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Enhancing effect of Labrafac Lipophile WL 1349 on oral bioavailability of hydroxysafflor yellow A in rats

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ABSTRACT

The objective of the present investigation was to clarify the mechanism by which Labrafac Lipophile WL 1349 (WL 1349) enhanced the oral bioavailability (BA) of hydroxysafflor yellow A (HSYA), the representative low permeable hydrophilic (biopharmaceutic classification system (BCS) Class III) drug. HSYA–phospholipid complex was prepared, and dissolved into WL 1349 with a certain surfactant to form a stable oil solution. Oral administration of HSYA aqueous solution at a dosage of 4.5 mg/kg resulted a low plasma HSYA concentration with C_{max} and $AUC_{0-8\,h}$ values of 0.105 μg/ml and 10.29 μg min/ml, respectively. HSYA–phospholipid complex oil solution with the same administration and dosage increased the plasma HSYA concentration significantly with C_{max} and $AUC_{0-8\,h}$ values of 2.063 μg/ml and 381.145 μg min/ml, respectively. The results showed that WL 1349 could improve oral absorption of HSYA remarkably. Bioavailability investigations were performed to show WL 1349 dosage independent from HSYA absorption within the dosage from 1 ml/kg to 9 ml/kg. The test of bile duct ligation in rats showed that the oil solution containing WL 1349 did not result in detectable plasma HSYA concentration, but HSYA aqueous solution had the same $AUC_{0-8\,h}$ as the bile duct was not ligated. The in vitro lipolysis experiments of WL 1349 showed that WL 1349 was emulsified by deoxycholate, and then was hydrolyzed to fatty acids and monoglycerides by pancreatic lipase rapidly. The lipolysis products of WL 1349, caprylic acid, capric acid and caprylic and capric acid monoglycerides all improved the BA of HSYA in vivo. The results above indicated the emulsifying by bile, and hydrolysis to fatty acids and monoglycerides by pancreatic lipase was one of the enhancing mechanisms of HSYA–phospholipid complex oil solution absorption.

1. Introduction

Many hydrophilic drugs such as bisphosphonate drugs, proteins, peptides, and peptide-like drugs are poorly absorbed from the gastrointestinal tract (GIT), among the various classes of biopharmaceutic classification system (BCS), peroral delivery of these Class III drugs is partially or completely hindered due to their poor intestinal permeability (Legen et al., 2005). Many drug molecules show poor permeability because of their unfavorable physicochemical and chemical features, which are difficult to change, thus an external excipient may be added to increase permeation transiently. It has been reported that association of hydrophilic drugs with oil carriers in the absence of aqueous phase can protect against intestinal proteases, improve stability and change cell permeability, and may improve absorption in GIT (New and Kirby, 1997). In present study, absorption enhancement of representative low permeable hydrophilic model drug of BCS class III, hydroxysafflor yellow A (HSYA), was investigated in bioavailability (BA) test by the preparation of HSYA–phospholipid complex oil solution.

The flower of the safflower plant, *Carthamus tinctorius* L., has been widely used in traditional Chinese medicine for treatment of cerebrovascular and cardiovascular diseases. The extracts of the flower contain yellow and red pigments including hydroxysafflor yellow A, safflor yellow B, safflomin A, safflomin C, and other chemicals (Liu et al., 2004, 2006). HSYA, the main chemical component of the safflower yellow pigments, has been demonstrated to antagonize platelet activating factor receptor binding and thus used to treat some dysaemia diseases, such as myocardial ischemia, cerebral ischemia, coronary heart disease, and cerebral thrombosis (Wang et al., 2006). HSYA is highly soluble in water (its water solubility is about 0.28 mg/ml, 25 °C) but slightly soluble in oil (Zhang et al., 2006), having very poor intestinal membrane permeability resulting in low oral BA. There is only injection of HSYA which has been reported (Shi, 2004; Wei et al., 2005) and no oral dosage forms have been mentioned by far. Labrafac Lipophile WL 1349 (WL 1349), caprylic and capric acid triglycerides, one of the medium chain triglycerides (MCT), have been used as oil system in emulsions (Devani et al., 2005). In the literature, MCT preferred in

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lipid-based formulations was used to enhance the bioavailability of poorly water-soluble drug (Grove et al., 2006, 2007; Dahan and Hoffman, 2007; Hauss, 2007), however, the effect of water-soluble drug has not been studied, meanwhile, the absorption enhancing mechanism of MCT has not been studied clearly either. It has been reported that phospholipids complex can increase the lipophilicify of lipophilic drug (Yanyu et al., 2006), but the hydrophilic drug–phospholipid complex has not been studied. In this study, HSYA–phospholipid complex was prepared to increase its lipophilicify, and dissolved into WL 1349 with a certain surfactant to form a stable oil solution with the purpose of enhancing the oral BA of HSYA, and the possible enhancing mechanism of WL 1349 to HSYA was studied.

