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Lipid-based formulations to enhance oral bioavailability of the poorly water-soluble drug anethol trithione: Effects of lipid composition and formulation

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ABSTRACT

This study has explored the use of lipid-based formulations to enhance the oral bioavailability of the poorly water-soluble drug anethol trithione (ATT), and compared the performance of different formulations. Two groups of lipid-based formulations, sub-microemulsion (SME) and oil solution, were prepared using short (SCT), medium (MCT) and long (LCT) chain triglycerides respectively; aqueous suspension was used as the reference formulation. In vitro and in vivo studies were conducted to investigate the impact of lipid composition and formulation on drug absorption. In vitro digestion was used to analyze lipid digestion rates and drug distribution/solubilization. After in vitro digestion, the performance rank order for drug solubilization was SCT < MCT < LCT. SME formulations were digested more rapidly in vitro than oil solutions. The bioavailability of the drug from different formulations was investigated in rats. All six lipid-based formulations enhanced drug absorption compared to the aqueous suspension. For the SMEs, which were rapidly digested, in vivo bioavailability increased in accordance with the increase of solubilization data obtained by in vitro digestion, with the rank order SCT-SME < MCT-SME < LCT-SME. For the oil solutions, which were digested more slowly, there was no significant difference in drug bioavailability for the different formulations. In conclusion, lipid-based formulations can enhance the oral bioavailability of ATT, and for this BCS class II drug, both the lipid composition and type of lipid formulation are likely to govern in vivo performance.

1. Introduction

In recent years, one of the most popular approaches for improving the oral bioavailability of poorly water-soluble drugs has been through the use of lipid-based drug delivery systems (Hauss et al., 1998), including oil solutions/suspensions, emulsions, or self-microemulsifying drug delivery systems (Pouton, 2000). This approach has led to the successful marketing of medicinal products including cyclosporin A (Neoral®), ritonavir (Norvir®) and saquinavir (Fortovase®). The success of these delivery systems is due to the selection of an appropriate vehicle and a rational delivery system design (Dahan and Hoffman, 2008). To date, however, lipid-based formulations comprise only a small fraction of commercially available drug products (Strickley, 2007). One reason for the low uptake of such formulations is that development strategies remain largely empirical. This is in part due to the lack of simple in vitro tests that accurately predict in vivo performance. In vitro lipid digestion methods have recently been proposed by several groups as a means to select appropriate lipid vehicles and to rationalize formulation design (Kossena et al., 2003; Ljusberg-Wahren et al., 2005; Porter and Charman, 2001; Zangenberg et al., 2001). Increasingly, researchers have focused on drug distribution/solubilization during lipid digestion (Kaukonen et al., 2004a, 2004b). Many studies have revealed that the lipid component of the delivery system has a great influence on its capability to enhance absorption (Dahan and Hoffman, 2008), and in some studies the in vitro data correlated well with the in vivo drug performance (Dahan and Hoffman, 2007).

In the present study the poorly water-soluble and lipophilic compound, anethol trithione (ATT, 5-(*p*-methoxyphenyl)-3H-1,2-dithiole-3-thione, molecular weight: 240.35, the chemical structure is shown in Fig. 1), was chosen as a model drug. ATT can increase salivary secretion in drug-induced xerostomia (Bagheri et al., 1997) and significantly inhibit carcinogenesis by increasing the activity of electrophile detoxification enzymes (Egner et al., 1994; Reddy et al., 1993). The drug is also prescribed as an adjunctive therapy for cholecystitis, gallstone, indigestion, acute/chronic hepatitis, and is marketed in many countries including France, Germany and China. ATT has high lipophilicity (log *P* = 3.8, Boudeville et al.,

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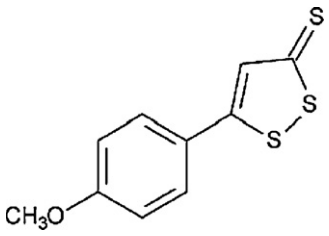

Fig. 1. Chemical structure of ATT.

1996) and high intestinal permeability (P_{app} is (2–3) × 10^{-4} cm/s, approximately, data from rat intestinal single-pass perfusion model in our laboratory), but the water solubility of ATT is extremely low (0.38 μg/ml, Jing et al., 2006). The drug can be classified as a BCS (biopharmaceutics classification system) class II compound. Low solubility limits ATT dissolution and bioavailability, and common solubilization methods (e.g. using cosolvent) have not been successful with this drug.

