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Preparation and evaluation of a microemulsion for oral delivery of berberine

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The principal aim of this study was to develop an oral microemulsion formulation of berberine in order to improve its bioavailability. The Microemulsion was prepared with pharmaceutically acceptable ingredients such as oleic acid, Tween 80 and PEG400. Phase diagrams were drawn to elucidate the phase behavior of systems, which were composed of Tween 80 as surfactant and PEG400 as cosurfactant. A single isotropic region, considered to be a bicontinuous microemulsion, was detected in the pseudo ternary phase diagrams. The berberine-loaded microemulsion was characterized by viscosity, refractive index, electrical conductivity and particle size. *In vivo* pharmacokinetic profile and oral bioavailability were also investigated in rats. The optimized formulation was as follows: 15 wt.% oleic acid, 17 wt.% Tween-80, 17 wt.% PEG400, and 51 wt.% water. The formulated microemulsion was found to be relatively uniform in size (24.0 nm). The *in vivo* study indicated that the bioavailability of the oral berberine-loaded microemulsion formulation was 6.47 times greater than that of the berberine tablet suspensions. The results suggest that the microemulsion is a promising oral drug delivery system for berberine.

1. Introduction

Berberine ($[C_{20}H_{18}NO_4]^+$), a kind of isoquinoline alkaloid, is one of the major constituents of the Chinese herb Rhizoma coptidis. It has been clinically applied as antibacterial agent for many years because of its reliable effect. Berberine has a broad antifungal spectrum, which could inhibit various kinds of bacteria, fungi, viruses, etc. In recent years, much attention has focused on pharmacological effects of berberine, such as inhibiting tumors, relieving symptoms of diabetes or improving cerebral ischemia (Guo and Xia 2006; Wang and Lin 2004; Zheng and Xu 2004; Zhou et al. 2002). Compared with synthetical drugs, berberine is a kind of natural product and has fewer adverse reaction and lower toxity. The LD_{50} (rat, ip) and LD_{50} (rat, oral) of berberine are 66 mg/kg and 393 mg/kg, respectively. However, berberine is suggested to be poorly absorbed after oral administration. Hu reported the peak blood concentration of rabbit was in the range of μmol/L after given oral berberine (50 mg/kg). The oral bioavailability in beagle dog of berberine is less than 5%. Ding suggested that the drug level in rat blood was only 10 ng/ml after continuous oral administration for a week (Hu et al. 1997; Song et al. 2005; Ding et al. 2004). Therefore, it is essential to design a novel formulation of berberine to improve its oral bioavailabity and therapeutic effect.

Microemulsions are thermodyamically stable systems which are constituted of surfactant, co-surfactant, oil phase and aqueous phase. The particle size in microemulsions is between 10–100 nm. As a potentially excellent vehicle candidate for bioactive molecules, microemulsions can increase solubility of bioactive materials, and can also improve absorption through human gut membranes because microemulsions have extremely low surface tension. Drug could directly contact the gastrointestinal epithelium cell, consequently bioavailability could significantly be improved (Lawrence and Rees 2000; Humberstone and Charman 1997; O'Driscoll 2002; Swenson and Curatolo 1992).

The purpose of the present study was to formulate an oral delivery system for berberine using O/W microemulsions. The absorption of berberine from the microemulsion formulations was compared to that of commercially available tablet suspensions.