2. Materials and methods

2.1. Materials

WL 1349 was a gift from Gattefosse Corp., HSYA was purchased from Chang-sha-ke-luo-ma Medicine Technique Ltd., purity >90%, and phospholipids was purchased from Tai-wei-yao-ye Ltd., containing approximately 75–80% (w/w) of soybean phospholipids. The other chemical reagents were of analytical grade or better and were used as received.

2.2. Animals

Sprague–Dawley rats, male, weighing 200–250 g were fed standard laboratory chow and had free access to water. Food was withheld from the rats for 12 h before they used in BA and absorption enhancing mechanism experiments.

2.3. Preparations of HSYA oil solution

The required amount of HSYA and phospholipids (the weight ratio was 1:3) were placed in a 100 ml round-bottom flask and dissolved in tetrahydrofuran. After tetrahydrofuran was evaporated under vacuum at 40 °C, the dried residues of HSYA–phospholipid complex were collected and placed in the desiccator overnight, then stored in room temperature.

The sufficient quantum of HSYA–phospholipid complex was added into a required amount of WL 1349 with Span-80, which was taken as stabilizer and emulsifier. The oil solution was vibrated at 60 °C until it became clear, and then cooled to room temperature, and the HSYA–phospholipid complex oil solution was obtained.

2.4. Solubility determination of HSYA in WL 1349

Solubility determination of HSYA and phospholipids complex in WL 1349 was carried out by adding excessive HSYA, the physical mixture of HSYA and phospholipids or phospholipids complex to 5 ml WL 1349 in sealed glass containers. The liquid were vibrated for 24 h at 25 °C, and centrifuged to remove excessive HSYA (12,000 rpm, 10 min). 1 ml of the supernatant was mixed with 9 ml of methanol, 20 μl of the resulting solution was injected into a HPLC system. The stationary phase, C_{18} column (4.6 mm × 150 mm, 5 μm), was kept at 40 °C. The mobile phase was consisted of methanol:acetonitrile:0.05 M KH_2PO_4 solution = 25:2:73. The flow rate was 0.8 ml/min. The effluent was monitored at 403 nm.

2.5. In vitro lipolysis experiments of WL 1349

0.1 g of WL 1349 was added to 5 ml medium containing 5 mM deoxycholate and 1.25 mM L-α-phosphatidylcholine was continuously stirred gently (100 rpm) and heated (37 °C). Fresh pancreatin extract was prepared by adding 1 g of porcine pancreatin lipase to 5 ml distilled water, stirring for 15 min followed by centrifugation. About 3.5 ml of the pancreatin extract (1000 IU/ml) was added into the medium and stirred for 30 min and initiated the enzymatic digestion of the formulation. During the lipolysis process of WL 1349, free fatty acids were liberated and consequently the pH decreases. The enzymatic digestion process was observed by acid–base titration taking phenolphthalein as indicator, 0.05 M NaOH as titrant, and the solution turning red as the end point of titration.

2.6. BA studies in rats

2.6.1. Chromatography

The plasma concentrations of HSYA were determined by HPLC. A mobile phase consisting of methanol:acetonitrile:0.05 M KH_2PO_4 = 25:2:73 was pumped through the C_{18} column (4.6 mm × 150 mm, 5 μm) kept at 40 °C at a flow rate of 0.8 ml/min. The effluent was monitored at 403 nm.

2.6.2. Preparation of plasma samples and validity

The rats were anaesthetized with aether, and 300–400 μl blood was taken from the eyeground vein. The plasma was obtained after centrifugation (4000 rpm, 10 min) and stored at −20 °C before being analyzed.

100 μl of 6% perchloric acid was added to100 μl of the thawed plasma. The mixture was shaken for 3 min before centrifugation (12,000 rpm, 10 min). 20 μl of the supernatant was injected for HPLC analysis.

This method was validated by adding various quantities of HSYA to blank plasma of rats. The concentrations of HSYA were 0.03 μg/ml, 0.075 μg/ml, 0.3 μg/ml, 0.75 μg/ml, 1.5 μg/ml, and 3.0 μg/ml. The calibration was critical to the entire analytical procedure in testing the linearity, precision, and accuracy of the method.

