Evaluation of Intestinal Absorption of Ginsenoside Rg₁ Incorporated in Microemulsion Using Parallel Artificial Membrane Permeability Assay

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In the present study, ginsenoside Rg₁ (Rg₁), a naturally occurring drug which is hardly absorbed in gastrointestinal (GI) tract due to its high hydrophilicity and low membrane permeability, was incorporated in different compositions of water-in-oil microemulsions (MEs). And parallel artificial membrane permeability assay (PAMPA) that have been mainly utilized for the evaluation of *in vitro* permeability of early drug candidates was introduced in present study, as well as rat *in vivo* pharmacokinetics and *in vitro* permeability measurements, to investigate the effect of w/o ME on Rg₁ absorption. Correlation between various models as mentioned above was further performed to estimate the feasibility of PAMPA in the application of pharmaceutical preparation studies. After being administrated intraduodenally to rats, most of MEs can enhance the intestinal absorption of Rg₁ to various extents with relative bioavailability (F_{re}) ranging from 268 to 1270% using drug solution as control. This enhanced absorption of Rg₁ may be related to its increased membrane permeability induced by ME as exhibited in the PAMPA and rat *in vitro* permeability measurements. Meanwhile, rat *in vivo* pharmacokinetics–PAMPA correlation ($r^2=0.6082$) is significant ($p<0.05$) for ME, representing a potential prospect for the application of PAMPA in the study of pharmaceutical preparation in some conditions.

Key words water/oil microemulsion; bioavailability; everted intestinal sac; parallel artificial membrane permeability assay

Being an isotropically clear and thermodynamically stable colloidal dispersions with droplet size smaller than 100 nm, microemulsion (ME) was first introduced by Hoar and Schulman in 1943.¹⁾ Recently, there has been an increasing interests in the development of water-in-oil (w/o) ME as a novel oral drug delivery system incorporating hydrophilic biomacromolecule with poor absorption such as peptide^{2,3)} and protein⁴⁾ in terms of its pharmaceutical advantages in stability, sterility, enhanced absorption and favorable curative effect.⁵⁾ The enhanced absorption offered by w/o ME may be related to the surfactant-induced membrane fluidity and thus permeability changes.^{6,7)} However, the mechanism of the enhanced absorption remains still largely unknown for w/o ME on the whole. Derived from the lack of full dilution capabilities with water, w/o ME may transform into coarse emulsion, suspension or break into two much more phases accompanied with precipitation when diluted in the gastrointestinal tract,⁷⁾ which make some parameters (such as droplet size,⁸⁾ drug release,⁹⁾ refractive index, electrical conductivity and viscosity) that had been applied in evaluating other colloidal system unsuitable for reflecting the quality of w/o ME efficiently. So far, approaches applied to explore the absorption profiles of w/o ME in gastrointestinal (GI) tract were mainly confined to Caco-2 cells monolayer, animals *in vitro* (intestinal membrane permeability) and *in vivo* (pharmacokinetics) models.

Parallel artificial membrane permeability assay (PAMPA) has been developed as a non-cell-based assay that measures passive permeability of new chemical entities for screening during early stages of drug discovery since its introduction by Kansy *et al.* in 1998.¹⁰⁾ This assay utilizes a simple phospholipid-coated filter, which has been usually prepared by impregnating a porous filter with a solution of lipids or other biological membrane constituents, to separate two compartments mimicking the passive transport of drug in GI tract. Despite absence of enzymes and transporters, PAMPA at-

tracts considerable interests in recent years in terms of its versatility, speed, inexpensive cost, straightforward data on the passive permeation, and higher precision between laboratories comparing to cell culture studies such as Caco-2 cell monolayer that is a relatively lower throughput and labor-intensive test. And in practice, passive permeation is the major route for passage of compounds from the GI tract into the body. Therefore, PAMPA has been taken as an alternative to cellular models for the earliest absorption, distribution, metabolism, excretion (ADME) primary screening of research compounds when correlating passive permeability with *in vivo* oral absorption.^{11–14)} Furthermore, some modifications in membrane compositions make PAMPA capable of predicting total passive permeability (including both transcellular and paracellular pathway) of drug across intestinal membrane¹⁵⁾ and passive permeability of drug through the blood–brain barrier.¹⁶⁾ In addition, since no living cells are involved, PAMPA can also be performed in some non-biological circumstances such as a wide pH range of incubation solution,¹⁷⁾ and addition of surfactants¹⁸⁾ or solubilizers (including organic solvents and excipients)¹⁹⁾ to the incubation solution, which suggests PAMPA may be also used in the pharmaceutical formulation in some conditions.²⁰⁾

