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Self-Microemulsifying Drug Delivery Systems (SMEDDS) for Improving In Vitro Dissolution and Oral Absorption of Pueraria Lobata Isoflavone

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ABSTRACT The aim of our investigation was to develop and characterize self-microemulsifying drug delivery systems (SMEDDS) of Pueraria lobata isoflavone to improve its in vitro dissolution and oral absorption in beagle dogs. SMEDDS consisted of oil (ethyl oleate), a surfactant (Tween 80), and a cosurfactant (Transcutol P). In all the SMEDDS, the level of Pueraria lobata isoflavone was fixed at 20% w/w of the vehicle. The in vitro self-microemulsification properties and droplet size analysis of SMEDDS were studied following their addition to water under mild agitation. A pseudoternary phase diagram was constructed identifying the efficient self-microemulsification region. From these investigations, an optimized formulation was selected and its dissolution and bioavailability were compared with a tablet formulation in beagle dogs. The in vitro dissolution rate of puerarin from SMEDDS was more than threefold faster than that from Yufengningxin tablets (Pueraria lobata isoflavone tablets). A 2.5-fold increase in the relative bioavailability was observed for the SMEDDS compared with Yufengningxin tablets. The absolute bioavailability of the SMEDDS was 82.32±15.51%, which was significantly improved compared with that of Yufengningxin tablets. These results demonstrate the potential of SMEDDS as an efficient way of improving the oral absorption of Pueraria lobata isoflavone.

KEYWORDS SMEDDS, Puerarin, Pueraria lobata isoflavone, Yufengningxin tablet, In vitro dissolution, Oral bioavailability

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INTRODUCTION

More recently, studies on oral dosage forms using self-microemulsifying drug delivery systems (SMEDDS) have been performed for the purpose of improving the solubility, dissolution, and oral absorption of poorly water-soluble

drugs (Itoh et al., 2002; Kim et al., 2000). Commercially available drugs that use this SMEDDS include cyclosporin A, as well as preparations of ritonavir and saquinavir (HIV protease inhibitors), and the usefulness of this system has also been demonstrated clinically (Cooney et al., 1998; Porter & Charman, 2001).

SMEDDS consist of a mixture of drugs, oils, and surfactants, and the gentle mixing of these ingredients in aqueous media can generate microemulsion droplets (with a mean droplet size $\leq$100 nm) of solubilized drugs (Pouton, 1997). It is considered that such SMEDDS may improve the absorption of drugs by rapid self-microemulsification in the stomach, with the microemulsion droplets subsequently dispersing in the gastrointestinal tract, thereby allowing them to be readily absorbed (Shah et al., 1994).

Pueraria lobata is a traditional Chinese medicinal herb. In China, its crude extract has been used for the treatment of hypertension, senile ischemic cerebrovascular disease, and angina pectoris (Zeng et al., 1979). Studies of its pharmacology and clinical applications have shown that the active constituents in the extract are isoflavones, mainly puerarin. This medicine is known to dilate coronary arteries, decrease myocardial oxygen consumption, and improve microcirculation in both animals and humans suffering from cardiovascular disease (Fan et al., 1984; Li & Yang, 1997; Liu et al., 2000).

Commercially available Pueraria lobata isoflavone tablets (also named Yufengningxin tablets) are orally active and well tolerated. However, the oral bioavailability of the tablets has not been reported until now. In our previous studies, the dissolution of Yufengningxin tablets was found to be poor.

In this study, a SMEDDS formulation of Pueraria lobata isoflavone was developed to increase the dissolution rate and consequently improve the oral bioavailability.

MATERIALS AND METHODS

Materials

Puerarin and Pueraria lobata isoflavone were provided by Luyin Pharmaceutical Co. (Yantai, China). Yufengningxin tablets (containing 13 mg purarin and other Pueraria lobata isoflavones in one tablet lot no. 020105) were manufactured by Beijing Tongrentang Pharmaceutical Co. (Beijing, China). Transcutol P (diethylene glycol monoethyl ether) was obtained from Gattefosse (Westwood, NJ). All other chemicals and solvents were purchased from Yuwang Co. (Shangdong, China).