2.6.3. Pharmacokinetic of HSYA in rats

The sufficient quantum of HSYA and the phospholipids complex were both diluted, by distilled water into 0.45 mg/ml (solution A) and 1.8 mg/ml (solution B, the concentration of HSYA equals to 0.45 mg/ml). In HSYA–phospholipid complex oil solution, the concentration of phospholipids complex in WL 1349 was 6 mg/ml (solution C, the concentration of HSYA equals to 1.5 mg/ml).

Pharmacokinetics studies were carried out after oral administration of the HSYA aqueous solution, HSYA–phospholipid complex aqueous solution and HSYA–phospholipid complex oil solution to rats. Eighteen male rats (body weight 200–250 g) divided randomly into three groups were fasted for 12 h with free access to water. The three groups of rats received each of the test preparations with the HSYA dosage of 4.5 mg/kg. All experiments were carried out at the same time of the day to exclude the influences by circadian rhythm. At 15 min before administration, a control blood sample (300–400 μl) was taken from the eyeground vein. After oral administration of the test solutions, 300–400 μl blood sample were collected from the eyeground vein at 8 min, 15 min, 30 min, 60 min, 90 min, 120 min, 180 min, 240 min, 300 min, 360 min, and 480 min. The plasma samples were detected according to the method in Section 2.6.2.

2.7. The influence of the dosage of WL 1349 on the absorption of HSYA

The HSYA–phospholipid complex oil solutions with different concentrations of HSYA, 0.5 mg/ml, 1.5 mg/ml, and 4.5 mg/ml, were prepared. Eighteen male rats (body weight 200–250 g) divided randomly into three groups, and each group was orally administered of HSYA–phospholipid complex oil solutions as above, with the HSYA dosage of 4.5 mg/kg and the WL 1349 dosages of 1 ml/kg, 3 ml/kg,

and 9 ml/kg. HSYA aqueous solution was taken as control group, and the relative bioavailability was calculated to investigate the relationship between the absorption of HSYA and WL 1349 dosage.

2.8. BA study after bile duct ligation in rats

Male Sprague–Dawley rats weighing 200–250 g were used. Twelve animals were divided in to two groups controlled at 22 ± 1 °C and maintained in an alternating 12-h light/12-h dark cycles, and were allowed free access to water. HSYA aqueous solution (solution A) was orally administered to one group of rats with the dosage of 4.5 mg/kg, HSYA–phospholipid complex oil solution (solution C) was orally administered to the other group of rats with the dosage of 18 mg/kg (equal to 4.5 mg/kg HSYA). The two groups without bile duct ligation (BDL) test were taken as control groups and received BDL test after a washout period of 1 week.

BDL was performed as described previously (Miyake et al., 2006; Tavakoli et al., 2006; Lee et al., 2007). In brief, under ether anesthesia, the common bile duct was ligated with 3–0 silk and sectioned between the ligatures. The midline abdominal incision was closed with catgut. HSYA aqueous solution and the oil solution, with the same dosage (4.5 mg/kg) and administration, were orally administered to each group of rats 2 h later after bile duct ligation, when bile was exhausted.

2.9. The influence of lipolysis products of WL 1349 on absorption of HSYA in rats

The influence of the lipolysis products of WL 1349 containing caprylic acid, capric acid and caprylic acid, and capric acid monoglycerides on absorption of HSYA in rats was investigated. The sufficient quantum of HSYA–phospholipid complex was added into the lipolysis products, respectively, and the concentrations of HSYA were all 1.5 mg/ml. Eighteen male rats (body weight 200–250 g) divided randomly into three groups, and each group was orally administered one of the drug preparation with the dosage of 4.5 mg/kg, respectively. The BA was calculated to investigate the influence of lipolysis products of WL 1349 on absorption of HSYA.

2.10. Pharmacokinetics and statistical analysis

The time to reach maximum HSYA concentration, T_{max}, and the maximum plasma HSYA concentration, C_{max}, were determined from the authentic plasma HSYA concentration versus time data. The area under the plasma HSYA concentration versus time curve (AUC_{0-tn}) and the area under the first-moment curve ($AUMC_{0-tn}$) after administration of the test preparations were calculated using the linear trapezoidal rule up to the last measured HSYA plasma concentration. The mean residence time (MRT) was calculated by $AUMC_{0-tn}/AUC_{0-tn}$. All values are expressed as their means ± their standard deviations (S.D.).