For BCS class II compounds, which have high intestinal permeability properties, the extent of absorption is dictated by the dissolution properties of the molecule in gastrointestinal fluids (Dahan and Hoffman, 2008). The purpose of this study was to design different lipid-based formulations intended to enhance the solubility and oral bioavailability of ATT, and to compare the performance of different formulations. Short (SCT), medium (MCT) and long (LCT) chain triglycerides were used to prepare two types of formulation (sub-microemulsion, SME; and oil solution); aqueous suspension was chosen as the reference formulation. An in vitro digestion model was used to investigate the effect of lipid composition and type of formulation on drug solubilization and lipid digestion. The bioavailability of the drug from the tested formulations was investigated in rats. Finally, comparison of in vivo and in vitro data allows predictions to be made concerning the impact of lipid composition and formulation on the drug absorption.

2. Materials and methods

2.1. Chemicals

Anethol trithione (ATT) was from the Zhangjiagang Fengda Pharmaceutical Co. (Zhangjiagang, Jiangsu, China), p-hydroxy anethol trithione (ATX) was from Chengdu Kinna Bio & Pharma Co. (Chengdu, Sichuan, China), and Rutaecarpine was from Shanghai Winherb Co. (Shanghai, China). Soybean oil (LCT) and triglycerides of caprylic/capric acid (MCT) were purchased from Tieling Beiya Medical Oil Co. (Tieling, Liaoning, China). The fatty acid composition of soybean oil consists mostly of long chain (C18) fatty acid, including linoleic acid, oleic acid, and saturated fatty acid, etc. MCT is triglycerides of medium chain length fatty acid (C8/C10). Tributyrin (SCT) was from Beijing Xizhong Chemical Plant (Beijing, China). All the lipids were used as obtained and the initial free fatty acid content of SCT, MCT and LCT was less than 1.0%, 0.22%, 0.20%, respectively. Labrasol was kindly given by Gattfosse (Lyon, France). Cremophor EL was from BASF AG (Ludwigshafen, Germany), and Lecithin (Epikuron 200, containing about 92% of soy phosphatidylcholine, PC) was from Lucas Meyer (Hamburg, Germany). Taurocholic sodium (NaTC) was obtained from Beijing Bio-lab Materials Institute and Porcine pancreatic lipase (40,000 IU/g) was from Shanghai Kayon Biological Technology Co., Ltd. (Shanghai, China). Tris-(hydroxymethyl) aminomethane, maleic acid, calcium chloride, carboxymethyl cellulose sodium (CMC-Na), magnesium chloride and sodium chloride were all purchased from the Sinopharm Chemical Reagent Co. (Shanghai, China). Ethanol, methanol, acetonitrile (Tedia Company, OH, USA) were HPLC grade. Water purification used a Milli-Q (Millipore, Billerica, MA, USA) water purification system. All other chemicals were of analytical reagent grade.

Table 1
Compositions of the ATT-loaded SMEs.

	LCT-SME	MCT-SME	SCT-SME
Composition (%, w/w)			
ATT	0.072	0.072	0.072
LCT	7.2		
MCT		7.2	
SCT			7.2
Glycerol	0.36	0.36	
Labrasol	1.4	1.4	
Cremophor EL			0.72
Water q.s.	100	100	100

2.2. Animals

Male Sprague–Dawley rats (body weight 300 ± 25 g) were obtained from the Medical Animal Test Center of Shanghai Institute of Materia Medica (Shanghai, China). All experiments were performed according to the Shanghai Institute of Materia Medica guidelines for experimental animal care. The rats were fasted for 12 h prior to the experiment and had free access to water.

2.3. Preparation of formulations

Compositions of the ATT-loaded SMEs are given in Table 1. These SMEs were prepared using emulsification followed by high-pressure homogenization. Briefly, ATT was dissolved in the mixture of oil and surfactant (either Cremophor EL or Labrasol). The mixture was then heated at 50 °C. The hot oil phase was dispersed in water at the same temperature, and a coarse emulsion was formed using a high shear dispersing emulsifier-Ultra Turrax® T25 (IKA, Staufen, Germany) at 12,000 rpm for 5 min. The hot coarse emulsion was then homogenized under 400 bar at 50 °C with a high-pressure homogenizer (NS1001L, GEA, Sala Baganza, PR, Italy) for six cycles. ATT oil solutions (10 mg/g) were prepared by dissolving the drug into LCT, MCT or SCT. The components were mixed at 50 °C using a magnetic stirrer to obtain clear oil solutions. The suspensions (0.7 mg/ml) were prepared as follows: At first, the drug powder (35 mg) was ground with a small amount of 0.5% carboxymethyl cellulose in a mortar, and then the remainder of 0.5% carboxymethyl cellulose (total volume 50 ml) was gradually added with grinding to obtain the suspension. The average particle size (n = 100) was 8.5 ± 2.7 μm measured by an optical microscope (CKX41; Olympus, Tokyo, Japan).