Preparation of SMEDDS

In the SMEDDS, the level of Pueraria lobata isoflavone was constant (i.e., 20% w/w of the vehicle). Components of SMEDDS (oils, surfactant, cosurfactant, and drug) were accurately weighed into screw-capped glass vials and heated at 40°C in a water bath to facilitate the solubilization, and then vortex mixed until uniformly distributed. At this level, the fill volume of a size 0 capsule contained 118 mg Pueraria lobata isoflavone (i.e., 22 mg puerarin) for bioavailability studies, and a size 1 capsule (i.e., 13 mg puerarin) was used for dissolution studies.

Phase Separation Study

Each SMEDDS (0.05 mL) was added to a glass test tube containing 5 mL distilled water at 25°C. After 1 min vortex mixing, each mixture was stored for a period of 2 hr and phase separation was observed visually. Mixtures exhibiting a negligible phase separation during the 2-hr period were used for subsequent study.

Visual Observations and Droplet Size Analysis

A visual test to assess the self-microemulsification properties was adopted in this study (Khoo et al., 1998). Using a standard USP XXIV dissolution apparatus 2, 1 mL of each formulation was added dropwise to 200 mL purified water at 37°C. Gentle agitation was provided by a standard stainless-steel dissolution paddle rotating at 60 rpm. The particle size distribution was determined immediately using a Brookhaven apparatus (90 Plus Particle Sizing Software, Ver. 2.31). The values of the mean microemulsion droplet diameters were compared. A tendency to microemulsify spontaneously, microemulsion droplet formation, and also the final appearance of the microemulsion were monitored. The tendency to form a microemulsion was judged as "good" when droplets spread easily in water and formed a fine microemulsion that was clear or slightly brown in appearance, and it was

judged "bad" when the corresponding performance was poor or there was less clear microemulsion formation, or emulsion formation. A phase diagram was constructed identifying the good self-microemulsifying region. All studies were repeated three times, with similar observations being made with the repeats.

Dissolution Studies

Dissolution studies were performed using USP XXIV, Dissolution Apparatus 1 with 900 mL distilled water, pH 6.8 phosphate buffer, and 0.1 mol/L HCl at 37±0.5°C being used as dissolution media. The speed of the basket was adjusted to 100 rpm. An aliquot (0.5 mL) of sample was collected at designated times and analyzed for puerarin by high-performance liquid chromatography (HPLC) (Li et al., 2002). An equivalent volume (0.5 mL) of fresh dissolution medium was added to compensate for the loss due to sampling.

Bioavailability Studies

Six male beagle dogs, 2.5 to 3 years old and weighing approximately 12 kg each, were used in the study. They were cared for and used in accordance with the Guidelines for Animal Experiments in China.

The bioavailability of two formulations of Pueraria lobata isoflavone, an optimized self-microemulsifying formulation and a Yufengningxin tablet formulation, as well as an intravenous formulation of puerarin were compared in beagle dogs. The study had an open, randomized, cross-over design. Six fasted beagle dogs each received the three formulations on separate occasions. For the intravenous group, each beagle dog was given 30 mg puerarin bolus infusion dissolved in a sodium chloride injection solution (6 mg/mL). Blood samples (2 mL) were withdrawn prior to dosing and at 5, 10, 20, 30, 45, 60, 90, 120, and 180 min after dosing. For the oral group, the SMEDDS group was given three SMEDDS capsules (66 mg puerarin), and the tablet group was given five Yufengningxin tablets (65 mg puerarin). Blood samples were taken prior to dosing and at 10, 20, 30, and 45 min, and 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, and 12 hr after dosing and transferred to 2 mL polypropylene centrifuge tubes. Serum was separated by centrifuging at 4000 g for 10 min. The samples were stored frozen at −20°C until HPLC analysis of puerarin. Specimens were thawed and allowed to reach room temperature before analysis.