2. Investigations, results, and discussion

2.1. Phase diagram discussion

There were 3 oils (oleic acid, isoptopyl myristate and ethyl oleate), 2 surfactants (Polysorbate-80, Triton X-100) and 3 co-surfactants (ethanol, PEG400 and propylene glycol) were selected to prepare berberine microemulsion. Among them, Triton X-100 could not form stable microemulsion with other components. When Polysorbate-80 (Tween-80)/PEG 400 was used as a combined emulsifier, a maximum 10% (w/w) isoptopyl myristate (IPM) was solubilized, compared with 20% oleic acid (OA) and 15%

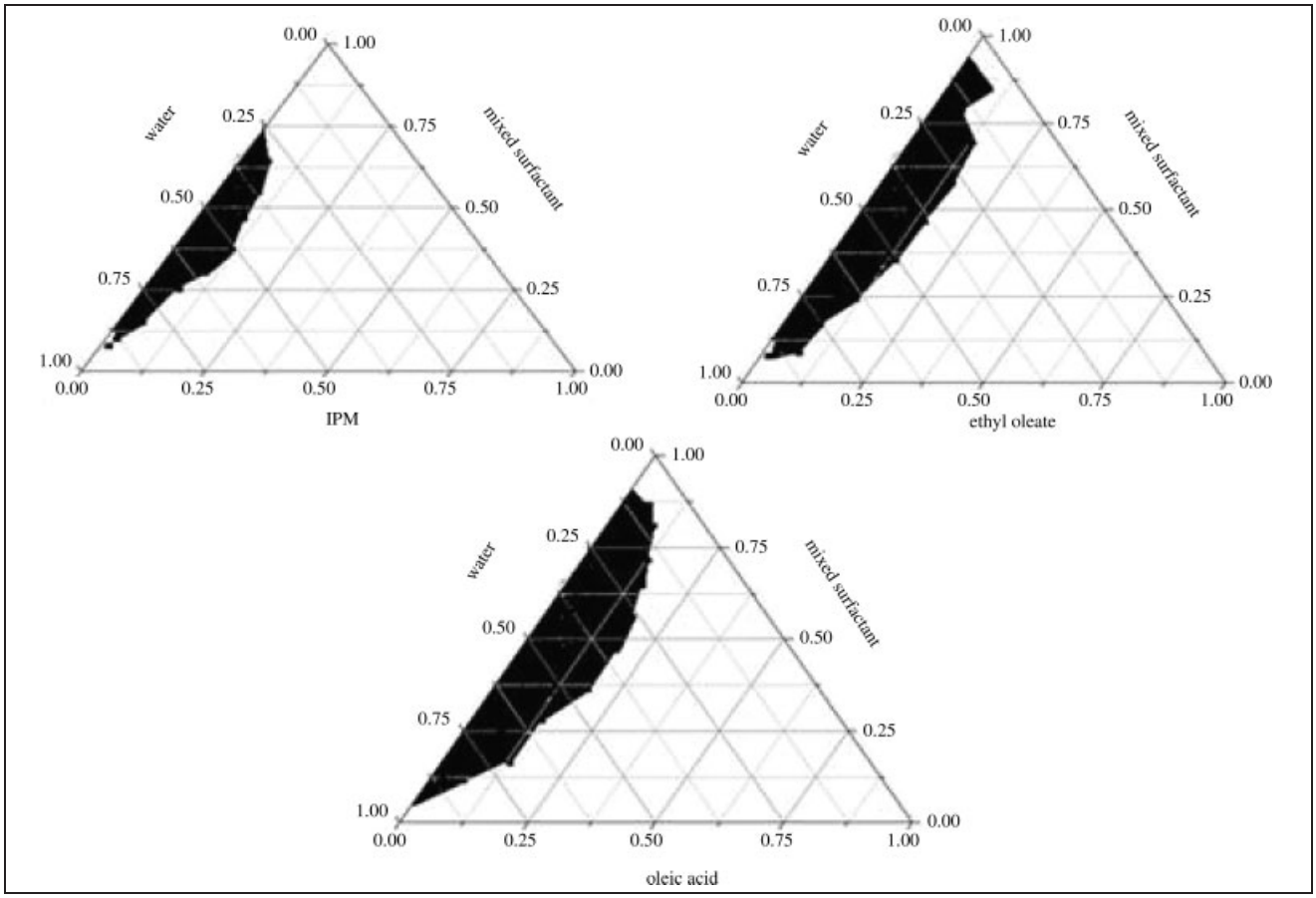

Fig. 1: Pseudoternary phase diagrams of systems consisted of Tween80/PEG400/water with different oil phase at 25 °C

