Disposition of Lipid-Based Formulation in the Intestinal Tract Affects the Absorption of Poorly Water-Soluble Drugs

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Solvent Green 3 (SG), a model poorly water-soluble compound, was orally administered to rats with soybean oil emulsion or the Self-microemulsifying drug delivery system (SMEDDS) composed of Gelucire44/14. The bioavailability of SG after oral administration with SMEDDS was 1.7-fold higher than that with soybean oil emulsion. The intestinal absorption of lipid-based formulations themselves was evaluated by the *in situ* closed loop method. The effect of lipase and bile salt on their absorption was also evaluated. SMEDDS itself was rapidly absorbed in the intestine even in the absence of lipase and bile salt, and the absorption was increased by the addition of lipase and bile salt. On the other hand, no soybean oil emulsion was absorbed in the absence of lipase and bile salt. However, mixed micelle prepared from emulsion by incubating soybean oil emulsion with lipase and bile salt was rapidly absorbed through the intestine. Without lipase and bile salt, SG was not absorbed after administration with soybean oil emulsion. Therefore, we concluded that the degradation of soybean oil emulsion was needed for SG to be absorbed through the intestine. Furthermore, we investigated the intestinal absorption of SG after oral administration to rats whose chylomicron synthesis were inhibited by pretreatment with colchicine. Colchicine completely inhibited the intestinal absorption of SG after administration with each lipid-based formulation, suggesting that SG was absorbed from the intestine *via* a lymphatic route. Absorption of the dosage formulation should be paid attention when poorly water-soluble drugs are orally administered with lipid-based formulation.

Key words poorly water-soluble drug; intestinal absorption; lipid-based formulation; Self-microemulsifying drug delivery system

Recently, drug discovery strategies, based on the technology of combinatorial chemistry and high throughput screening, have often produced compounds with poor water-solubility.[1,2] Most of those compounds will drop out as candidates at the pre-formulation or formulation study stage because of their low intestinal absorption despite their high pharmacological activity *in vitro*. Therefore, the investigation of new dosage formulations for poorly water-soluble compounds will contribute to drug development.

Since a new oral dosage formulation for an immunosuppressant drug (cyclosporine A), named "Neoral^{R}", was developed and used clinically,[3] lipid-based formulations[4,5] to improve the oral absorption of poorly water-soluble drugs have received considerable attention in the pharmaceutical industry. Neoral^{R} contains various ingredients to form an emulsion in the intestinal tract and works as a Self-microemulsifying drug delivery system (SMEDDS).[6] This system is regarded as an advanced system of lipid-based formulations. As SMEDDS generally consists of lipid(s) and surfactant(s), these components may affect the absorption mechanism of poorly water-soluble drugs. However, the disposition of lipid-based formulation components in the intestinal tract has not yet been clarified.

In this study, we investigated the role of lipid-based formulation components for the intestinal absorption of poorly water-soluble drug by focusing on the disposition of the formulation after oral administration.

MATERIALS AND METHODS

Materials Soybean oil was from Wako Pure Chemicals Co., Ltd. (Osaka, Japan). Lipase from the porcine pancreas (EC 3.1.1.3: 220 U/mg protein) and Solvent Green 3 (SG) were from Sigma Chemicals Co., Ltd. (St. Louis, MO, U.S.A.). Tween 80 and sodium taurocholate were obtained from Nacalai Tesque Co., Ltd. (Kyoto, Japan). Gelucire 44/14^{R}, the mixture of saturated polyglycolized glyceride and mono- or di-fatty acid esters of polyethylene glycol (Lauroyl macrogol-32 glycerides), was a kind gift from Gattefosse (Gennevilliers Cedex, France). All other chemicals used were of analytical grade.

Preparation of Lipid-Based Formulations SG (M.W. 418.5), an anthraquinone derivative, was used as a poorly water-soluble model compound. This compound is a poorly water-soluble dye, and its logP value was 7.40. Soybean oil emulsion was prepared by the conventional method.[7] Briefly, 3 mg of SG was added to 0.5 ml of soybean oil preheated at 60 °C in a hot water bath and dissolved completely. 0.25 ml of Tween 80 and 4.25 ml of distilled water were added to the oil and sonicated at 20 W for 5 min using an Ultrasonic Disrupter UD-201, Tomy Co. (Tokyo, Japan). For SMEDDS, 3 mg of SG was dissolved in 5 ml of Gelucire 44/14^{R} preheated at 60 °C in a hot water bath. It was then cooled down immediately before use because Gelucire 44/14^{R} becomes semi-solid at room temperature.