The concentration of puerarin in serum was determined by HPLC using the method previously reported (Li et al., 2002). A Selectosil C_{18} column (250 × 4.6 mm i.d.; 5 μm; Phenomenex) and a mobile phase consisting of methanol–water (1:3 v/v) were used. Quantification was performed at a wavelength of 250 nm. Six percent perchloric acid (100 μL) was added to serum (100 μL) in a 2-mL polypropylene centrifuge tube. The samples were vortexed for 2 min to precipitate serum proteins, and then centrifuged for 10 min at 12,000 g. Finally, 50 μL aliquots of the supernatant were injected directly onto the HPLC column. The assay was linear over a puerarin concentration range of 0.052–10.300 μg/mL. The mean recovery was 86.5%, with a coefficient of variation below 7%.

The pharmacokinetic parameters associated with the intravenous and oral formulations were calculated from the serum concentration–time data using a noncompartmental model. The area under the whole serum concentration–time curve ($AUC_{0\text{-}T}$) was calculated using the trapezoidal rule. The maximum serum concentration (C_{max}) and the time to reach the peak serum concentration (T_{max}) were obtained directly from the experimental data. The pharmacokinetic parameters for different formulations were compared using Student's *t*-test. The absolute bioavailabilities of puerarin in the oral formulations were calculated from the $AUC_{0\text{-}T}$ data relative to that following IV administration (correcting for the difference in dose). Values were reported as mean ± SD, and the data were considered statistically significant at $p < .05$.

RESULTS AND DISCUSSION

Characterization of SMEDDS

SMEDDS form fine oil-in-water (O/W) microemulsions with only gentle agitation upon their introduction into aqueous media. Because the free energy required to form a microemulsion is very low, the formation is thermodynamically spontaneous (Kim et al., 2000).

Visual testing measured the apparent spontaneity of microemulsion formation. A series of SMEDDS were prepared and their self-microemulsifying properties were observed. A pseudoternary phase diagram was constructed to identify the self-microemulsifying regions and also to establish the optimum

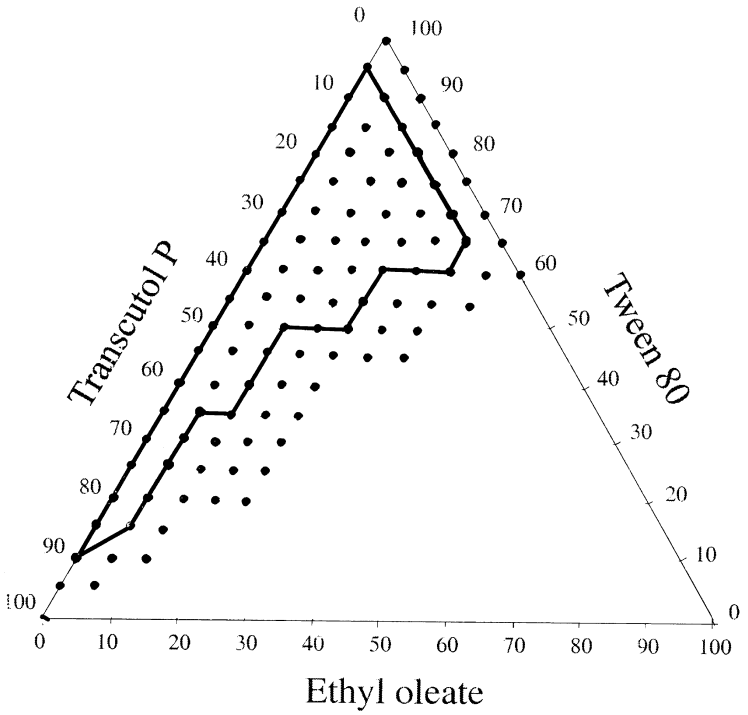

FIGURE 1 **Pseudoternary Phase Diagram Indicating the Efficient Self-Microemulsification Region. (Key: The Region of Efficient Self-Microemulsification is Bounded by the Solid Line, and the Filled Circles Represent the Compositions which were Evaluated.)**