ethyl oleate (EO). The ternary phase diagram is shown in Fig. 1. Generally speaking, increasement of oil content in microemulsion may provide a greater opportunity for drug solubilization. Thus, the further study on maximum drug solubilization was performed. In OA/Tween-80/PEG400/water system, the maximal solubility of berberine was 9.48 mg/ml at 25 °C, compared with 2.29 mg/ml in ethyl oleate/Polysorbate-80/PEG400/water. Furthermore, the area of microemulsion region based on different co-surfactants was determined. Compared with ethanol and propylene glycol, the biggest area in the diagrams appeared with PEG400 as a co-surfactant. Therefore, the optimal formulation of microemulsion consisted of OA (15%), Tween-80 (17%), PEG400 (17%) and water (51%).

2.2. Physicochemical characterization

The physicochemical characteristics of the developed microemulsion were determined. It had low viscosity (2.11 cPa · s). The investigated microemulsion system containing the non-ionic surfactant mixture, oil, and water showed electroconductive behavior in spite of its non-ionic nature. The electrical conductivity was 125.5 μΩ measured by a conductivity meter (Nanjing, China). From the results of viscosity and electroconductivity, it could be concluded that the microemulsion system was of the O/W type. The refractive index of the developed system was similar to the refractive index of water (1.363). The mean particle diameter of berberine microemulsion droplets was 24.0 nm. The particle size and distribution of berberine microemulsion is shown in Fig. 2. The morphological characterization of berberine microemulsion was shown in Fig. 3. In general, the berberine microemulsion droplets were round, uniform, and well-dispersed. The appearances were transparent with visible sky-blue opalescence.

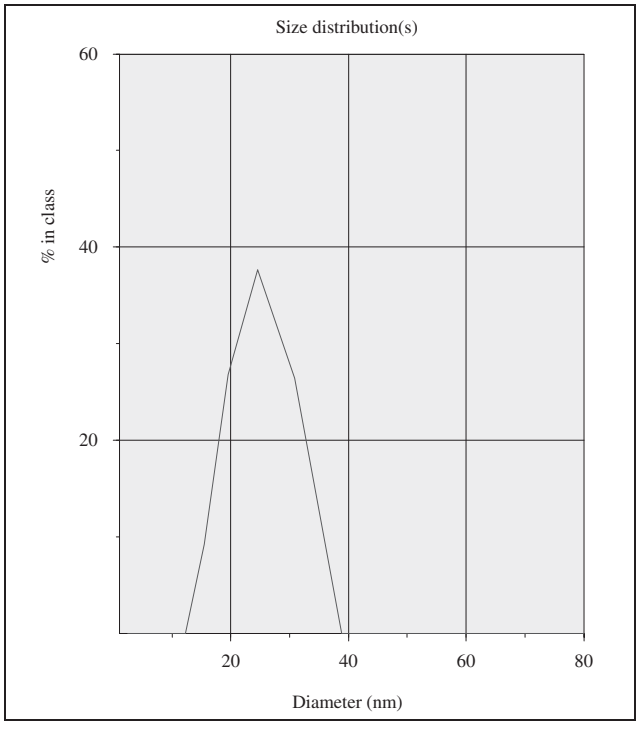

Fig. 2: Particle size and distribution of berberine microemulsion measured by PCS