***In Vivo* Oral Administration to Rats** All animal experiments were approved by the Animal Experimentation Committee of Osaka University of Pharmaceutical Sciences. Male Wistar rats weighing 300—350 g were obtained from Japan SLC, Inc. (Shizuoka, Japan). The day before the experiment, the rats were lightly anesthetized with ether, and were implanted surgically with a combination of Phicon (Fuji Systems Ltd., Tokyo, Japan) and PE50 (Clay Adams, Parsippany, NJ, U.S.A.) in a catheter, which was inserted into the right jugular vein for blood sampling. The catheter was externalized through the back of the neck and secured. The rats were fasted 18 h before experiment, but drinking water was supplied *ad libitum*. One milliliter of soybean oil emulsion or

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SMEDDS containing SG was orally administered to rats (dose of SG: 600 μg/rat). Blood samples (0.4 ml) were withdrawn from the jugular vein at a predetermined time interval for 8 h. Separately, 120 μg of SG dissolved with dimethysulfoxide was intravenously injected and blood samples were withdrawn in a similar manner to the oral administration experiment to calculate bioavailability. Blood samples were transferred into heparinized tubes and centrifuged at 10000 rpm for 4 min. The plasma was kept at −20 °C until assay.

Stability of Formulations in the Simulated Intestinal Fluids The stability of lipid-based formulations in the small intestinal tract was evaluated by the method of Nishihata[8] with some modifications. Briefly, 0.2 ml of each formulation was added to 4 ml of simulated intestinal fluids composed of 10 mM sodium taurocholate and 375 U/ml pancreatic lipase in phosphate-buffered saline (pH 6.5), and the mixture was incubated at 37 °C. At a predetermined time, 0.2 ml of acetic acid (50 v/v%) was added to terminate the reaction. The amounts of non-esterified fatty acids (NEFA) liberated from triglyceride in the formulations were determined to evaluate the degraded amount of formulation using NEFA Test Wako (Wako Pure Chemicals, Osaka, Japan).

Preparation of Mixed Micelle from Emulsion Four hundred microliters of soybean oil emulsion was added to 2 ml of simulated intestinal fluid, and the mixture was incubated at 37 °C for 1 h. This sample was then centrifuged at 160000 ***g*** for 30 min. The lower transparent layer was taken and used as mixed micelle for the following experiment.

Evaluation of Intestinal Absorption of Lipid-Based Formulations The intestinal absorption of lipid-based formulations in rats was determined by the *in situ* closed loop method. In brief, under pentobarbital anesthesia, the rat abdomen was incised and a 10 cm loop was made in the proximal jejunum. Both bile duct and pancreatic duct were ligated to avoid the secretion of bile juice and pancreatic lipase into the loop. After washing the luminal side of the loop with physiological saline, 2 ml of each formulation without SG was injected into the loop. The glyceride concentration containing each dosage formulation was adjusted to 10 mg/ml. At a predetermined time, the luminal side of the loop was flushed with 20 ml of physiological saline. This washing solution was collected as a sample. Triglyceride concentration in a sample was determined using Triglyceride E Test Wako (Wako Pure Chemicals, Osaka, Japan) to measure the amount of formulation remaining in the intestinal loop.

Effects of Lipase and Bile Salt on the Intestinal Absorption of Poorly Water-Soluble Drug The effects of bile salt and pancreatic lipase on the intestinal absorption of poorly water-soluble drug were also evaluated by the *in situ* closed loop method. Briefly, under pentobarbital anesthesia, the intestinal loop was made by the same method as above and 2 ml of each formulation including SG (0.3 mg/ml) was injected into the loop with or without bile salt (10 mM) and lipase (375 U/ml). Blood was withdrawn from the jugular vein at 0.5, 1 and 2 h after administration. A blood sample was transferred into heparinized tubes and centrifuged at 10000 rpm for 4 min. The plasma was kept at −20 °C until assay.