concentrations of oil, surfactant, and cosurfactant. The phase diagram of the systems containing Tween 80, Transcutol P, and ethyl oleate was shown in Fig. 1. The efficiency of microemulsification was good when the surfactant concentration was more than 35%. It was observed that increasing the concentration of the cosurfactant, Transcutol P, within the self-microemulsifying region increased the spontaneity of the self-microemulsification process. When a cosurfactant was added to the system, it further lowered the interfacial tension between the O/W interface and also influenced the interfacial film curvature, which

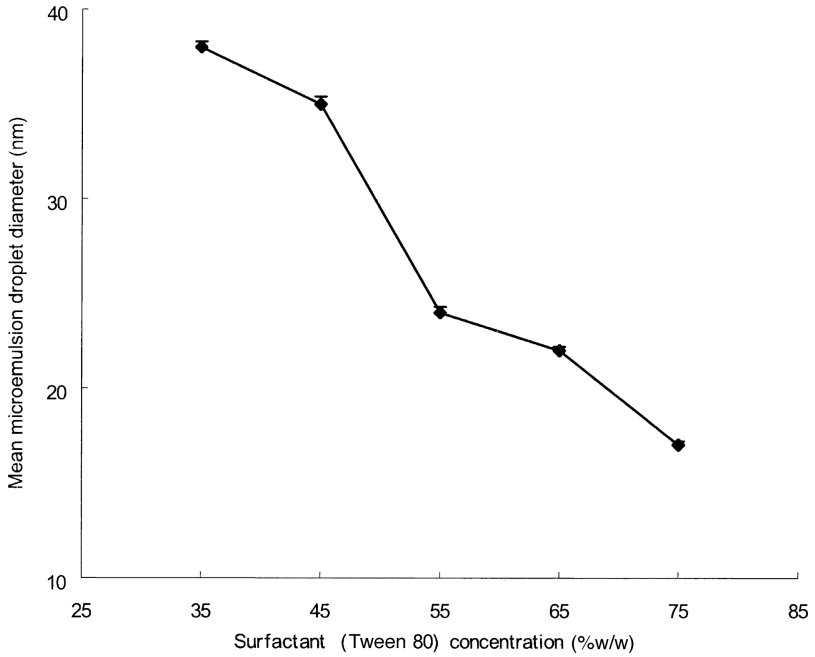

FIGURE 2 **Effect of Surfactant (Tween 80) Concentration on Mean Microemulsion Droplet Diameter (Mean ± SD, *n* = 3).**

thereby readily deformed around the oil droplets (Eccleston, 1992).

Droplet Size Analysis

When the concentration of oil was 10% and the cosurfactant was present, the effect of the surfactant concentration in the self-microemulsifying systems on the droplet size distribution was presented in Fig. 2. In SMEDDS, increasing the surfactant concentration (from 35 to 75%) decreased the mean droplet size. Visual observations indicated that at higher levels of surfactant, the spontaneity of the self-microemulsification process was increased. This may be due to excess penetration of water into the bulk oil causing massive interfacial disruption and ejection of droplets into the bulk aqueous phase (Pouton, 1997).

The effect of the cosurfactant (Transcutol P) concentration on the droplet size distribution in SMEDDS was similar to that of the surfactant (Tween 80) at concentrations of Transcutol P from 0 to 15%. When a cosurfactant was added (in addition to surfactant) to the