Being one of main ingredient of *Panax notoginseng* saponins (PNS), ginsenoside Rg₁ (Rg₁) (molecular weight=801) is a hydrophilic naturally occurring drug that is hardly absorbed in GI tract, which transports across Caco-2 cell monolayer by a passive diffusion.²¹⁾ In present study, w/o ME incorporating Rg₁ was prepared, and PAMPA measurement, rat *in vitro* model (everted intestinal sac), rat *in vivo* model (pharmacokinetics) model were utilized to evaluate the influence of ME on Rg₁ absorption. Correlation between various models as mentioned above was further performed to estimate the feasibility of PAMPA applied in the pharmaceutical preparation.

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MATERIALS AND METHODS

Materials SP (Soybean phospholipid) was purchased from Shanghai Taiwei Pharmaceutical Industry Co., Ltd. Labrafil M 1944 CS[®] (oleoyl macroglycerides), Maisine 35-1[®] (glyceryl monolinoleate) and Lauroglycol FCC[®] (propylene glycol laurate) were kindly provided from Gattefossé (France). Miglyol 812 (caprylic/capric triglyceride), polyethylene glycol 400 (PEG 400), Imwitor 600[®] (polyglyceryl-3 polyricinoleate), Imwitor 780 K[®] (isostearyl diglyceryl succinate), Imwitor 380[®] (glyceryl cocoate/citrate/lactate), Imwitor 312[®] (glyceryl laurate) were obtained from Sasol (Springfield, NJ, U.S.A.). SQO (sorbitan sesquioleate), isopropyl palmitate (IPP), 2-ethyl hexyl palmitate (2EHP) and isopropyl myristate (IPM) were from Shanghai Zhirou Co. (Shanghai, China). *N*-Dodecane was bought from Fluka (Chemie AG, Buchs, Switzerland). *Panax notoginseng* saponins (PNS) was purchased from Kunming Pharmaceutical Co. (Kunming, China). Ginsenoside Rg₁ (Rg₁) was purchased from National Institute for the Control of Pharmaceutical and Biological Products, China. MultiScreen 96-Well Filtration plate (Millipore Corporation, Bedford, MA, U.S.A.). All other chemicals and reagents were of analytical or HPLC grade as appropriate.

Microemulsion Preparation It is essential to acquire a high drug loading capacity for w/o ME because of low permeability and poor absorption of Rg₁ in GI tract. To assess w/o ME region, pseudoternary phase diagrams were established by titration of a series of mixtures of drug solution (Rg₁ concentration 110 mg/ml) and various oils (Labrafil M 1944 CS[®], Maisine 35-1[®], Miglyol 812, isopropyl palmitate, Lauroglycol FCC[®], IMWITOR 380[®], IMWITOR 312[®], oleic acid, isopropyl myristate or 2-ethyl hexyl palmitate) with a blend of surfactants (SP, Imwitor 780 K, Imwitor 600, SQO or Span 80) and co-surfactants (EtOH, PEG 400 or propylene glycol) (surfactant/co-surfactant ratio: 0.5, 1.0, 1.5 or 2.0) until a transparent w/o ME was obtained. The area enclosed by the w/o ME existence field was analyzed among different compositions to obtain appropriate formulation.

Determination of Rg₁ by HPLC High performance liquid chromatography (HPLC) was carried out using a YMC ODS C₁₈ column (5 μ m, 4.2 i.d. $\times$ 150 mm, Japan) with a liquid chromatograph (LC-10AT, Shimadzu, Japan) and a UV-VIS detector (SPD-101, Shimadzu, Japan) at 203 nm. A mobile phase with the speed of 1 ml/min consisting of acetonitrile : 20 mM NaH₂PO₄ (25 : 75, v/v) was used for chromatographic resolution.

