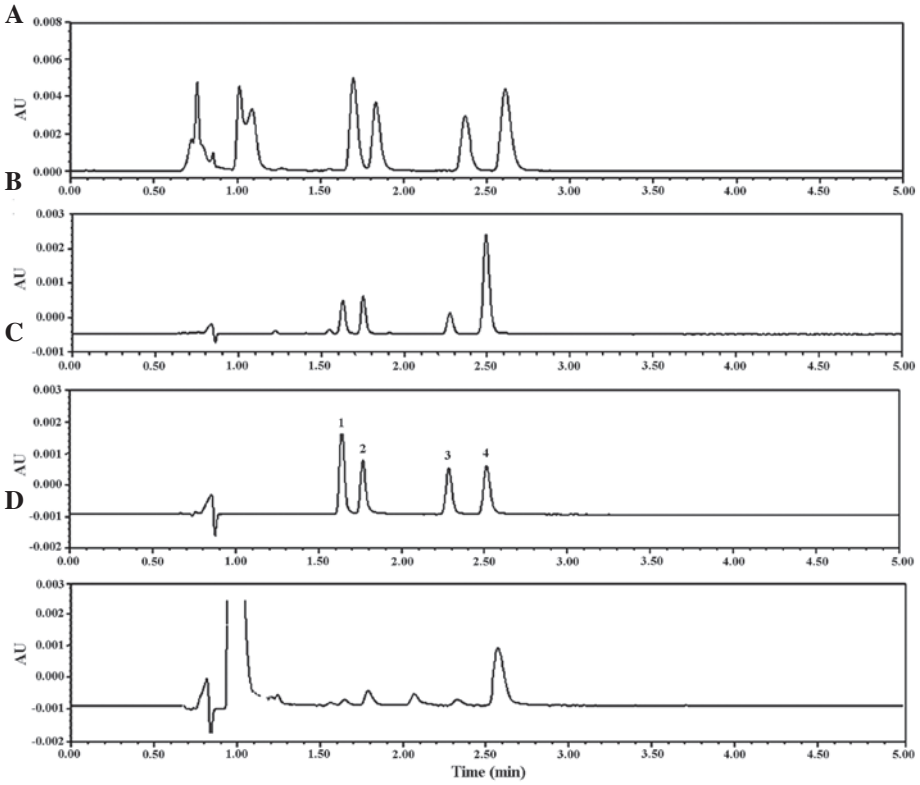


Figure 2. Ultra performance liquid chromatography for the determination of specificity. (A) Blank Caco-2 experiment; (B) mixed reference in pH 7.4 phosphate buffer; (C) four standards, 1-4 is representative of jatrorrhizine, coptisine, palmatine and berberine, respectively; (D) perfusion sample.

at the same time-points. The samples were then centrifuged at 13,800 x g for 10 min, and the supernatants were analyzed using the validated UPLC method (n=6).

The P-gp inhibitor verapamil is considered a useful tool as a cocktail drug to investigate drug transportation (29,31,44-46). Caco-2 cells with a resistance >350 Ω were selected for the transport experiments in the present study. Following 21 days incubation in Transwell plates, 100 μmol·l^{-1} of verapamil and 30 μmol·l^{-1} of BER, COP, PAL and JAT solutions were added to the AP or BL side, respectively. The plates were then incubated at 37˚C in an oscillating water bath agitated at 50 r·min^{-1}. Subsequently, 100 μl samples were collected at 30, 60, 90 and 120 min from the AP or BL side, with an equal volume of blank Hanks' solution collected at the same time-points (n=6). The samples were centrifuged at 13,800 x g for 10 min, and the supernatants were measured using the validated UPLC method. The Papp (1x10^{-5} cm·s^{-1}) was calculated as follows: Papp = (dQ / dt) / (A x C), where dQ / dt (μg·s^{-1}) is the rate of drug transportation; A is the surface area of the cell monolayer (cm^2); and C is the initial concentration of the drug in the administered side (μg·ml^{-1}).