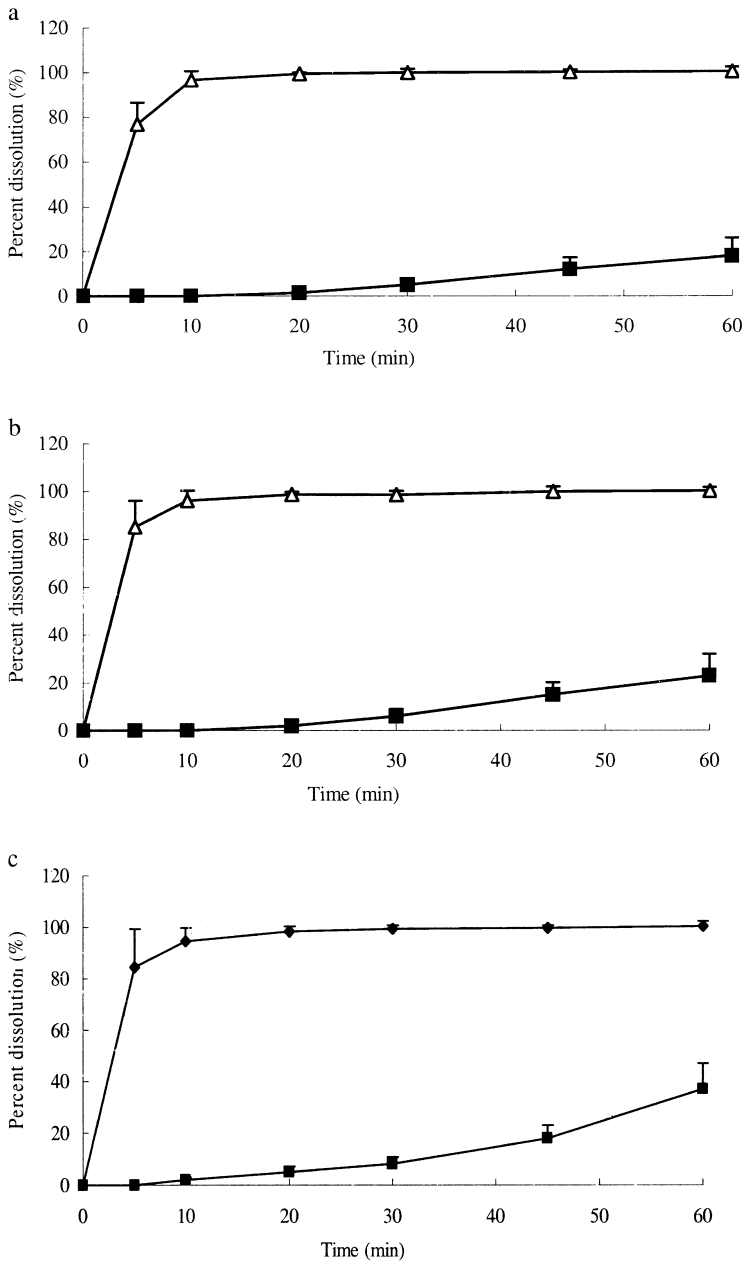

FIGURE 3 **Dissolution Profiles of Puerarin from SMEDDS (△) and a Yufengningxin Tablet Formulation (■) in Various Dissolution Media at 37°C. (a) Distilled Water, (b) pH 6.8 Phosphate Buffer, and (c) 0.1 M HCl. Bars Represent SD. Each Point Represents the Mean ± SD (n = 6).**