2.4. Characterization of formulations

Particle size and the polydispersity index of the ATT-SMEs were determined by photon correlation spectroscopy using Nicomp 380 ZLS (Particles Sizing Systems, Santa Barbara, CA, USA). Particle sizes were recorded as intensity distributions.

2.5. In vitro digestion

2.5.1. Digestion condition

Digestion experiments were conducted using an in vitro lipid digestion model (Sek et al., 2001) with modifications. Briefly, each oil solution or SME (containing 210 mg lipid and 2.1 mg ATT) was added to the reaction vessel and made up to 19 ml with respective digestion buffer to obtain the digestion medium which consisted of 50 mM Tris-maleate (pH 7.5), 150 mM NaCl, 5 mM $CaCl_2$, 5 mM NaTC/1.25 mM PC (conditions broadly representative of fasted state

intestinal conditions (Dahan and Hoffman, 2008; Kaukonen et al., 2004a, 2004b)). Experiments were initiated by the addition of 1 ml of porcine pancreatic lipase solution (4000 tributyrin units/ml), and the initial volume of the digestion system was 20 ml. 1 M NaOH was added in the digestion system using micro burette at proper rate to maintain the pH at 7.50 ± 0.05 (start or stop the titration when pH goes beyond this range). Titrant volumes were read at predetermined times. The digestion system was agitated by a magnetic stirring apparatus during the whole process and the delay in response of pH to base solution addition was less than 1.5 s.

2.5.2. Sample preparation and HPLC analysis of in vitro digestion samples

Digestion experiments were conducted over 35 min at 37 °C, after which 2× 5 ml aliquots of the post-digestion mixture were ultracentrifuged (302,000 × *g*, 30 min, 37 °C, CP100MX centrifuge, P55ST2 rotor, Hitachi, Tokyo, Japan) to separate the mixture into a pellet phase, an aqueous phase, and an oil phase (after digestion and centrifugation, only the LCT oil solution had oil phase, other five formulations were digested completely and had no oil phase). Then, each phase was dissolved in 10 ml of tetrahydrofuran-acetonitrile (1:3 (v/v), as demulsifer) and subsequently diluted to 50 ml before injection onto the HPLC column. The HPLC condition is described below. The minimum quantifiable concentration was 20 ng/ml, the R.S.D. of precision was less than 1% and the accuracy was 98–102% for $n = 6$ replicates.

2.5.3. Lipid digestion rate

The curve of cumulative volume of NaOH (1 M) required for titration at different time-points was plotted. By recording the volume of NaOH added, we could calculate the extent of lipid digestion indirectly. When the cumulative volume of NaOH versus the time curve reached the stagnation phase (and there was no oil phase after centrifugation), we supposed the lipid was digested completely, and this volume of NaOH was denoted as $V_{100\%}$. The cumulative volume of NaOH used at one time point was denoted as V_t. We used the ratio of V_t to $V_{100\%}$ to calculate the extent of lipid digestion. The time required for 50% digestion (i.e., the time when $V_t/V_{100\%} = 50\%$) was used to evaluate the lipid digestion rate, denoted as T_{50}.

2.6. Bioavailability studies in rats

The fasted rats were divided into seven groups (six rats in each group) for the in vivo bioavailability study. Seven ATT formulations were respectively administered to the rats in each group by oral gavage at a dose of 6.75 mg/kg. The lipid dose administered to the animals was 675 mg/kg (190–220 mg lipid per rat) and the gavage volume of SMEs was 9.4 ml/kg (2.7–3.0 ml per rat). Blood samples (0.5 ml) were withdrawn by retro-orbital venous plexus puncture into heparinized tubes at designated time intervals (Suspension, SMEs and SCT oil solution: 5 min pre-dose, 0.5, 1.1, 1.8, 2.5, 4, 6, and 8 h post-dose; LCT and MCT oil solutions: 5 min pre-dose, 0.5, 1.1, 1.8, 2.5, 3.5, 4.5, 5.5, and 8 h post-dose). Plasma was separated by centrifugation for 5 min at 2000 × *g* and stored at −20 °C prior to ATT and ATX analysis by HPLC as described below.