Absorption evaluation of the four alkaloids by perfusion in rat intestine. The perfusion solution was prepared using 20 μg·ml^{-1} phenol red Krebs-Ringer buffer solution (7.8 g NaCl; 0.35 g KCl; 0.37 g $CaCl_2$; 0.32 g $NaH_2PO_4 \cdot 2H_2O$; 0.02 g $MgCl_2$; 1.37 g $NaHCO_3$ and 1.4 g glucose (per liter of H_2O), in order to obtain 20 μg·ml^{-1} of 58.82 μg·ml^{-1} for BER, 51.35 μg·ml^{-1} for COP, 46.36 μg·ml^{-1} for PAL and 62.18 μg·ml^{-1} for JAT.

The intestinal perfusion experiments were performed, as described previously (41,47,48). Six SD male rats weighing 300-320 g were reared in the Guang' An Men Hospital Experimental Animal Center (Beijing, China), and were maintained under an alternating 12 h light-dark cycle with *ad libitum* access to water. The jejunum and ileum of the rats were collected under anesthesia using intraperitoneal injection of 20% urethane (Sigma-Aldrich) solution at a dose of 5 ml·kg^{-1}. For the 10 cm jejunum section, the pylorus was selected as a starting point, and 10 cm down from the starting point was considered the terminal point. A sterile polyethylene pipe was inserted through a small hole from the starting point (inlet) to the terminal point (outlet). The intestinal content was flushed with saline at 37˚C and replaced with perfusate at 37˚C. The inlet and outlet pipes were connected with a pool using a peristaltic pump at a flow rate of 1 ml·min^{-1} for the perfusion circulation. For the 10 cm ileum section, the ileocecal was selected as the terminal point for the outlet, and 10 cm up from the terminal was selected as the starting point for the inlet. A sterile polyethylene pipe was inserted from the starting point (inlet) to the terminal point (outlet). The same wash and perfusion program was performed, as described for the jejunum perfusion. A total of 0.6 ml of each sample was collected at after 0.5, 1.0, 1.5, 2.0, 3.0 and 4.0 h, and an equal volume of Krebs-Ringer solution (20 μg·ml^{-1} phenol red) was added to the pool immediately following removal. The removal of the perfused solution was used to determine the quantity of phenol red and the absorption of the four alkaloids (n=6). The inner diameter of the small intestine (R) was determined for calculation of the surface area of the bowel perfusion (A). A blank sample was collected from the drug-free perfusion test solution, which contained 20 μg·ml^{-1} phenol red.

The percentage of absorption (A %) for each drug was calculated using the following formula: A (%) = (initial concentration C0 x volume of the drug V0 - Ct x Vt) / (initial concentration C0 x volume of the drug V0) x 100%. The perfusion fluid volume was normalized to the concentration of phenol red. The A % was obtained following administration of the drugs for 4 h. The absorption rate constant (Ka) was obtained from the slope of the regression equation, calculated by the logarithmic of the remaining doses in the pool versus time.

Method validation for the phenol red: The phenol red assay was performed using an ultraviolet-visible 2102-PC spectrophotometer (Unico, East Dayton, NJ, USA) at a wavelength of 559 nm, with no interference verified by the blank intestine perfusion solution. The concentration of phenol red was evaluated by the linearity via diluting the standard of phenol red with Krebs-Ringer solution to produce the following concentrations: 10, 20, 30, 40, 50 and 60 $\mu g \cdot ml^{-1}$, there is a good linearity by regression of the concentration (x) and absorbance values (y) at range of 10.12-60.72 $\mu g \cdot ml^{-1}$. The linearity equation was Y = 0.015x + 0.0419 with a correlation coefficient of 0.9994. The precision and stability of the experiment were evaluated using the RSD% <5% during the analysis, which was compliant with the phenol red determination.

Statistical analysis. Statistical analysis was performed using Microsoft Excel 2007 software (Microsoft Corporation, Redmond, WA, USA). Data are presented as the mean ± standard deviation. The difference between the groups was analyzed using a one-way analysis of variance. P<0.05 was considered to indicate a statistically significant difference.