Solid phase extraction (SPE) cartridges (strata-X, Phenomenex) were conditioned under vacuum with 1 ml of methanol and 1 ml of water. Then, 200 μ l of plasma samples were mixed with 4 μ l of 85% (v/v) phosphoric acid, and centrifuged at 9000 *g* for 10 min. One hundred microliters of supernatants were applied to SPE cartridges and drawn through under low vacuum. Washed cartridges with 1 ml of 5% (v/v) methanol aqueous solution, then elute them with 1 ml of methanol and evaporated to dryness under the stream of nitrogen at 40 °C. Finally, reconstituted the residues in 100 μ l mobile phase and 10 μ l was injected into HPLC.

Bioavailability Studies after Intraduodenal Administration in Rat Male Sprague-Dawley rats weighing 250–300 g were anesthetized lightly with diethyl ether after a 20 h

fasting, and then drug solution (Rg₁ concentration 41.04 mg/ml) and different composition of w/o MEs were administered intraduodenally (id) to the rats at a dose volume of 4 ml/kg (dose, see Table 2). After the small surgery on the duodenum in the rats, muscle and skin of the main incision were sutured carefully. Blood samples were collected from caudal vein at 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, and 12 h after id administration, and the rats have already been postanesthetic recovery at that time. Plasma samples were obtained by centrifugation at 4000 *g* for 10 min after the blood collection and the plasma concentration of Rg₁ was determined by HPLC. Pharmacokinetic behavior of Rg₁ after intravenous (iv) administration (13.68 mg/kg) through caudal vein in rats was also conducted to evaluate the absolute bioavailability of both drug solution and microemulsion after id administration.

Model-independent methods based on the theory of statistical moments were used to characterize the pharmacokinetics of Rg₁ after id administration of solution or w/o ME. *AUC* (area under the plasma concentration *versus* time curve) and *MRT* (mean residence time) were calculated by numerical integration under the trapezoidal rule as described.²²⁾ Because this was observed over a limited period of time, extrapolation to an infinity time was carried out using a monoexponential equation. The absolute bioavailability (*F*) and relative bioavailability (*F_{re}*) of Rg₁ was calculated through dividing $AUC_{id}/X_{0,id}$ by the $AUC_{iv}/X_{0,iv}$, and dividing $AUC_{ME}/Dose_{ME}$ by the $AUC_{solution}/Dose_{solution}$ respectively.

Transport of Rg₁ across Everted Intestinal Sacs Everted intestinal sac experiments were performed according to the method described by Deshmukh with some modification.²³⁾ Male Sprague-Dawley rats weighing 220–250 g were fasted overnight with free access to water until the rats were anaesthetized with urethane. Then, a middle abdominal incision was made and jejunum was taken quickly. After the underlying mesenterium was removed, the jejunum was flushed with ice-cold Tyrode's solution to remove intestinal contents. The jejunum was gently stretched and cut into segments (10 cm long each). Each of the sacs was carefully everted with a glass rod. One end was ligated, the other end was ligated with a conical rubber stopper that housed one port for removal and addition of the serosal fluid, and another port for continuous supply for 5% CO₂ and 95% O₂ throughout the experiment.

After being filled with 2 ml Tyrode's solution as serosal fluid (inner compartment), the sacs were incubated in 25 ml mucosal fluid (outer compartment) that was obtained by diluting w/o MEs with Tyrode's solution (1 $\rightarrow$ 20, v/v) (D-ME) at 37 °C in a water bath. Five hundred microliters of the serosal fluid inside the sac was taken at predetermined times (15 min, 30 min, 45 min, 60 min) followed by immediate replacement with fresh Tyrode's solution of the same volume (500 μ l). All samples were reserved at –20 °C until analysis by HPLC. The viability of the intestinal membrane during the course of the experiments was monitored by measuring the activity of lactic acid dehydrogenase (LDH) released from the intestinal mucosa. Enhanced rate (*ER*) of Rg₁ transport across rat intestine was calculated as followed equation to evaluate the influence of the ME on Rg₁ absorption.