Inhibition Effect of Chylomicron Synthesis on the Intestinal Absorption of Drugs *via* Lymphs A surgical catheter, implanted into the jugular vein of rats by the same method as above, was used for this experiment. Colchicine, which is known as an inhibitor of chylomicron synthesis, dissolved in PBS (pH 7.4) was intravenously administered at a dose of 5 mg/kg body weight.[9—11] One hour after colchicine injection, each lipid-based formulation including SG was orally administered. Blood was then withdrawn and a plasma sample was obtained as described above.

Determination of SG Concentration in Plasma Eight hundred microliters of PBS (pH 7.4) was added to a plasma sample (200 μl). Then, 2 ml of chloroform was added and the mixture was vigorously shaken for 10 min. After centrifugation at 3000 rpm for 10 min, the organic phase was transferred into another tube and evaporated *in vacuo*. Methanol (150 μl) was added for reconstitution and 50 μl of aliquot was injected into HPLC.

HPLC Condition SG was assayed by reversed phase HPLC on a LiChrospher^R 100 RP-18 column (250×4.0 mm, 5 μm). The HPLC consisted of a PU-980 pump, UV-970 UV-VIS spectrophotometric detector (JASCO Co., Tokyo, Japan) and C-R6A integrator (Shimadzu Co., Kyoto, Japan). The mobile phase was acetonitrile (100%) and was run at a flow rate of 1 ml/min. The UV–VIS detector was set at 644 nm.

Calculation of Pharmacokinetic Parameters The area under the SG concentration–time curve (*AUC*). *AUC*s was calculated to include the last measurement of SG concentration–time data by the linear trapezoidal method, and *AUC*s extending beyond the last data sampling point (only for *in vivo* experiments) were estimated by dividing the final concentration–time data by the terminal slope. C_{max}, T_{max} and mean residence time (*MRT*), were calculated based on the moment analysis method.

Data Analysis All values are expressed as the mean± S.E. Statistical analysis was performed using the Mann–Whitney *U* test. The level of significance was taken as $p<0.05$.

RESULTS

Increased Absorption of SG by Lipid-Based Formulations Figure 1 shows the SG concentration in plasma after *p.o.* administration with soybean oil emulsion or SMEDDS. Pharmacokinetic parameters are also listed in Table 1. No SG was detected after administration in suspension form (data not shown), whereas both lipid-based formulations enhanced the intestinal absorption of SG. When SG was orally admin-

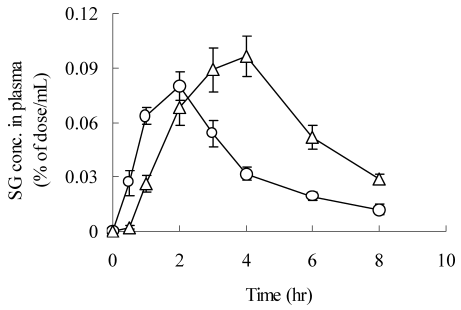

Fig. 1. Time Course of SG in Plasma after *p.o.* Administration (600 μg) to Rats

Circles: soybean oil emulsion. Triangle: SMEDDS. Data represent the mean±S.E. of 4 experiments.

Table 1. Pharmacokinetic Parameters of SG after *p.o.* Administration (600 μg) to Rats

	Soybean oil emulsion	SMEDDS
C_{max} (% of dose/ml)	0.080±0.008	0.097±0.011*
T_{max} (h)	1.750±0.217	4.200±0.179*
AUC (% of dose×h/ml)	0.332±0.026	0.548±0.056*
MRT (h)	3.581±0.230	4.784±0.107*

$*p<0.05$ *vs.* soybean oil emulsion group.

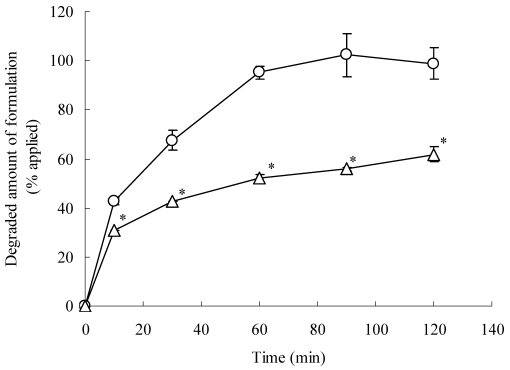

Fig. 2. Degraded Amount of Lipid-Based Formulations in Simulated Intestinal Fluid