Results

UPLC validation for determination of the four alkaloids. No interference was detected in either the Caco-2 cell model or the intestinal perfusion fluid model from PBS, Hanks' solution, or Krebs-Ringer solution with phenol red (Fig. 2). The RSD% for the inter-assay and inner-assay were <5.33 and 8.58%, respectively. The alkaloids were relatively stable in the medium of the Caco-2 cell model and intestinal perfusion fluid, with an RSD% <4.04% within 24 h. The recovery of the four alkaloids was at a range between 95.46 and 100.99%. The LOD (S/N ≥3) and the LOQ (S/N ≥10) were 4.50 and 15.01 $ng \cdot ml^{-1}$, respectively. All data was compliant with the requirement of analytical method validation (49,50).

UPLC validation for determination of the apparent partition coefficient. The $P_{o/w}$ of BER was significantly higher, compared with the three other alkaloids, which may be due to the polarity of BER. The $P_{o/w}$ of the four alkaloids were higher in 0.1 $mol \cdot l^{-1}$ HCl, compared with in pH 7.4 PBS (Table I).

UPLC validation for determination of permeability. Validation of the PAMPA plate model using poor and good absorptive drugs is shown in Table II, the results of which were concordant with previous literature (35,41). These results suggested that a PAMPA plate may be used to investigate the penetration and transportation of the four alkaloids.

Table I. Octanol-water partition coefficient of the four alkaloids from *Coptis*.

Compound	HCl (0.1 $mol \cdot l^{-1}$)	Phosphate buffer solution (pH 7.4)
COP	0.65±0.0046	0.079±0.0075
PAL	0.78±0.020	0.082±0.0024
JAT	0.84±0.033	0.080±0.0025
BER	1.43±0.036[a]	0.213±0.017

Data are presented as the mean ± standard deviation (n=3). [a]P<0.05, BER vs. JAT. PAL, palmatine; COP, coptisine; JAT, jatrorrhizine; BER, berberine; HCL, hydrochloride.

Table II. Permeability assessment of four tool drugs ($x10^{-6}$ $cm \cdot s^{-1}$).

Tool drug	Plate 1	Plate 2
Furosemide	0.64±0.046	0.65±0.039
Hydrochlorothiazide	0.48±0.052	0.59±0.13
Caffeine	11.21±0.29	11.31±0.048
Metoprolol	5.35±0.32	5.61±0.48

Data are presented as the mean ± standard deviation (n=6).

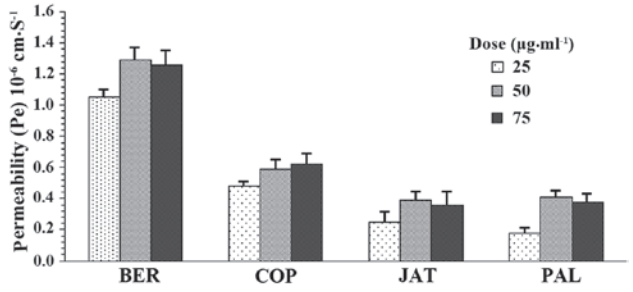


Figure 3. Permeability of the four alkaloids from *Coptis*. Error bars represent the data presented as the mean ± standard deviation BER, berberine; COP, coptisine; PAL, palmatine; JAT, jatrorrhizine.

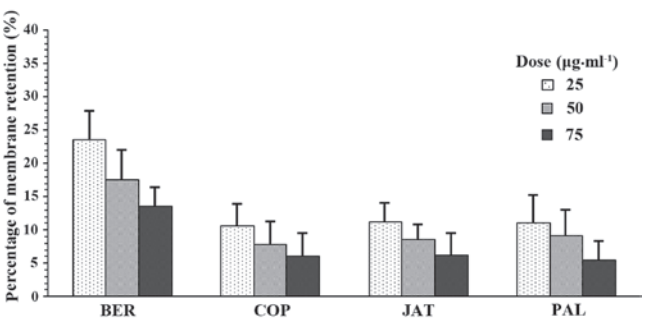


Figure 4. Membrane retention of the four alkaloids from *Coptis*. Error bars represent the data presented as the mean ± standard deviation BER, berberine; COP, coptisine; PAL, palmatine; JAT, jatrorrhizine.