Table 1. Composition (% w/w) and R_{g1} Concentration (mg/ml) of w/o Microemulsion

Samples	Composition			R _{g1} concentration (mg/ml)
	Surfactant/co-surfactant (1/1) (w/w)	Oil (w/w)	Drug solution (w/w)	
ME I	SP/EtOH (31.20%)	Labrafil M 1944 CS® (44.11%)	24.69%	35.04
ME II	SQO/EtOH (45.47%)	Maisine 35-1® (45.62%)	8.91%	10.50
ME III	SP/EtOH (45.66%)	Miglyol 812 (44.86%)	9.38%	18.28
ME IV	SP/EtOH (47.79%)	IPP (43.39%)	8.82%	12.38
ME V	SP/EtOH (35.09%)	Lauroglycol FCC® (43.11%)	21.80%	33.11
ME VI	SP/EtOH (35.14%)	IMWITOR 380® (43.67%)	21.19%	36.83
ME VII	SP/EtOH (45.10%)	IMWITOR 312® (31.43%)	23.48%	32.03
ME VIII	SP/EtOH (41.39%)	Oleic acid (38.19%)	20.42%	25.61
ME IX	SP/EtOH (52.50%)	IPM (32.01%)	15.49%	21.61
ME X	SP/EtOH (66.71%)	2EHP (24.55%)	8.73%	11.00

Notation: SP and SQO indicate soybean phospholipid and Sorbitan sesquioleate, respectively.

$$ER = \frac{C_{60 \text{ min, ME}} \cdot C_{0 \text{ min, control}}}{C_{0 \text{ min, ME}} \cdot C_{60 \text{ min, control}}}$$

Where $C_{60 \text{ min, control}}$ and $C_{60 \text{ min, ME}}$ are the concentration of R_{g1} in serosal fluid at 60 min when drug solution (control) and D-MEs were used as mucosal fluids respectively. $C_{0 \text{ min, control}}$ and $C_{0 \text{ min, ME}}$ are the initial concentration of R_{g1} in mucosal fluids (drug solution or D-MEs).

PAMPA Method The following protocol was applied to measuring P_e values. A 10% solution (w/v) of egg lecithin (phosphatidyl choline >98%, lipid) in dodecane⁽¹⁰⁾ was obtained as artificial membrane solution by ultrasound. Each donor filtration plate well was then impregnated with 10 μ l of this solution carefully, avoid touching with pipet tip in case of damage of the membrane. Immediately after the application of the artificial membrane, each well of donor plate was introduced 150 μ l of phosphate buffer (pH 7.4) containing R_{g1} at 11 mg/ml, or MEs which was diluted with phosphate buffer (pH 7.4) (D-ME) or not (ME) (R_{g1} concentration in MEs refers to Table 1). Then the drug-filled donor plate was placed into the acceptor plate that had been prefilled with phosphate buffer (pH=7.4, 300 μ l) as acceptor solution, making sure the underside of the membrane was in contact with the buffer in all wells. After plate lid was replaced, the resulting assembled donor-acceptor plates were placed in a sealed container with wet paper towels to avoid evaporation, and incubated at room temperature for 2 h under constant light shaking (50 rpm), following which R_{g1} concentration in the acceptor solutions were determined by HPLC. Permeability of R_{g1} transporting across the artificial membrane was calculated according to the equation:

$$P_e = \frac{V_D \cdot V_A}{(V_D + V_A) \cdot A \cdot t} \times \ln \frac{C_{\text{equilibrium}}}{C_{\text{equilibrium}} - C_{\text{acceptor}}}$$

Where P_e is the effective permeability coefficient ($\text{cm} \cdot \text{s}^{-1}$), V_D and V_A are volume of donor and acceptor compartment respectively (ml), A =effective filter area (0.24 cm^2), t is incubation time for the assay, C_{acceptor} is the concentration of the compound in the acceptor compartment at the completion of the assay, and $C_{\text{equilibrium}}$ is the concentration of compound at theoretical equilibrium.