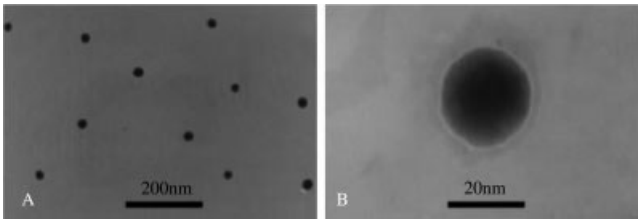

Fig. 3: Transmission electron microscope photographs of berberine microemulsions

2.3. Bioavailability and pharmacokinetic study

The mean berberine concentration in the blood after oral administration of multi-dose berberine microemulsion and suspensions of berberine tablets as a reference was illustrated in Fig. 4. The concentration-time curve of berberine microemulsion was significantly different from that of berberine suspensions. The concentration-time data of the two preparation were best fitted to a two-compartment model with a weight of $1/C^2$, and the relevant pharmacokinetic parameters such as C_{max}, T_{max}, AUC_{0-24}, elimination constant of the central compartment (K_{10}), the absorption constant from gastrointestinal (GI) to central compartment (Ka), mean residence time (MRT) and relative bioavailability (Fr) are given in the Table.

The C_{max} of tablet suspension formulation was 0.394 mg/ml after 8.3 h, whereas it was 4.169 mg/ml for the microemulsion formulation after 7.3 h. $AUC_{0-\infty}$, maximum plasma concentration (C_{max}) and the corresponding time (T_{max}) of microemulsion formulations were significantly different from conventional tablets ($p < 0.05$). The values of $AUC_{0-\infty}$ and C_{max} for the microemulsion formulation increased 6.468- and 10.58-fold, and T_{max} decreased by 1 h compared with that of the tablet formulation. The relative bioavailability of microemulsion formulation to the tablet group was 647%. The results indicated that the microemulsion formulation significantly increased the rate and extent of absorption of berberine. The enhanced absorption efficiency may be explained as follows: (1) the huge specific surface area of the microemulsion droplets (mean droplet size 24 nm), (2) the stability of the microemulsion in the gastrointestinal tract (Spernath and Aserin 2006; Araya et al. 2005; Spernath et al. 2007; Shiokawa et al. 2005), (3) promoted microemulsion of lymphatic transport through transcellular pathway (Gershanik and Benita 2000). A statistically significant difference between the two formulations was found from the ANOVA analysis. The results suggested that there was a significant difference between the pharmacokinetic parameters of berberine microemulsion and berberine tablet suspensions.

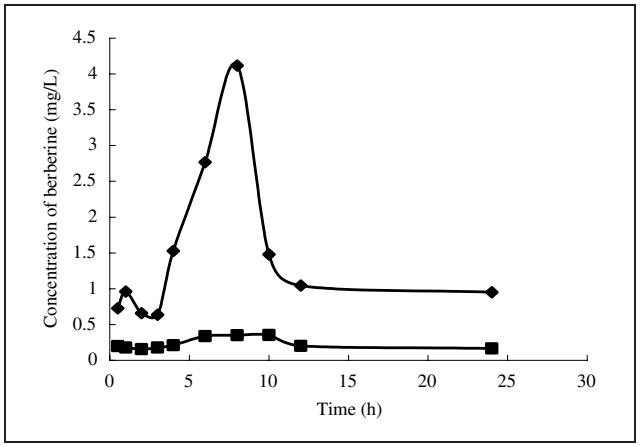

Fig. 4: Mean berberine blood concentrations upon oral administration of berberine microemulsion (□), and berberine tablet suspensions (■)

Table 1: Summary of pharmacokinetic parameters of berberine in blood of rats upon oral administration of berberine microemulsions and berberine tablet suspensions

Parameter	Microemulsion (50 mg/kg)	Tablets (50 mg/kg)
C_{max} (mg/l)	4.169	0.394
T_{max} (h)	7.333	8.333
$AUC_{(0-t)}$ (mg/l · h)	34.879	5.392
$AUC_{(0-\infty)}$ (mg/l · h)	64.647	8.312
$MRT_{(0-t)}$ (h)	10.693	11.009
$MRT_{(0-\infty)}$ (h)	32.655	22.662
$t_{1/2\alpha}$ (h)	58.175	6.86
$t_{1/2\beta}$ (h)	58.521	19.166
CL/F (l/h/kg)	0.856	5.928
V1/F (l/Kg)	2.685	108.842
Relative bioavailability	647%	