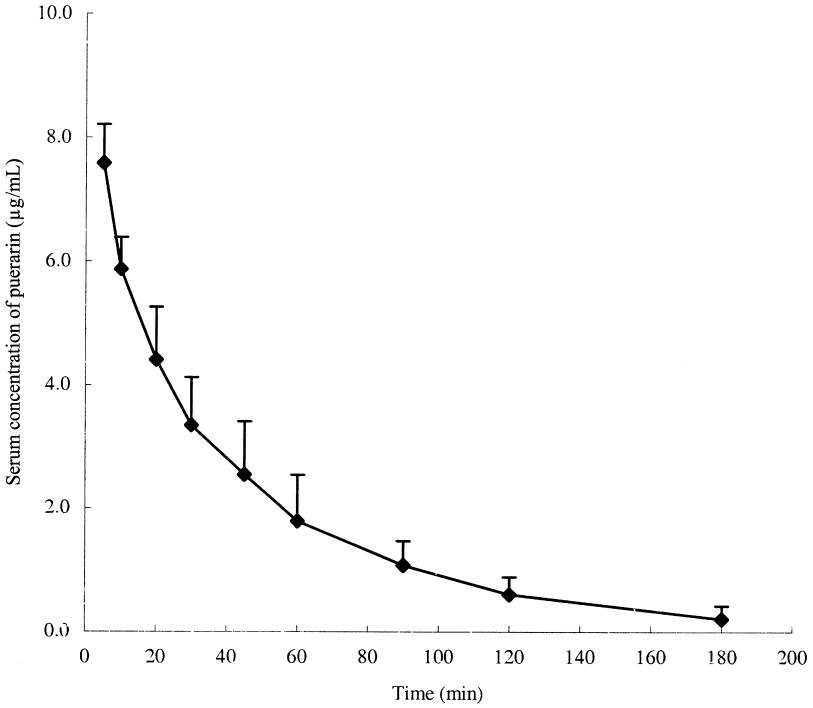

FIGURE 4 **Mean Serum Concentration–Time Profile of Puerarin After Intravenous Administration to Beagle Dogs (2.7 mg/kg). Each Data Point is an Average Value, and the Error Bars Represent the Standard Errors (n = 6).**

system, it lowered the interfacial tension, fluidized the hydrocarbon region of the interfacial film, and decreased the bending stress of the interface (Eccleston, 1992). In the case of self-microemulsifying drug delivery systems, a decrease in droplet size was observed with an increase in the cosurfactant concentration of Transcutol P from 0 to 15%, after which the droplet size was slightly increased. Gao et al. (1998) reported similar observations with microemulsion systems containing Captex-355, Cremophor-EL, Transcutol, and saline.

Release of Puerarin from the SMEDDS

The release of puerarin from the SMEDDS (Tween 80 : Transcutol P : ethyl oleate, 65:25:10) and

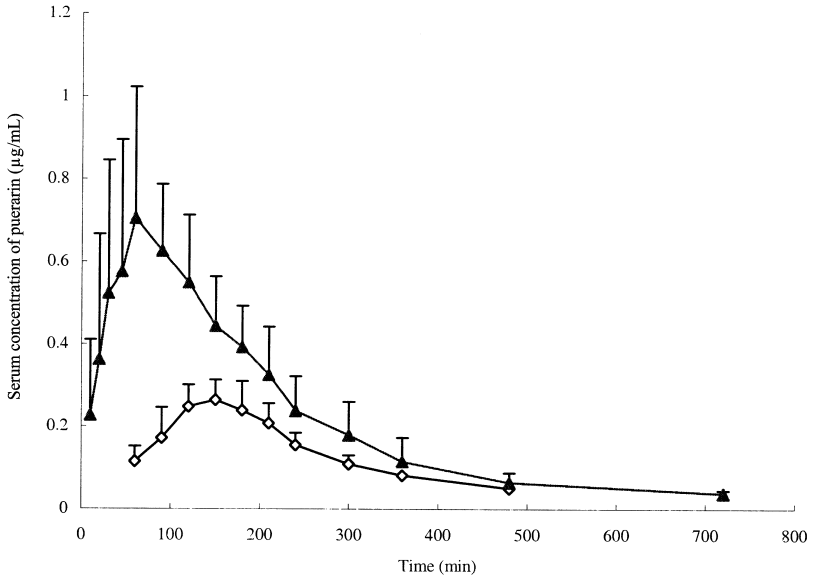

FIGURE 5 **Mean Serum Concentration–Time Profiles of Puerarin After Oral Administration to Beagle Dogs. Each Data Point is an Average Value, and the Error Bars Represent the Standard Errors (n = 6). Formulation Type: (◇) Tablet, (▲) SMEDDS.**