Statistical Analysis Values are expressed as mean $\pm$ S.D. The difference among mean values was analyzed with two-

tailed Student's *t*-test and comparisons among more than three groups were performed by using one-way ANOVA. $p < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Microemulsion Formulations In preparation of w/o ME, much more drug solution could be dissolved in most kinds of oils as mentioned in the text when SP was used as main surfactant than that was dissolved in oils when Imwitor 780 K, Imwitor 600, Span 80, and SQO were used as main surfactants. And considering favourable safety and biocompatibility offered by SP, it was selected and further mixed with EtOH (a co-surfactant) as the surfactant/co-surfactant at a ratio of 1/1 (w/w) in MEs. Thus, ten MEs were developed by titrating a mixture of drug solution and oils with SP/EtOH (1/1) until a clear and transparent appearance was obtained. The composition of MEs was showed in Table 1 without lower R_{g1} concentration than 10 mg/ml. Otherwise, difficulties in administrating intraduodenally to rats and performing PAMPA precisely for ME could be brought when Maisine 35-1® was used as oil phase due to its relatively high melting point causing solidification of the w/o ME at room temperature after addition of the SP/EtOH. SQO was then substituted for SP/EtOH in the ME to prepare a liquid w/o ME using Maisine 35-1® as oil phase. Droplet size of MEs as presented in Table 1 was below 100 nm (e.g. $17.8 \pm 1.7 \text{ nm}$ for ME I).

Pharmacokinetics and Everted Intestinal Sac Studies Pharmacokinetic behavior of w/o ME was experimented in rats after id administration. Although absolute bioavailability (F) of various w/o MEs was lower than 20%, most of them can enhance intestinal absorption of R_{g1} to various extents with relative bioavailability (F_{re}) ranging from 268 to 1270% in comparison to drug solution (Table 2), as well as promote the permeation of R_{g1} across intestinal membrane as shown in the everted intestinal sac studies (Table 3). Before rat everted sac studies, it is essential to dilute w/o MEs with Tyrode's solution because of its incompatibility of continuous oil phase with biological membrane. Low extent of dilution for w/o ME in mucosal fluid may induce the damage to intestinal membrane due to a relatively high concentration of surfactants in mucosal fluid, whereas high extent of dilution for w/o ME leads to the low concentration of R_{g1} in serosal fluid, and thus resulting in the inability to be quantitated.

Table 2. Model-Independent Pharmacokinetic Parameters of Rg₁ after Intravenous Administration (iv) of Drug Solution (Control), and Intraduodenal Administration (id) of Drug Solution and Different Composition of w/o Microemulsion to Rats

Samples	Dosing routes	Pharmacokinetics parameters				F (%)	F _{re} (%)
		MRT (h)	X ₀ (mg·kg ⁻¹)	AUC (μg·ml·h ⁻¹)	AUC/X ₀ (g·ml·h ⁻¹)		
Control	iv	1.55±0.39	13.68	37.98±3.67	2.776±0.2683	100	—
Solution	id	7.12±3.64	164.2	6.920±0.55	0.04214±0.0034	1.52	100
ME I	id	5.31±1.97	140.2	48.25±15.33	0.3442±0.1094*	12.4	817
ME II	id	2.85±1.34	42.01	22.41±7.78	0.5334±0.1852*	19.2	1270
ME III	id	2.04±0.64	73.11	8.252±2.30	0.1129±0.0315*	4.07	268
ME IV ^{a)}	id	—	49.52	—	—	—	—
ME V	id	2.03±0.49	132.4	33.25±4.80	0.2511±0.0363*	9.05	596
ME VI	id	2.16±0.81	147.3	65.62±16.82	0.4455±0.1142*	16.1	1060
ME VII	id	1.35±0.21	128.1	44.56±3.44	0.3479±0.0269*	12.5	826
ME VIII	id	2.44±0.88	102.4	20.71±0.51	0.2022±0.005*	7.28	480
ME IX	id	2.09±0.66	86.43	24.44±2.24	0.2828±0.0259*	10.2	671
ME X ^{a)}	id	—	43.99	—	—	—	—

a) Pharmacokinetic parameters for ME IV and ME X can not be obtained because concentration of Rg₁ in plasma is too low to be determined. Values are given as the mean±S.D. (n=3). * Significantly different from control (p<0.05). F%=[(AUC_{id}/X_{0, id})/(AUC_{iv}/X_{0, iv})]×100%. F_{re}%=[(AUC_{ME}/X_{0, ME})/(AUC_{solution}/X_{0, solution})]×100%.