Table III. Effects of different concentrations of the four alkaloids on the survival rate of Caco-2 cells.

	Survival rate (%)				
Compound	10 μmol·l^{-1}	20 μmol·l^{-1}	30 μmol·l^{-1}	60 μmol·l^{-1}	100 μmol·l^{-1}
BER	99.20	97.80	97.57	98.18	96.32
COP	99.56	98.25	98.02	98.45	97.23
PAL	99.35	98.06	97.88	97.67	96.18
JAT	99.38	98.79	98.34	98.01	97.72

BER, berberine; COP, coptisine; PAL, palmatine; JAT, jatrorrhizine.

Table IV. Apparent permeability coefficient of the four alkaloids in a Caco-2 cell model.

		Papp (x10^{-6} cm/s)				
Compound	Papp direction	10 μmol	20 μmol	30 μmol	60 μmol	100 μmol
BER	(AP-BL)	2.59	3.53	2.85	2.98	2.68
	(BL-AP)	25.18	31.30	20.81	25.30	25.90
	(BL-AP)/(AP-BL)	9.72	8.87	7.30	8.49	9.66
COP	(AP-BL)	1.79	1.97	2.24	2.49	2.55
	(BL-AP)	16.05	18.81	20.03	22.83	23.80
	(BL-AP)/(AP-BL)	8.97	9.55	8.94	9.17	9.33
PAL	(AP-BL)	1.46	1.82	2.03	2.25	2.39
	(BL-AP)	11.51	14.89	15.67	18.38	20.89
	(BL-AP)/(AP-BL)	7.88	8.18	7.72	8.17	8.74
JAT	(AP-BL)	1.46	1.59	1.78	2.04	2.37
	(BL-AP)	9.50	10.86	12.32	15.80	20.80
	(BL-AP)/(AP-BL)	6.51	6.83	6.92	7.75	8.78

Papp, apparent permeability coefficient; BER, berberine; COP, coptisine; PAL, palmatine; JAT, jatrorrhizine; AP, apical; BL, basolateral.

The Papp and R% of BER, COP, JAT and PAL are shown in Fig. 3 and 4. The Papp of BER was at between 1.06 and 1.33x10^{-6} cm·s^{-1} under three concentrations, which was significantly higher than COP (0.49-0.64x10^{-6} cm·s^{-1}), PAL (0.15-0.32x10^{-6} cm·s^{-1}) and JAT (0.23-0.36x10^{-6} cm·s^{-1}). The R% of BER was also significantly higher than the three other alkaloids.

Bidirectional transportation and the effect of the P-gp transporter. The survival rate of the Caco-2 cells following treatment with the alkaloids was determined using an MTT assay. The survival rate (%) of all of the cells was >97.5% (Table III), and there was no effect on Caco-2 activity following treatment with 10-100 μmol·l^{-1} of each alkaloid.

Verification of the Caco-2 monolayer cell model was performed using the propranolol cocktail drug. The Papp values of propranolol and fluorescent yellow were 1.29x10^{-5} and 5.17x10^{-7} cm·s, respectively, which was concordant with the findings of a previous study (51). The resistance value of the Caco-2 cells was 350-450 Ω, which indicated that the Caco-2 cell monolayer model was successfully validated (40-42,50,52,53).

The validated Caco-2 cell monolayer model was used to determine the bidirectional transportation of the four alkaloids at various concentrations (AP-BL or BL-AP). The Papp results are shown in Table IV. The Papp (AP-BL) and Papp (BL-AP) of BER were similar as the dose increased, indicating that other alkaloids did not affect the transportation of BER. The Papp (AP-BL) and Papp (BL-AP) of BER linearly increased with increasing dose (10-100 μmol·l^{-1}) (Fig. 5). The concentration of the four alkaloids was higher on the BL-AP side in the Caco-2 cell monolayer model, compared with the AP-BL side, which may be caused by carrier-mediated transportation of BER from the BL side to the AP side. The cumulative concentration of BER versus time is shown in Fig. 5, Papp (BL-to-AP) was increased in a dose-dependent linear manner.