The HSYA aqueous solution was taken as control experiment to calculate the relative BA (F_{rel}). The relative BA values were calculated using the following formula:

$$\text{BA}\ (\%) = \frac{\text{AUC preparation}}{\text{AUC aqueous solution}} \times \frac{\text{Dos. aqueous solution}}{\text{Dos. preparation}}$$

3. Results and discussion

3.1. Solubility enhancement of HSYA in WL 1349 by phospholipid complex

It has been reported that phospholipids complex can increase the lipophilicify of lipophilic drug, but the hydrophilic drug–phospholipid complex has not been studied. In this study, phospholipid complex was prepared to improve the lipophilicify of HSYA. Table 1 shows that phospholipid complex can increase the solubility of HSYA in WL 1349, and the solubility in WL 1349 is enhanced higher by phospholipid complex than by the physical mixture of HSYA and phospholipid.

Table 1
The solubilities of HSYA and complex in WL 1349

Samples	Solubility (mg/g)
HSYA	ND[a]
The physical mixture of HSYA and phospholipid	0.0545 ± 0.016
HSYA–phospholipid complex	0.115 ± 0.025

[a] ND: HSYA cannot be detected.

3.2. In vitro lipolysis experiments of WL 1349

WL 1349, a kind of MCT, may be digested and absorbed after emulsified by endogenous emulsifier such as bile and hydrolyzed by pancreatic lipase (Fernandez et al., 2007; Ljusberg-Wahren et al., 2005), and lipolysis products containing fatty acids and monoglycerides can be absorbed by body directly.

The solution was titrated by 0.05 M NaOH, 30 min after hydrolyzed by pancreatic lipase. 1.5 ml of 0.05 M NaOH was exhausted when the solution turned red. The results indicated that WL 1349 would be hydrolyzed to fatty acids with the emulsification of bile rapidly, and the existence of WL 1349 in vivo was in the form of lipolysis products. The absorption enhancement of WL 1349 to HSYA may be achieved by its lipolysis products.

3.3. BA studies in rats

HSYA in plasma could be completely separated under analytical conditions with standard curves linearly ranging from 0.03 μg/ml to 3 μg/ml (r = 0.9996) (Fig. 1). The results attained from the method recoveries of high, middle, and low concentrations were 89.21%, 83.59%, and 83.03%, respectively. The R.S.D. in days were 1.9%, 1.4%, and 1.2%, the R.S.D. intra-day were 2.9%, 2.2%, and 1.7%, respectively, showing satisfying recoveries and R.S.D. in days or intra-days, and the lowest detection limit was 0.009 μg/ml.

Fig. 2 shows the mean plasma concentration–time of the HSYA aqueous solution, HSYA–phospholipid complex aqueous solution and HSYA–phospholipid complex oil solution, equivalent to 4.5 mg/kg of HSYA, after oral administration to rats (n = 6). The average value of C_{max} was 2.063 μg/ml after oral administration of oil solution with a T_{max} of about 24 min (Table 2). However, the average value of C_{max} was 0.606 μg/ml after applying the same administration route of HSYA–phospholipid complex aqueous solution with about 20 min as T_{max}, and C_{max} was 0.105 μg/ml, T_{max} was 45 min after administration of HSYA aqueous solution. Other parameters were obtained by linear trapezoidal rule. It showed that HSYA–phospholipid complex oil solution, could enhance the

Table 2
Main pharmacokinetics parameters of HSYA after oral administration of HSYA aqueous solution, HSYA–phospholipid complex aqueous solution, and HSYA–phospolipid complex oil solution in rats (n = 6)

Parameters	HSYA	Complex	Oil solution
C_{max} (μg/ml)	0.105 ± 0.034	0.606 ± 0.15	2.063 ± 1.125
T_{max} (min)	45 ± 10	20 ± 6	24 ± 7
MRT (min)	106.4 ± 6.4	294.3 ± 22.1	177.6 ± 15.4
$AUC_{0-8\,h}$ (μg min/ml)	10.209 ± 0.073	80.11 ± 24.54	381.145 ± 208.584
BA, F_{rel} (%)	100	778 ± 238	3700 ± 2000