Table 3. Enhanced Rate (ER) of Rg₁ Transporting across Rat Everted Intestinal Sacs Induced by Diluted Microemulsion (1→20) (D-ME), and P_e Values of Rg₁ in PAMPA Analysis for Microemulsion Diluted (1→10) (D-ME) or Not (ME)

Samples	ER (D-ME)	P _e (×10 ⁻⁸ cm/s)	
		ME	D-ME
Control	1	8.61±2.26	8.61±2.26
ME I	2.45	5.39±0.32	18.3±4.14*
ME II	5.02	24.9±7.46*	21.4±2.92*
ME III	3.94	0.490±0.05*	4.07±0.89*
ME IV	1.02	1.18±0.75*	25.1±7.60*
ME V	2.28	5.25±2.70	80.8±4.52*
ME VI	2.18	21.5±2.82*	23.1±1.44*
ME VII	2.47	9.99±2.12	12.3±6.56
ME VIII	1.45	11.6±1.19	2.96±1.39*
ME IX	1.02	0.903±0.38*	6.06±1.81
ME X	3.08	3.86±1.14*	45.9±17.0*

Drug solution containing Rg₁ at 1.1 mg/ml and 11 mg/ml was used as controls for ER studies and P_e studies respectively. Values are given as the mean±S.D. (n=3). ER=(C_{ME}, 60min/C_{ME}, 0min)/(C_{control}, 60min/C_{control}, 0min). * Significantly different from control (p<0.05).

Therefore, diluted w/o ME (D-ME) (1→20, v/v) were used in the rat everted intestinal sac studies after some tries, and no evident damage to intestinal membrane was observed in 1 h during experiments as demonstrated by the determination of LDH. Not all the w/o MEs represent coincident and enhanced effects on Rg₁ absorption in both rat *in vivo* and *in vitro* models. Particularly, ME II and ME VI could increase Rg₁ bioavailability to 10 times higher than that of control (solution). In contrast, no pharmacokinetic parameters could be obtained for ME IV and ME X since the Rg₁ concentration in plasma is too low to be determined exactly. This disparate effect on the drug absorption among various ME formulations may be related to the difference of drug content loaded in MEs on the one hand, and on the other hand, composition of MEs could contribute to a large great extent.

Except surfactants that could interact with intestinal mucosa membrane causing increase of permeability of drug across intestinal membrane, some fatty acids and glycerides such as IPM, Miglyol 812, oleic acid and other monoglycerides may also enhance drug absorption by penetrating into

the intestinal membrane to induce disorder of the membrane and thus causing the increase of membrane fluidity,²⁴⁾ which is also observed by us that w/o MEs prepared in present study could increase the membrane fluidity of liposome as a model of the biological membrane using DPH (1,6-diphenyl-1,3,5-hexatriene) as a fluorescence probe that is incorporated in the inner apolar core of the membrane (data not shown). Meanwhile, some fatty glycerides were reported to promote the paracellular diffusion of drug through intracellular regulation of tight junction.²⁵⁾ Additionally, in an attempt to explore relationship between lipid components and drug absorption, solubility and dissolution of the lipophilic drug could be improved significantly by *in vitro* digestion products of some medium-long chain triglycerides,^{26–29)} which could be also demonstrated by the fact that much more cyclosporine oils (Miglyol 812 or olive oil) that had been digested *in vitro* could be absorbed in GI tract of rat with bile duct ligated than that could be absorbed without *in vitro* digestion.³⁰⁾ However, the lipid digestion may have no significant influence on the solubility and dissolution of Rg₁ because of its potent hydrophilicity, and our preliminary experiments showed that no evident change depending upon the *in vitro* digestion was observed for the solubility of the Rg₁ incorporated in MEs.

PAMPA Studies and Correlation In present study, egg lecithin dissolved in dodecane was used as model membrane barrier in the PAMPA method to mimic the high presence of phosphatidylcholine in mammalian membranes.²⁰⁾ Because lack of transporter- and pore-mediated permeability, PAMPA may underestimate the permeation value of some compounds which transport across membrane partly by transporter and/or paracellular routes. Thus, as an excellent bio-mimetic model, PAMPA only estimates the ability of molecules to permeate membrane by transcellular passive diffusion.³¹⁾ Since Rg₁ is known to transport across Caco-2 cell monolayer by a passive permeation,²¹⁾ comparative studies on correlation of PAMPA (P_e) with rat *in vivo* pharmacokinetics (F_{re}) and rat *in vitro* permeability (ER) were performed to explore the feasibility of PAMPA applied in the screen of pharmaceutical preparation studies. Because the concentration of Rg₁ in plasma for ME IV and ME X is too low to be determined, the corresponding F_{re} for both of them was then set to