The effects of verapamil on the bidirectional transport of the four alkaloids were determined using the Caco-2 cell monolayer. The Papp (AP-BL/BL-AP) was significantly decreased following treatment with verapamil (Table V), indicating that P-gp proteins may be involved in the efflux of the four alkaloids.

Intestinal perfusion in rats. The present study demonstrated that BER, COP, PAL and JAT were absorbed into the rat

Table V. Apparent permeability coefficient of the four alkaloids induced by verapamil.

Group	Papp ($x10^{-6}$ cm/s)		
	AP-BL	BL-AP	AP-BL/BL-AP
BER	2.85±0.02	20.81±0.19	7.30±0.02
BER and verapamil	8.93±0.18	17.90±0.26	2.00±0.20[a]
COP	2.24±0.12	20.03±0.42	8.94±0.36
COP and verapamil	7.25±0.16	16.16±0.37	2.23±0.28[a]
PAL	2.03±0.08	15.67±0.23	7.72±0.16
PAL and verapamil	6.35±0.27	15.94±0.46	2.51±0.21[a]
JAT	1.78±0.09	12.32±0.15	6.92±0.22
JAT and verapamil	7.62±0.18	13.98±0.38	1.83±0.28[a]

Data are presented as the mean ± standard deviation. [a]P<0.01, Papp of each drug compared with verapamil of each drug. BER, berberine; COP, coptisine; PAL, palmatine; JAT, jatrorrhizine; Papp, apparent permeability coefficient; AP, apical; BL, basolateral.

Table VI. Ka and absorption percentage of the four alkaloids from *Coptis* in the rat intestine.

Compound	Intestinal segment	Absorption percentage (A %)	Ka (h^{-1})
BER	Jejunum	12.11±1.15[a]	0.0351±0.0012[a]
	Ileum	9.82±0.89	0.0266±0.0024
COP	Jejunum	8.64±2.58	0.0235±0.0038
	Ileum	6.39±3.09	0.0196±0.0048
PAL	Jejunum	14.36±4.56	0.0358±0.0089
	Ileum	12.17±5.71	0.0323±0.0092
JAT	Jejunum	8.98±2.43	0.0219±0.0036
	Ileum	7.54±1.45	0.0198±0.0024

Data are presented as the mean ± standard deviation (n=6). [a]P<0.05, jejunum compared with ileum for each drug. BER, berberine; COP, coptisine; PAL, palmatine; JAT, jatrorrhizine; Ka, absorption rate constant.