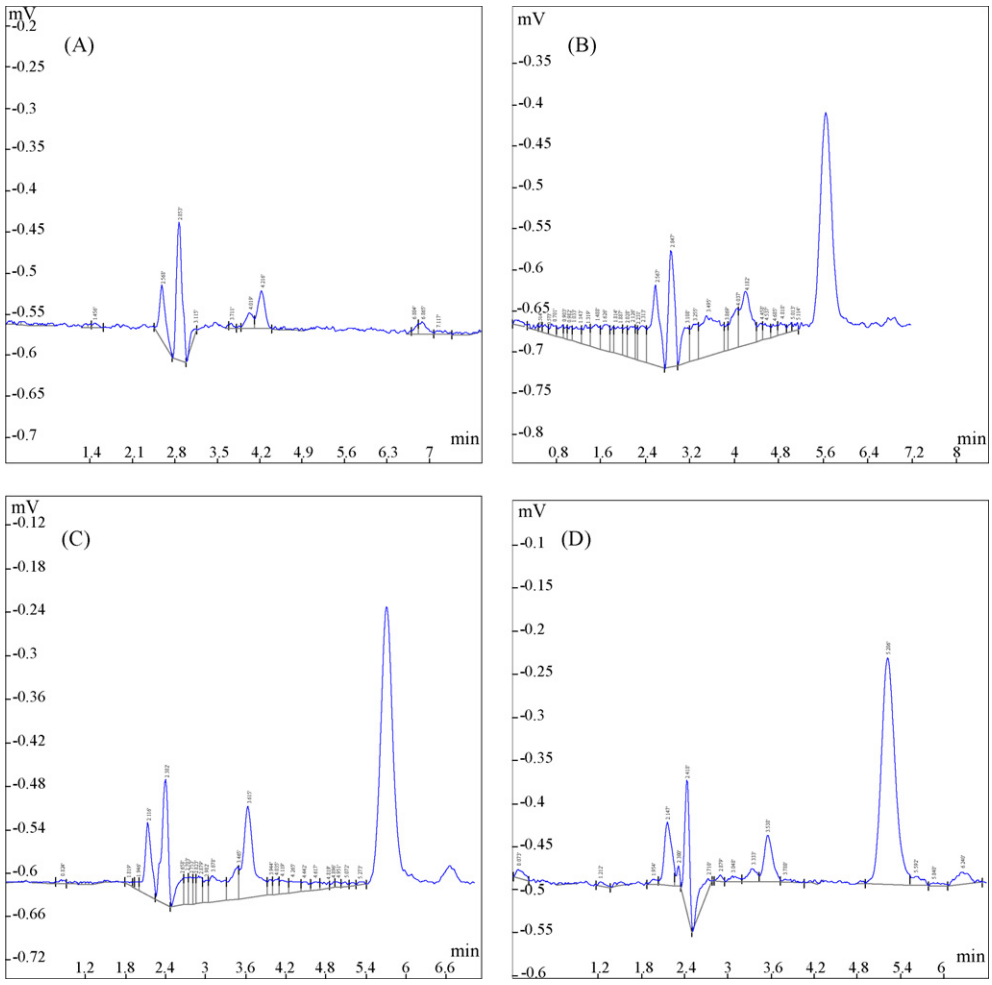

Fig. 1. Chromatogram of blank rat plasma (A), blank rat plasma spiked with HSYA (B), plasma after oral administration of HSYA aqueous solution (C), and plasma after oral administration of HSYA–phospholipid complex oil solution (D).

absorption of HSYA remarkably comparing with HSYA aqueous solution and phospholipid complex aqueous solution and achieving a much higher BA than HSYA and phospholipid complex. It indicated that in the preparation, WL 1349 could enhance oral absorption of HSYA significantly.

3.4. The influence of WL 1349 dosage on the absorption of HSYA

Fig. 3 shows the mean plasma concentration–time of HSYA–phospholipid complex aqueous solutions with different dosages of WL 1349 (the dosage of HSYA was all 4.5 mg/kg) after

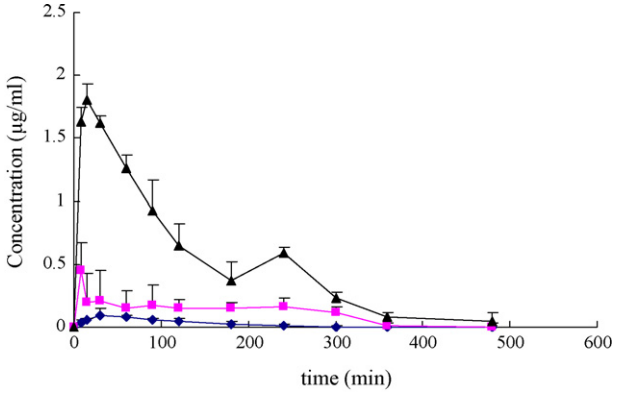

Fig. 2. Mean plasma concentration–time curves of HSYA in rats after oral administration of HSYA aqueous solution, HSYA–phospolipid complex aqueous solution and HSYA–phospolipid complex oil solution ($n = 6$). (▲) HSYA–phospholipid complex oil solution; (■) HSYA–phospholipid complex aqueous solution; (♦) HSYA aqueous solution.