Fig. 1. Comparison of Correlations among PAMPA-Rat *in Vivo*-Rat *in Vitro* Models Based on the Data from the 10 MEs

(A) Correlation between parallel artificial membrane permeability assay PAMPA (P_e) of diluted MEs (1→10, v/v) (D-ME) and rat *in vivo* studies (pharmacokinetics) (F_{re}). (B) Correlation between rat *in vitro* studies (everted rat intestinal sacs) (ER) for diluted MEs (1→20, v/v) (D-ME) and rat *in vivo* studies (F_{re}). (C) Correlation between PAMPA (P_e) of diluted MEs (1→10, v/v) (D-ME) and rat *in vitro* studies (ER) for diluted MEs (1→20, v/v) (D-ME). (D) correlation between (PAMPA) (P_e) of MEs (without dilution) and rat *in vivo* studies (F_{re}). For rat *in vivo* studies in (A), (B) and (C), the MEs were intraduodenally administrated to rat without dilution.

be 0 value to carry out the subsequent correlation studies.

A dilution of w/o ME by gastrointestinal fluid in GI tract is unavoidable, thus P_e values of D-MEs were first investigated. Hitherto, mechanisms of enhanced oral absorption for lipophilic drug offered by o/w ME have been comparatively clarified, such as improved solubility and dissolution,^{26–29} increased intestinal membrane permeability,³² or accelerated transport of lipophilic drug through the routes of lymph.³³ However, it is still largely unclear for w/o ME, one of the primary mechanisms is considered to be related to its increased intestinal membrane permeability of drug, the medium chain length fatty acid lipid components and other bioenhancers such as phosphatidylcholine (PC) that are commonly consisted in the w/o MEs have been shown to enhance transmembrane passage of drug across the digestive tract, thus improving the bioavailability.^{24,34} It was further confirmed in the present study as shown in Table 3, P_e (D-MEs) are mostly higher than that of drug solution (R_{g1} concentration 11 mg/ml) ($8.61 \pm 2.26 \times 10^{-8} \text{ cm/s}$), as well as rat *in vitro* permeability model (everted intestinal sac), suggesting the increased membrane permeability of R_{g1} offered by w/o ME. Otherwise, an additional process for drug released from the inner aqueous phase to the PAMPA membrane decreased the P_e values of most of w/o MEs in comparison to that of both D-MEs and solution (Table 3). However, the correlation of P_e - ER , P_e - F_{re} and ER - F_{re} are not significant ($p > 0.05$) for D-MEs, which may be attributed to the deviation in the data of both P_e (D-ME) and ER originating from the phase inver-

sion of w/o MEs after being diluted with buffer solution. For instance, the lipids precipitated from D-MEs after dilution may adhere to the membrane surface of the PAMPA or the intestinal mucosa of the rats everted intestinal sac, thus interfering with the accuracy and reproducibility of the results. On the other hand, a relatively large volume of w/o MEs (4 ml/kg) was dosed directly into the duodenum of rats thereby limiting the chances of phase inversion and magnifying absorption enhancing properties of the undiluted w/o ME, which might also result in the insignificant correlation between P_e , ER and F_{re} for the diluted microemulsion (D-ME). In contrast, undiluted w/o microemulsion (ME) that is homogeneous and isotropical systems may also reflect the effects of w/o MEs on drug permeability to some extent, and a better, significant correlation for w/o MEs could be observed between P_e and F_{re} ($r^2 = 0.6082$) ($p < 0.05$), suggesting the potential prospect for the application of PAMPA in the pharmaceutical research in some conditions, especially for the study of homogeneous and isotropical delivery system. Further studies is required to investigate the effect of administrated volume on the intestinal absorption and correlation between P_e and F_{re} for D-ME.

In conclusion, w/o microemulsion (ME) can enhance the intestinal absorption of a hydrophilic drug (R_{g1}) after id administration to rats, which may be due to the increased membrane permeability provided by w/o MEs as shown in PAMPA and rat everted intestinal sac studies. PAMPA may be introduced in the study of the pharmaceutical preparation to evaluate the membrane permeability in some conditions.

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