Mechanism of Phospholipid Complex and its SNEDDS Enhancing the Oral Bioavailability of Silybin

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ABSTRACT SUMMARY
The mechanisms of silybin–phospholipid complex (SIB–PC) and SIB-PC self-nanoemulsion drug delivery system (SIB-PC-SNEDDS) enhancing the oral bioavailability of SIB were studied by using dynamic in vitro digestion model, transport across Caco-2 cells, measurement of cell membrane fluidity and tight junction protein, in situ perfusion and intestinal lymphatic transport experiment.

INTRODUCTION
Enhanced bioavailability of BCS II to BCS IV drugs by preparing into phospholipid complex (PC) has been demonstrated by pharmacokinetics and activity studies conducted in animals as well as human beings. In order to improve the poor dispersion of phospholipid complex in GI tract, which could limit the release and absorption of the complexed drug, self-nanoemulsifying drug delivery system (SNEDDS), have been developed to increase the bioavailability further. However, the mechanisms of the improvement on the absorption and bioavailability of phospholipid complex and SNEDDS still need to be clarified.

In this study, flavonolignan silybin, which has been widely used in the treatment and prevention of chronic inflammatory liver disorders, was chosen as a model drug. After oral administration, SIB-PC gives plasma levels significantly higher than those found after the administration of silybin or silymarin to rats or to humans.

In vitro dynamic lipolysis was investigated to study the digesting process of the formulation. Caco-2 and single pass intestinal perfusion in rats was used to compare the permeability and absorption rate among SIB, SIB-PC and SIB-PC-SNEDDS. Lymphatic transport of SIB-PC and SIB-PC-SNEDDS was measured using an unconscious rat model with three duct-cannulated (jugular vein, mesenteric lymphatics and duodenum).

EXPERIMENTAL METHODS
Silybin–phospholipid complex (SIB–PC) was prepared. The composition of SNEDDS was optimized with ternary phase diagrams.

Complexation efficiency was measured to evaluate the complex formation of SIB-PC. The solubility and the dissolution of silybin in SIB-PC and SIB-PC-SNEDDS were measured. The droplet size/distribution and ζ-potential of SNEDDS were determined with Nicomp™ 380 ZLS Zeta Potential/Particle sizer.

An established model with minor adjustments was employed to characterize the in vitro lipolysis of SIB-PC and SIB-PC-SNEDDS. The extent of lipolysis was defined as the percentage of triglycerides digested in vitro lipolysis experiments. Transport studies were performed at 37°C on mature Caco-2 cell monolayers from apical to basolateral side and The apparent permeability coefficient was calculated.

The effect of SIB-PC and SIB-PC-SNEDDS on membrane fluidity of Caco-2 cells was measured. The effect of SIB-PC and SIB-PC-SNEDDS on the expression of ZO-1 (tight junction protein) was evaluated by western blotting.

The in situ single pass perfusion studies were performed. The methods of simultaneous perfusion in two segments were utilized to reduce the number of rats used in the experiment. The absorption rate constant (K_a) and apparent permeability coefficients (P_app) were calculated.

The intestinal lymphatic transport studies were performed. The extent of lymphatic transport was calculated using the concentration of drug found in each lymph sample, multiplied by the volume of the lymph produced per hour, and expressed as a cumulative percentage of the dose. Plasma concentrations versus time data for SIB for individual rats were analyzed by standard non-compartment analysis using the computer program DAS2.0.

RESULTS AND DISCUSSION
The complexation efficiency of phospholipid complex was 98.2% ±3.5%. The average droplet size and zeta potential of SNEDDS dispersed in water was 92.0 ± 5.3 nm and 14.02 ± 7.12 mV, respectively. Fig.1 showed the release profiles of silybin from SIB and SIB-PC in 0.1M HCl and phosphate buffer saline (pH 6.8). It could be concluded that the solubility and release of SIB could be improved significantly by SIB-PC.

As shown in Fig.2, the lipolysis curves of SIB-PC and phospholipid, SIB-PC-SNEDDS and blank SNEDDS, were quite similar. The result indicated that lipolysis of PC and SNEDDS depended mostly on the property of lipid excipients used in the formulation.

As shown in Fig.3, SIB-PC and SIB-PC-SNEDDS noticeably increased the transport of SIB across Caco-2 cells at 37°C at concentrations of 10, 20 and 30 μg/ml.
The ratio of Papp BL-AP/Papp AP-BL of SIB was 17.43 (P < 0.01 = . Compared with SIB, the ratio of Papp BL-AP/Papp AP-BL of SIB-PC and SIB-PC-SNEDDS decreased significantly, and the effect of Cyclosporin A on the transport was also reduced, suggesting SIB-PC and SIB-PC-SNEDDS could significantly inhibit the efflux of P-gp.

By taking the fluorescence anisotropy of DPH, changes in the fluidity of the cell membrane upon exposure to different drugs was assessed (Fig. 8, n=5). The result showed that the SIB-PC significantly increased the membrane fluidity at the hydrophobic core of the bilayer. No significant difference was observed between SIB and SIB-PC-SNEDDS.

Caco-2 monolayers were treated with SIB, SIB-PC or SIB-PC-SNEDDS for 2 h, and then the amount of ZO-1 was detected using western-blotting. After treatment with SIB-PC-SNEDDS, ZO-1 was lower than that of control, which indicated that SIB-PC-SNEDDS might lead to loss of tight junction integrity of Caco-2 cell monolayers.

The absorption characteristics of SIB, SIB-PC and SIB-PC-SNEDDS in four different intestinal segments (duodenum, jejunum, ileum and colon) were studied. SIB-PC-SNEDDS and SIB-PC presented higher Ka and Papp values than SIB (P < 0.01) in all the intestinal segments, and SIB-PC-SNEDDS > SIB-PC in jejunum, ileum and colon (P < 0.05 or P < 0.01).

The extent of lymphatic transport of SIB was determined after intraduodenal administration. Fig. 4 compared the cumulative SIB amount of lymphatic transport versus time for the three formulations. The extent of transport after 8 h was SIB-PC-SNEDDS > SIB-PC > SIB (P < 0.05 = . The concentration of portaly absorbed SIB as a function of time following intraduodenal administration of SIB, SIB-PC and SIB-PC-SNEDDS was illustrated in Fig. 5. The relative bioavailability SIB-PC and SIB-PC-SNEDDS was 1265.9% and 1802.5%, respectively.

CONCLUSION
Phospholipid complex and its SNEDDS

Fig. 4 The cumulative transport of SIB in mesenteric lymph following duodenal administration to 

Fig. 5 Mean plasma concentration versus time profiles of SIB following duodenal administration to 

significantly improve the hydrophilicity and dissolution of SIB, and the drug in precipitation during digesting could keep a high energy amorphous form, which might display better dissolution and absorption. The possible reasons for phospholipid complex promoting the intestinal absorption include the inhibition of P-gp efflux, improvement of membrane fluidity and lymphatic absorption. For PC-SNEDDS, the mechanisms include the inhibition of P-gp efflux, open of cell tight junctions and enhancement lymphatic absorption.

REFERENCES