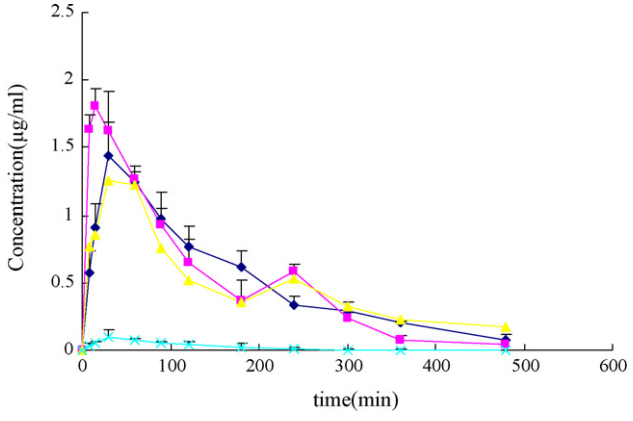

Fig. 3. Mean plasma concentration–time curves of HSYA in rats after oral administration of HSYA aqueous solution and HSYA–phospholipid complex oil solution with different dosages (1 ml/kg, 3 ml/kg, 9 ml/kg) in rats ($n = 6$). (×) HSYA aqueous solution; (♦) HSYA–phospholipid complex oil solution 1 ml/kg; (■) HSYA–phospholipid complex oil solution 3 ml/kg.

Table 3
Main pharmacokinetics parameters of HSYA after oral administration of HSYA aqueous solution and HSYA–phospolipid complex oil solution with different dosages (1 ml/kg, 3 ml/kg, and 9 ml/kg) in rats ($n = 6$)

Parameters	HSYA	1 ml/kg	3 ml/kg	9 ml/kg
C_{max} (μg/ml)	0.105 ± 0.034	1.499 ± 0.482	1.657 ± 0.275	0.991 ± 0.202
T_{max} (min)	45 ± 10	32 ± 18	12 ± 4	60 ± 40
MRT (min)	106.4 ± 6.4	138.6 ± 19	144.6 ± 14.8	148 ± 25
$AUC_{0-8\,h}$ (μg min/ml)	10.209 ± 0.073	269.998 ± 11.765	289.256 ± 22.342	288.962 ± 28.805
BA, F_{rel} (%)[a]	100	2624 ± 114	2811 ± 217	2807 ± 279

[a] The relative BA between every two dosages have no significant difference ($P > 0.05$).

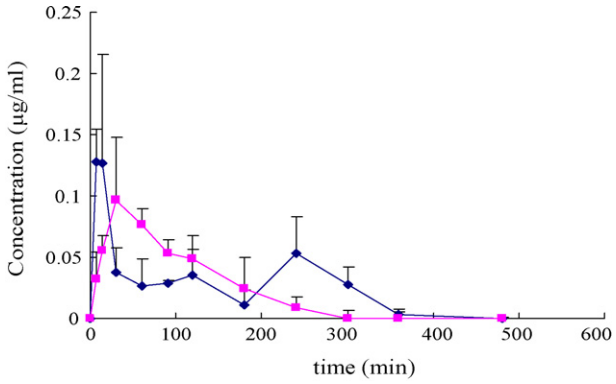

Fig. 4. Mean plasma concentration–time curves of HSYA in rats after oral administration of HSYA aqueous solution on the condition of ligating bile duct and not ligating ($n = 6$). (■) HSYA concentration of the rats not ligating bile duct; (♦) HSYA concentration of the rats ligating bile duct.

oral administration to rats. The pharmacokinetic parameters, T_{max}, C_{max}, MRT, and $AUC_{0-8\,h}$, obtained oral administration of HSYA aqueous solution and HSYA–phospholipid complex oil solutions are given in Table 3. It shows that with the different dosages of WL 1349, 1 ml/kg, 3 ml/kg, and 9 ml/kg, the BA are 2624%, 2811%, and 2807%, and there is no significant difference between every two dosages ($P > 0.05$). It indicates that the absorption of HSYA was not enhanced as the dosage of WL 1349 increased and WL 1349 dosage independent from HSYA absorption within the dosage from 1 ml/kg to 9 ml/kg.