Circles: soybean oil emulsion. Triangles: SMEDDS. Data represent the mean±S.E. of 3 experiments. $*p<0.05$ *vs.* soybean oil emulsion group.

istered to rats with soybean oil emulsion, SG in plasma was detected from 1 h after administration without lag time. Then, after reaching the maximum concentration (C_{max}) at 2 h after administration, the SG concentration in plasma was gradually decreased. On the other hand, SG concentration in plasma increased after approximately 1 h of lag time and reached C_{max} around 4 h after administration with SMEDDS. C_{max} value after administration of SMEDDS was significantly higher than that of soybean oil emulsion. Also, both T_{max} and *MRT* values after administration of SMEDDS were greater than those of soybean oil emulsion. The bioavailability of SG after SMEDDS administration (0.263±0.027) significantly increased 1.7-fold more than that with soybean oil emulsion (0.159±0.012).

Stability of Lipid-Based Formulations in the Intestinal Tract The production amount of NEFA from a lipid-based formulation in the simulated intestinal fluid was periodically measured to evaluate the stability of each formulation in the intestinal tract. Figure 2 shows the degradation amount of lipid-based formulations in the simulated intestinal fluid. Soybean oil emulsion shows comparable rapid degradation in the simulated in intestinal fluid and more than 40% of the initial amount decreased within 10 min, thereafter, almost all the formulation degraded within 60 min. On the other hand, less than 60% of SMEDDS decreased within 2 h although approximately 30% was broken down within 1 h. Thus, SMEDDS was more stable than soybean oil emulsion in simulated intestinal fluid.

Intestinal Absorption of Lipid-Based Formulations The intestinal absorption of lipid-based formulations was compared using the *in situ* intestinal loop method. Changes in the amount of lipid-based formulation remaining in the loop are shown in Fig. 3. Almost all the soybean oil emulsion

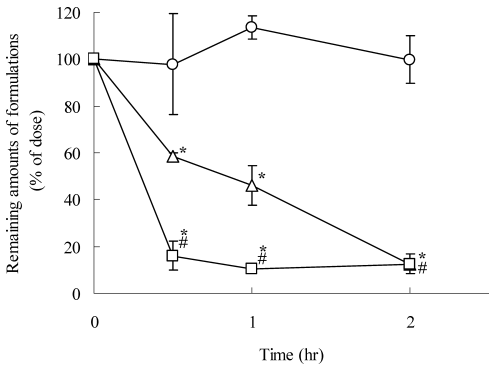

Fig. 3. Remaining Amount of Lipid-Based Formulations in the Intestinal Loop

Circles: soybean oil emulsion. Triangles: SMEDDS. Squares: mixed micelle. Data represent the mean±S.E. of 4 experiments. $*p<0.05$ *vs.* soybean oil emulsion group. $^{\#}p<0.05$ *vs.* SMEDDS group.

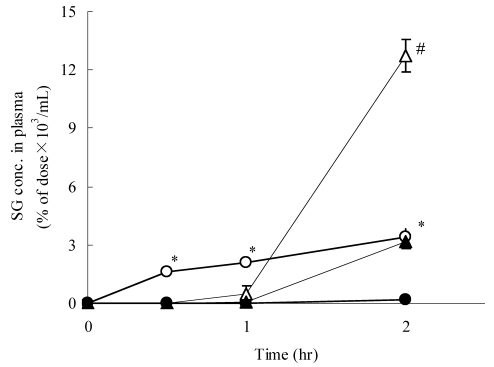

Fig. 4. SG Concentration in Plasma after Administration of Soybean Oil Emulsion or SMEDDS into Intestinal Loop

Open circles: soybean oil emulsion (+lipase and bile). Open triangles: SMEDDS (+lipase and bile). Closed circles: soybean oil emulsion (−lipase and bile). Closed triangles: SMEDDS (−lipase and bile). Data represent the mean±S.E. of 4 experiments. $*p<0.05$ *vs.* soybean oil emulsion with −lipase and bile condition. $^{\#}p<0.05$ *vs.* SMEDDS with −lipase and bile condition.

injected was recovered even 2 h after administration. On the other hand, the remaining SMEDDS gradually decreased and less than 20% of the initial level was recovered from the loop at 2 h after administration.