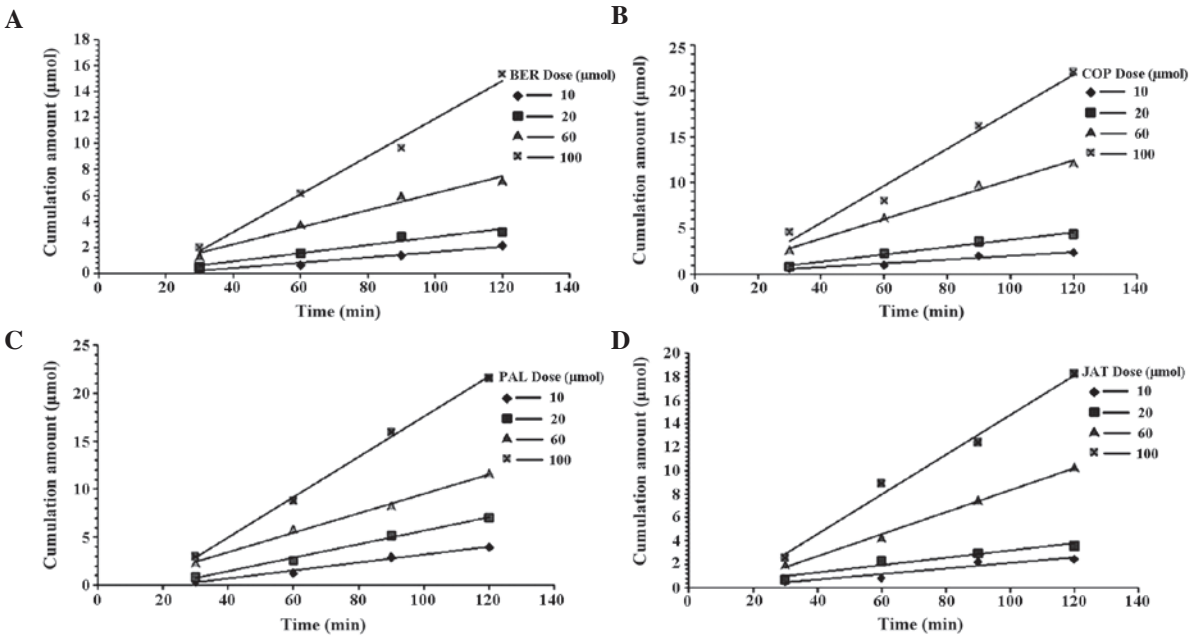


Figure 5. Time course for the basolateral-to-apical cumulative amount of the four alkaloids from *Coptis* across the Caco-2 cell monolayer (n=6). The representative cumulative levels of (A) BER, (B) COP, (C) PAL and (D) JAT are shown. BER, berberine; COP, coptisine; PAL, palmatine; JAT, jatrorrhizine.

jejunum and ileum. The level of absorption of the compounds was markedly higher in the jejunum than in the ileum (Table VI). The A % of the four alkaloids were small. The absorption rate of BER was significantly higher in the jejunum than in the ileum ($P<0.05$).

Discussion

The hydrophilic or lipophilic ability of a drug can be determined by measuring its Papp, which is an important parameter for the prediction of passive drug diffusion. The solubility of a drug in n-octanol is similar to that in biofilm, and n-octanol may be used to simulate the lipid barrier of the digestive tract. In addition, 0.1 $mol \cdot l^{-1}$ HCl and phosphate buffer (pH 7.4) may be used to simulate the environment of the stomach and intestines (54). The results of the present study demonstrated that the $P_{o/w}$ of the four alkaloids were significantly higher in 0.1 $mol \cdot l^{-1}$ HCl than in PBS (pH 7.4). Furthermore, the $P_{o/w}$ of BER was significantly higher than the three other alkaloids in 0.1 $mol \cdot l^{-1}$ HCl and PBS (pH 7.4).

PAMPA is a high throughput tool used for passive diffusion drug screening. The Papp (AP-BL) of the four alkaloids was between 0.15 and $1.33x10^{-6}$ $cm \cdot s^{-1}$; and a drug with a Papp $>1.5x10^{-6}$ $cm \cdot s^{-1}$ is considered to be highly penetrative; therefore, the Papp of the four alkaloids assessed in the present study indicated poor permeability. In addition, the Papp of the four alkaloids from BL-to-AP was significantly higher than from AP-to-BL.

The present study demonstrated that the quantity of BER migrating through the Caco-2 cell monolayer model increased linearly with increasing concentration. The Papp value of BER was markedly dependent on concentration, indicating that passive diffusion was responsible for the transport of BER from AP-to-BL. In the presence of verapamil, the speed of transportation and the Papp of BER were significantly increased on the AP-to-BL surface, and significantly decreased on the BL-to-AP surface. These results indicated that P-gp may be involved in the efflux transportation of the alkaloids, which is concordant with previous findings (29-31,41). The Ka for the P-gp carrier of BER was >100 μmol.

The rat intestinal perfusion experiments demonstrated that the four alkaloids were absorbed in the jejunum and ileum. The absorption of the four alkaloids was higher in the jejunum, compared with in the ileum. The cumulative percentage of intestinal absorption for the four alkaloids within 4 h was >15.75%. These results suggested that gastrointestinal absorption was weak due to the poor permeability and absorption of BER, COP, PAL and JAT.

In conclusion, in combination with a previous metabolic investigation of BER in the liver (51), the present study indicated that weak permeability, poor absorption, rapid P-gp efflux and rapid hepatic metabolism leads to the low oral bioavailability and low plasma concentrations of BER, COP, PAL and JAT, and may affect their pharmacological functions.

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