3.5. BA study after BDL in rats

The test of BDL in rats was performed to investigate the absorption of HSYA aqueous solution and HSYA–phospholipid complex oil solution without bile. Bile, endogenous emulsifier, may have influence on the absorption enhancement of HSYA–phospholipid complex oil solution.

The plasma HSYA concentration versus time profiles following oral administration of HSYA aqueous solution is shown in Fig. 4. The pharmacokinetic parameters, T_{max}, C_{max}, MRT, and $AUC_{0-8\,h}$, obtained following administration of HSYA aqueous solution are given in Table 4. The C_{max} and T_{max} values given in table are the mean of individual values of six rats. The administration of HSYA solution on the conditions of bile duct ligation and not ligation had resulted in C_{max} and $AUC_{0-8\,h}$ values of 0.105 μg/ml and 10.209 μg min/ml, and 0.109 μg/ml and 10.011 μg min/ml, respectively. It indicated that bile had no influence on the absorption of HSYA aqueous solution, since the values of $AUC_{0-8\,h}$ were the same basically. Fig. 5 shows the plasma HSYA concentration versus time profiles obtained following oral administration of HSYA–phospholipid complex oil solution to rats. The C_{max} and $AUC_{0-8\,h}$ values, obtained from the group whose bile duct was not ligated were significantly higher than that from other group whose bile duct was ligated. The group whose bile duct was ligated had no absorption basically and the plasma concentration could not be detected except in the 4th hour, having a much smaller absorbed peak. The results indicated that HSYA–phospholipid complex oil solution could be absorbed well with the participation of bile. According to the result in Section 3.2, WL 1349 was hydrolyzed by pancreatic lipase with the help of bile rapidly. So, it was possible that the lipolysis products of WL 1349 enhanced the absorption of HSYA in GIT.

Table 4
Effect of ligating bile duct on absorption of HSYA from HSYA aqueous solution ($n = 6$)

Parameters	HSYA aqueous solution	
	Not ligating bile duct	Ligating bile duct
C_{max} (μg/ml)	0.105 ± 0.034	0.109 ± 0.019
T_{max} (min)	45 ± 10	10 ± 3
MRT (min)	106.4 ± 6.4	142.5 ± 34.5
$AUC_{0-8\,h}$ (μg min/ml)	10.209 ± 0.073	10.011 ± 1.918
BA, F_{rel} (%)	100	98 ± 19

3.6. The influence of lipolysis products of WL 1349 on absorption of HSYA in rats

The plasma HSYA concentration versus time profiles following oral administration of HSYA aqueous solution and HSYA lipolysis products of WL 1349 solution are shown in Fig. 6. The pharmacokinetic parameters, T_{max}, C_{max}, MRT, and $AUC_{0-8\,h}$, obtained following administration of HSYA aqueous solution and HSYA lipolysis products solution are given in Table 5. The C_{max} and T_{max} values given in table are the mean of individual values of six rats. The administration of HSYA–phospholipid complex caprylic acid solution, complex capric acid solution and complex caprylic acid and capric acid monoglycerides solution had resulted in C_{max} and $AUC_{0-8\,h}$ values of 2.277 μg/ml and 606.943 μg min/ml, 0.708 μg/ml and

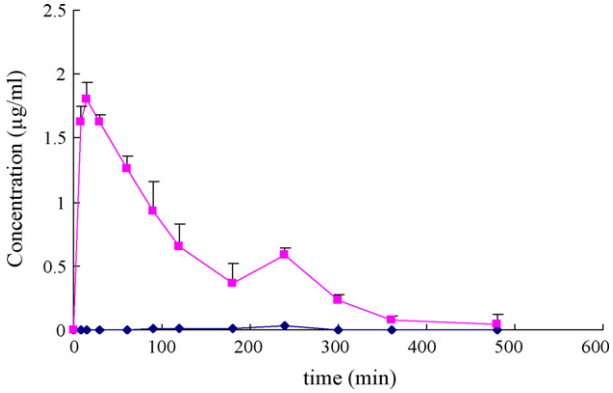

Fig. 5. Mean plasma concentration–time curves of HSYA in rats after oral administration of HSYA–phospholipid complex oil solution on the condition of ligating bile duct and not ligating ($n = 6$). (■) HSYA concentration of the rats not ligating bile duct; (♦) HSYA concentration of the rats ligating bile duct.