For comparison, mixed micelle was prepared by incubating soybean oil emulsion with simulated intestinal fluid and the intestinal absorption was also determined. As shown in Fig. 3, mixed micelle was rapidly eliminated and more than 80% of the initial level was absorbed through the intestine during the experiments.

The effects of lipase and bile salt on the intestinal absorption of SG are shown in Fig. 4. *AUC* values (0—2 h) after SG administration with lipid-based formulations into the intestinal loop are also listed in Table 2. For soybean oil emulsion, the intestinal absorption of SG was very low in the absence of lipid and bile salt in the loop. The *AUC* value, however, was increased 51-fold by the addition of lipase and bile salt to the loop. For SMEDDS, the *AUC* value even in the absence of lipase and bile salt was comparable to that in their presence after administration with soybean oil emulsion. Furthermore, the addition of lipase and bile salt significantly increased the *AUC* value (4.1-fold).

There is a possibility of an intestinal absorption mechanism *via* the lymphatic route for poorly water-soluble drugs.

Table 2. Effects of Lipase and Bile on the Absorption of SG from Intestinal Loop

	AUC (% of dose×h/ml)×100	
	Soybean oil emulsion	SMEDDS
−lipase and bile	0.008±0.000	0.165±0.066
+lipase and bile	0.407±0.047*	0.672±0.053*

*$p<0.05$ *vs.* −lipase and bile group.

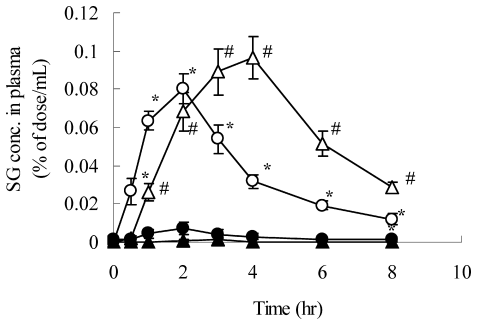

Fig. 5. Effects of Pretreatment with Colchicine on the Intestinal Absorption of SG after *p.o.* Administration (600 μg) to Rats

Open circles: soybean oil emulsion (no pretreatment). Open triangles: SMEDDS (no pretreatment). Closed circles: soybean oil emulsion (pretreatment). Closed triangles: SMEDDS (pretreatment). Data represent the mean±S.E. of 4 experiments. *$p<0.05$ *vs.* soybean oil emulsion with pretreatment group. #$p<0.05$ *vs.* SMEDDS with pretreatment group.

SG was orally administered to rats with a lipid-based formulation, with colchicine pre-dosed intravenously, to clarify the participation of the lymphatic route in the intestinal absorption of SG. In Fig. 5, the time course of SG concentration in plasma after administration to rats with or without pretreatment with colchicine is shown. When SG was administered with soybean oil emulsion, intestinal absorption was almost completely inhibited by pretreatment. Similarly, SG absorption was almost completely inhibited after administration of SG with SMEDDS.

DISCUSSION

Recently, the number of poorly water-soluble compounds as drug candidates has increased with the rapid progress in drug discovery techniques based on combinatorial chemistry and high throughput screening. The solubility of the compounds becomes a critical issue, when they are developed as oral drugs. As a strategy to overcome these problems, lipid-based formulation seems promising. In practice, many researchers have reported the successful improvement of intestinal absorption of poorly water-soluble drug by using lipid-based formulations such as emulsion[12] and SMEDDS.[13] However, the mechanism of intestinal absorption of poorly water-soluble drugs after oral administration of lipid-based formulations remains controversial.

SG, a model poorly water-soluble compound, was rapidly absorbed after administration with soybean oil emulsion, whereas it was gradually absorbed after a 1-h delay when administered with SMEDDS (Fig. 1, Table 1). It is known that SMEDDS is micro-emulsified on contact with digestive juice[14] after oral administration. Therefore, SG absorption may be delayed during the process of SMEDDS emulsification. Although SG was not absorbed at all after administration in suspension (data not shown), both lipid-based formulations remarkably enhanced SG absorption. The bioavailability of SG after oral administration to rats with soybean oil emulsion or SMEDDS was 15% and 26%, respectively. It has already been reported that the intestinal absorption of poorly water-soluble compounds such as celecoxib,[13] paclitaxel[15] and simvastatin[14] was enhanced by SMEDDS. SMEDDS used in this study is also a suitable lipid-based formulation for the oral administration of poorly water-soluble compounds. However, it is still unknown whether the absorption enhancing effects by SMEDDS is only caused by the improvement of the solubility of compound. Therefore, further investigation is necessary to clarify the mechanism of absorption enhancing effects by SMEDDS.