3. Experimental

3.1. Materials

Tween 80 was obtained from Surui Chemical (Shanghai, China). Ethyl oleate and oleic acid were purchased from Guangcheng Chemical Reagents Limited Company (Tianjin, China). Triton X-100 was purchased from Wuxi Haishuo Biology Co., Ltd (Wuxi, China). Isoptopyl myristate was purchased from Sinopharm Chemical ReAgent Co., Ltd, (Shanghai, China). Berberine was a gift from JinHua Pharmaceutical (Chengdu, China). HPLC-grade methanol and acetonitrile were purchased from Merck (Germany). All the other chemicals used were of analytical reagent grade. Double-distilled water was used throughout the whole study, which was purified by a Millipore Simplicity System (Bedford, USA).

3.2. Preparation of microemulsion

To find out optimal components in the formation of O/W microemulsions, two acceptable and compatible nonionic surfactants, namely Tween 80, Triton X-100, combined with ethanol, PEG 400 or propylene glycol as cosurfactant were chosen for the study. The oils employed in the present study were IPM, EO and OA. Surfactant was blended with cosurfactant to form a homogeneous mixture (Smix) of fixed weight ratios. The ratio of surfactant to cosurfactant was regarded as Km. The pseudo-ternary phase diagram of oil, Smix and water was plotted using water titration method. The phase behavior of system was studied at Km values of 1, 2 and 3. Briefly, mixtures of the oil with Smix were prepared in different vials at ratios (w/w) of 9 : 1, 8 : 2, 7 : 3, 6 : 4, 5 : 5, 4 : 6, 3 : 7, 2 : 8, 1 : 9, respectively. A small amount of water with 5% (w/w) increment was added into the vials. Upon water addition, the mixtures in each vial were stirred for 3–5 min. After equilibration, the mixtures were then evaluated by visual observation and polarizing microscope. In the phase diagram, microemulsion was the region of monophasic, clear and isotropic solutions that might also contain micellar solutions. Gels were claimed for those mixtures that did not show a change in the meniscus when tilted to an angle of 90°. All the experiments were performed at 25 °C (Ghosh et al. 2006; Wu et al. 2006; Subramanian et al. 2004).

The berberine-loaded microemulsion was prepared by spiking berberine powder directly into the blank microemulsions. The samples were tightly sealed and stored at ambient temperature, and their physical stability was determined by periodically observing the occurrence of phase separation.

3.3. Characterization of berberine-loaded microemulsion

The morphology of berberine-loaded microemulsion was examined using a transmission electron microscope (JEM-1230, Jeol, Japan). One drop of sample was deposited on a PVA-coated copper specimen grid and allowed to stand for 10 min after which any excess fluid was removed with filter paper. The grid was later stained with one drop of 3% phosphotungstic acid and allowed to dry for 5 min before examination.

The analysis of particle size was performed by photon correlation spectroscopy (which analyses the fluctuations in light scattering due to Brownian

motion of the particles) using a Zetasizer 3000 (Worcestershire, UK) which measures sizes between 3 and 3000 nm. The samples were filtered by a 0.2 μm polyvinylidene difluoride Millipore filter into a polystyrene disposable cuvette, with no further dilution. The average size was reported by the intensity distribution of each measurement. The wavelength was 633 nm, and light scattering was monitored at 25 °C, angle of 90°. Other parameters such as reflection index and viscosity were also investigated.