Table 5
Main pharmacokinetics parameters of HSYA after oral administration of HSYA aqueous solution and HSYA lipolysis products of WL 1349 solution in rats ($n = 6$)

Parameters	HSYA aqueous solution	Complex with caprylic acid	Complex with capric acid	Complex with monoglycerides
C_{max} (μg/ml)	0.105 ± 34	2.277 ± 0.906	0.708 ± 0.339	0.593 ± 0.287
T_{max} (min)	45 ± 10	1 15 ± 83	25 ± 6	55 ± 27
MRT (min)	106.4 ± 6.4	1 86 ± 42.6	139.7 ± 40.4	119.5 ± 20
$AUC_{0-8\,h}$ (μg min/ml)	10.209 ± 0.073	606.943 ± 322.447	96.427 ± 23.582	86.264 ± 61.040
BA, F_{rel} (%)	100	5945 ± 3133	945 ± 231	845 ± 597

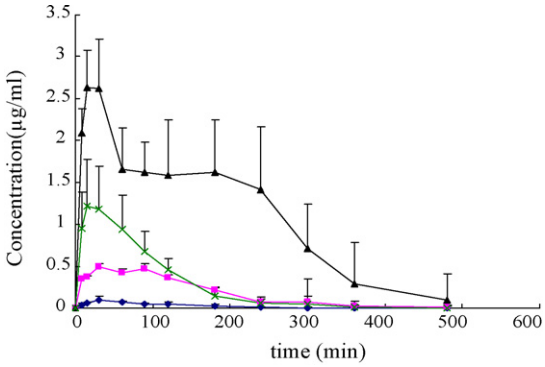

Fig. 6. Mean plasma concentration–time curves of HSYA in rats after oral administration of HSYA aqueous solution and HSYA lipolysis products of WL 1349 solution ($n = 6$). (▲) Complex with caprylic acid; (×) complex with capric acid; (■) complex with monoglycerides; (♦) HSYA aqueous solution.

96.427 μg min/ml, and 0.593 μg/ml and 86 μg min/ml, respectively. It showed that the three lipolysis products solution all could enhance the absorption of HSYA to some extent, with the relative BA of 5945%, 945%, and 845%, and the summary of them exceeded the BA of administration of HSYA–phospholipid complex oil solution (BA, 3700%) and the absorption enhancing effect of caprylic acid was the greatest. It has been reported that medium-chain fatty acids and medium-chain glycerides can enhance the intestinal absorption of drug (Constantinides et al., 1994, 1996; Sharma et al., 2005). The results above illustrated that the lipolysis products of WL 1349 could enhance the absorption of HSYA, and indicated that WL 1349 needed to be hydrolyze by pancreatic lipase and the lipolysis products played the role of absorption enhancement.

4. Conclusion

There are several barriers for the oral delivery of macromolecular and hydrophilic drugs. The major causes of low oral BA of these drugs are the luminal low membrane permeability (Prasad et al., 2003a,b). The investigation in this paper shows that phospholipid complex can increase the solubility of HSYA in oil and the permeability to some extent. It has been reported that oil carriers can improve the delivery of hydrophilic drug by affording projection against intestinal degradation and can improve the absorption of drug. HSYA–phospholipid complex WL 1349 oil solution is prepared and found that this oil solution can enhance the oral absorption of HSYA remarkably. Since it has been reported that the oil-based formulation can improve the absorption of drug, but its mechanism has not been explained clearly. In this protocol, BDL test, in vitro lipolysis experiments of WL 1349 and BA experiments of lipolysis products of WL 1349 on absorption of HSYA in rats were performed. The BDL test and in vitro lipolysis experiments of WL 1349 indicated that HSYA–phospholipid complex oil solution could be absorbed well with the participation of bile. Since WL 1349 was hydrolyzed rapidly by pancreatic lipase with the help of bile, it may be that the lipolysis products of WL 1349 enhanced the absorption of HSYA in GIT. The BA experiments of lipolysis products of WL 1349 on absorption of HSYA in rats showed that all the products of WL 1349 could enhance the BA of HSYA, and the summary effect of absorption enhancing was greater than WL 1349. It indicated the lipolysis products of WL 1349 played the role of absorption enhancement. It has been known that the lipolysis products of MCT can be absorbed directly by epithelial cell in GIT and they may enhance the penetration and permeability of membrane. The lipolysis products of WL 1349 may enhance the permeability of membrane and open the intercellular tight junction, and the absorption of HSYA was improved significantly. Further studies need to prove the possibility of this mechanism of absorption enhancing effect.

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