In simulated intestinal fluid including lipase and bile salt, both lipid-based formulations rapidly degraded (Fig. 2). Soybean oil emulsion degraded more rapidly than SMEDDS and was completely degraded within 2 h. Pancreatic lipase generally attacks and cleaves the ester bond between fatty acid and glycerol.[16] Soybean oil used as an oil phase of emulsion is mainly composed of triglyceride, the esters of glycerol and a long acyl-chain fatty acid such as linoleic acid. On the other hand, the predominant composition of SMEDDS used in this study was an ester of lauric acid and PEG-1500 or glycerol. Therefore, reactivity of the ester bond between lauric acid and glycerol or PEG-1500 against lipase might be lower than that between linoleic acid and glycerol.

The intestinal absorption of both lipid-based formulations was examined by the *in situ* closed loop method (Fig. 3). No soybean oil emulsion was absorbed during the 2-h experimental period when it was injected into the intestinal tract in the absence of lipase and bile salt; however, mixed micelle prepared by incubating soybean oil emulsion in the simulated intestinal fluid was rapidly absorbed. These results suggest that mixed micelle formed from emulsion can diffuse into the intestinal mucous layer and be absorbed from the intestinal wall, although emulsion itself cannot disperse into the mucous layer. Interestingly, SMEDDS was absorbed from the intestinal wall although it is one of the emulsion, indicating that SMEDDS may be similar to mixed micelle in structure or physicochemical characteristics. The size of a particular formulation, such as an emulsion, is considered one of the determining factors of intestinal absorption.[17] The size of mixed micelle formed from soybean oil emulsion and SMEDDS might be much smaller than that of soybean oil emulsion. Therefore, smaller particles might be absorbed. It is difficult to conclude from only the results of these experiments, but we speculate that constituents originating from lipid (oil) may play an important role in the diffusion of the formulation and/or its degradation products into the mucous layer. Further investigations are necessary to clarify this point.

SG absorption in the presence or absence of lipase and bile salt was examined to clarify the effects of formulation absorption on SG (Fig. 4, Table 2). The intestinal absorption of SG after administration with soybean oil emulsion in the absence of lipase and bile salt was very low; however, the addition of lipase and bile salt remarkably increased SG absorption. These results suggest that the degradation of soybean oil emulsion is needed for SG to be absorbed. On the

other hand, SG was absorbed even in the absence of lipase and bile salt when it was administered with SMEDDS. Moreover, the intestinal absorption of SG was increased by the addition of lipase and bile salt. This additional effect may occur by the effective emulsification of SMEDDS in the presence of lipase and bile salt.

As described above, there is a possibility that the disposition of lipid-based formulation plays an important role in the intestinal absorption of poorly water-soluble drugs; however, it is unknown whether SG is absorbed by the same mechanism as lipid-based formulation. When dietary lipid is absorbed, chylomicron formed in the epithelial cells transfers to the mesenteric lymph stream.[16] Lipid-based formulations are considered to be absorbed by the same route as dietary lipids. We performed an *in vivo* oral administration experiment of SG with a lipid-based formulation using rats whose chylomicron synthesis were inhibited by pretreatment with colchicine to clarify the absorption mechanism of SG (Fig. 5). Regardless of the kind of lipid-based formulation, SG was almost completely inhibited by pre-dosing with colchicine. This result suggests that SG is, more or less, absorbed *via* the lymphatic route. It is known that the absorption route of dietary lipid is generally dependent on the chain-length of the fatty acids of each lipid.[18] Therefore, the composition of the lipid-based formulation may affect the route of intestinal absorption of poorly water-soluble drugs.

In conclusion, SMEDDS significantly enhanced the intestinal absorption of poorly water-soluble drugs more than soybean oil emulsion as (1) poorly water-soluble drugs are concomitantly absorbed with lipid-based formulations and (2) the intestinal absorption of SMEDDS is higher than that of soybean oil emulsion. Therefore, the absorption of dosage formulation should be paid attention when poorly water-soluble drugs are orally administered with lipid-based formulations.

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