3.4. Determination of berberine in blood samples

Berberine level in blood was determined by reversed-phase HPLC. Briefly, exactly 100 μl of the plasma sample was mixed with 500 μl of acetonitrile, and vortexed for 1 min using a vortex mixer (IKA, Germany). The mixture was then centrifuged at 4 °C for 15 min (× 15000 rpm) using a highspeed frozen centrifuging machine (Shanghai, China). After filtered through a 0.45 μm microporous filter, 20 μl of supernatant were injected into the HPLC system. The HPLC system consisted of a LC-20AT VP solvent delivery system, a SPD-20A UV spectrophotometric detector, a column heater (Shimadzu, Japan), and a N2010 chromatographic integrator (Zhejiang, China). The mobile phase consisted of acetonitrile/0.05 M KH_2PO_4/triethylamine (50 : 50 : 0.5, v/v/v), and the wavelength was 356 nm. Separation was achieved by VP-ODS C_{18} reversed-phase column (4.6 × 250 mm, Kyoto, Japan) thermostated at 30 °C with a flow rate of 1.0 ml/min.
The method was proved suitable for berberine detection. Berberine was separated well from impurities in plasma extracts, with a retention time of 6.6 min. The limit of detection was 0.03 μg/ml, and the linear range was from 0.05–7.5 μg/ml ($r = 0.9999$). At concentrations of 0.1, 2.5, and 5 μg/ml, recoveries of spiked berberine from rats plasma were 98.3%, 101.3%, and 100.5%, intra-day precision was 3.41%, 3.02%, and 3.12%, inter-day precision was 3.63%, 4.50%, and 3.74%, respectively.

3.5. Pharmacokinetic study and bioavailability

The *in vivo* studies were carried out with male Sprague-Dawley rats (280–350 g) supplied by Medical Animal Test Center of Anhui Medical University. All animals were housed in conventional cages with free access to water and rodent chow at 20–22 °C with 12 h light-dark cycle. All procedures of laboratory animals involved were in accordance with National Institutes of Health guidelines. Rats were randomly divided into two groups; berberine microemulsion (group A), berberine tablet suspensions (group B), and each group consists of 6 rats. Group A received berberine microemulsions at a dose of 50 mg/kg. Group rats B received suspensions of berberine tablet at the same dose of 50 mg/kg. Berberine tablet suspensions were prepared as follows: first, berberine tablets were milled into powder, and then suspending the powders in 0.5% sodium methylcellulose solution. Prior to the experiment, the animals were fasted overnight but had free access to water. All of the two groups were treated by daily intragastric injection (ig) for continuous 4 days. In the fifth day morning, blood samples (approximately 500 μL) were collected into a heparinized microcentrifuge tube from the retro-orbital vein using a heparinized needle (18–20 size) at 0, 0.5, 1, 2, 3, 4, 6, 8, 12, 24 h timepoints, respectively. The samples were then subjected to centrifugation in a laboratory centrifuge for 15 min at 4 °C (× 10000 rpm) and plasma was collected into another microcentrifuge tube and stored at −20 °C until analysis.
The pharmacokinetic parameters were evaluated by software DAS2.0, a computer programme produced by drug research center of Shanghai University of Traditional Chinese Medicine. The area under the blood concentration-time curve (AUC) was calculated by the trapezoidal rule, while the maximum blood concentration (C_{max}) and the time of maximum blood concentration (T_{max}) were obtained directly by observation. The berberine plasma concentration-time curves after multiple oral dose administration were evaluated by two-compartmental model analysis using DAS 2.0 software. The AUC_{0-24h} was the area under the plasma concentration-time curve from time 0 to final observed concentration timepoint 24 h opun oral administration which was calculated using the linear trapezoidal rule. The $AUC_{24-\infty}$ is the terminal area under the plasma concentration-time curve from time 24 h to infinity, calculated by dividing the last observed plasma concentration by λz, where λz denotes the first order rate constant of the terminal phase. Relative bioavailability can be calculated according to Eq. (1):

$$\text{Relative bioavailability} = \frac{AUC_{test}}{AUC_{reference}} \times \frac{Dose_{reference}}{Dose_{test}} \times 100 \qquad (1)$$

The statistical significance of the difference between mean values was assessed by one-way analysis of variance (ANOVA). Statistical probability (p) values less than 0.05 were considered significantly different.

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