TABLE 1 **Pharmacokinetic Parameters Determined for Puerarin in Beagle Dogs Following IV Administration and Oral Administration of SMEDDS and a Yufengningxin Tablet Formulation of Pueraria Lobata Isoflavone (Mean±SD, $n=6$)**

Parameters	AUC (μg/mL·min)	C_{max} (μg/mL)	T_{max} (min)	Absolute bioavailability (%)
IV (30 mg)	84.15±10.04			
Yufengningxin tablet (65 mg)	61.50±16.60	0.29±0.05	155.0±22.6	33.02±7.31
SMEDDS (66 mg)	154.93±44.05[a]	0.77±0.27[a]	80.0±24.5[b]	82.32±15.51

[a] $p<.05$.
[b] $p<.01$.

Yufengningxin tablets was evaluated in distilled water, pH 6.8 phosphate buffer, and 0.1 mol/L HCl; the percentage of puerarin released from the SMEDDS was significantly higher than that from Yufengningxin tablets in all three dissolution media studied (Fig. 3). When distilled water and pH 6.8 phosphate buffer and 0.1 mol/L HCl were used as the media, the percentage release of puerarin from SMEDDS at 20 min was 99.4±0.6%, 98.7±1.0%, and 98.2±2.0%, respectively. No statistically significant differences were observed among the three different dissolution media ($p>.05$). The results showed that the developed formulation was not affected by the pH and ionic strength of the dissolution media over the pH range 1.0–6.8.

From the results, it is considered that the performance of SMEDDS depends on two main factors: 1) the ability of the self-microemulsifying mixture to form a microemulsion with uniform fine particle size droplets (i.e., <50 nm), and 2) the ability of SMEDDS to present the Pueraria lobata isoflavone in a solubilised and highly dispersed form, thereby overcoming the dissolution rate-limited step.

Bioavailability Studies

The serum profiles of puerarin in beagle dogs following intravenous administration of puerarin, oral administration of Yufengningxin tablets, and SMEDDS of Pueraria lobata isoflavone were compared. Based on the in vitro self-microemulsification properties, the SMEDDS containing Tween 80 (65%), Transcutol P (25%), and ethyl oleate(10%) was selected for bioavailability studies. The serum concentration versus time profiles were presented in Figs. 4 and 5, and the pharmacokinetic parameters were given in Table 1. The absorption of puerarin from the SMEDDS of Pueraria lobata isoflavone resulted in a 2.5-fold increase in bioavailability (as indicated by the AUC and C_{max} values, $p<.05$) compared with Yufengningxin tablets. Also the reduction in T_{max} from 155.0±2.6 min to 80.0±24.5 min was significant ($p<.01$).

It is well known that the main rate-limiting barrier for drug absorption is the single layer of intestinal epithelial cells that covers the luminal surface of the intestinal wall. For the majority of drugs, absorption occurs via passive transcellular transport and paracellular transport is limited due to the tight junctions between the cells (Artursson & Karlsson, 1991). Surfactants are also known to increase the permeability by disturbing the cell membrane (Swenson & Curatolo, 1992). In this study, the mechanism of enhancement in oral absorption by Pueraria lobata isoflavone SMEDDS involves the facilitation of diffusion and subsequent absorption of the drug from the GI tract from small droplets (22±0.3 nm; unpublished data) with large interfacial area. So the increased bioavailability seen following administration of SMEDDS was most likely due to an improvement in in vitro dissolution, the facilitation of diffusion and subsequent absorption of the drug via the GI tract from small droplets of oil with a large interfacial area, and inclusion of an absorption enhancer (Transcutol P).

In conclusion, the small particle size and the drug release rate were found to be important parameters for the evaluation of the efficiency of SMEDDS. In vivo absorption studies in beagle dogs of Pueraria lobata isoflavone, SMEDDS gave at least a 2.5-fold greater C_{max} and AUC than for the drug in Yufengningxin tablets. These data clearly demonstrate the utility of SMEDDS in improving the in vitro dissolution and oral absorption of Pueraria lobata isoflavone.

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