2.7. Analytical methods of plasma samples

Earlier work has shown that the majority of ATT is metabolized in vivo into p-hydroxy anethole trithione (ATX, with similar pharmacological activity to ATT) via *O*-demethylation in both animals (rats and rabbits) and humans; the plasma concentration of ATT is therefore low (Li et al., 2008). In the present study, we used the total plasma concentration of ATT + ATX to calculate pharmacokinetic data. The concentration of ATT and ATX in plasma was determined using a high performance liquid chromatography (HPLC) system.

Frozen plasma samples were thawed at room temperature prior to the extraction procedure. To each plasma sample (200 μl) was added 40 μl methanol, 20 μl internal standard solution (rutaecarpine, 1.225 mg/ml in methanol), and 1200 μl extraction agent (ethyl acetate: isopropyl alcohol, 95:5, v/v) and mixed for 2 min. After centrifugation (1600 × *g*, 5 min), the organic layer was transferred and evaporated under nitrogen at 40 °C. Samples were reconstituted with 100 μl of solvent mixture (methanol:water:acetonitrile, 60:30:10, v/v), and 50 μl of the resulted solution was injected into the HPLC system.

The HPLC system (Agilent 1200 series, California, USA) used comprised an autosampler (G1367B ALS), a pump (G1311A Quatpump), a column oven (G1316A Column), a diode array detector (G1315D DAD). HPLC conditions were: Zorbax Eclipse C18, 5 μm, 4.6 mm × 150 mm column (Agilent Co., USA), the mobile phase was methanol:acetonitrile:water (27:33:40, v/v), column temperature was 35 °C, the flow rate was 1 ml/min. ATT, ATX and the internal standard were detected at 346 nm. The peak elution time for ATX, internal standard, and ATT was 5.4, 8.7, and 12.2 min, respectively. The HPLC run time was 15 min.

Assay performance was validated using standard measures of linearity, precision, and accuracy. The correlation co-efficient (R^2) for the standard curves of the plasma samples were acceptable (>0.999 for both ATT and ATX). The minimum quantifiable concentrations for ATT and ATX were 10 ng/ml and 12 ng/ml respectively. For ATT and ATX in plasma samples, the relative standard deviation (R.S.D.) of precision was less than 6%, the accuracy was 94–104%, and the efficiency of extraction from plasma was 80–90% for $n = 6$ replicates.

2.8. Pharmacokinetic analysis

ATT + ATX plasma concentrations at different time-points for individual rats were analyzed (noncompartmental analysis model) using DAS Professional software version 2.0 (Anhui, China). We calculated peak plasma concentration of ATT + ATX (C_{max}), time to maximum plasma concentration (T_{max}), the mean residence time between 0 and 8 h ($MRT_{0-8\,h}$), the area under the concentration–time curve between 0 and 8 h ($AUC_{0-8\,h}$), and the area under the first moment of the concentration–time curve between 0 and 8 h ($AUMC_{0-8\,h}$). The $AUC_{0-8\,h}$ and $AUMC_{0-8\,h}$ were calculated by the DAS software using the linear trapezoidal rule method. $MRT_{0-8\,h}$ was calculated as the ratio of $AUMC_{0-8\,h}$ to $AUC_{0-8\,h}$.

2.9. Statistical analysis

All values were expressed as means ± standard deviation (SD). Statistically differences in AUC, C_{max}, T_{max}, and $MRT_{0-8\,h}$ were determined by ANOVA followed by Tukey's test for multiple comparisons at a significance level of $P = 0.05$. All statistical analysis was performed using Graphpad Instat for windows version 3.05 (GraphPad Software, Inc., CA, USA).

3. Results

3.1. Characterization of SME

The size distribution intensity values and polydispersity index (P.I.) values for LCT-SME, MCT-SME and SCT-SME are shown in Table 2. The ATT contents in LCT-SME, MCT-SME and SCT-SME were determined to be 0.70% (w/w), 0.71% (w/w) and 0.71% (w/w) respectively, close to the theoretical drug content of 0.72% (w/w).

Table 2
The particle size analysis for three SMEs.

	Mean particle size[a] (nm)	Polydispersity index
LCT	292.2 ± 3.5	0.187 ± 0.037
MCT	230.6 ± 7.0	0.106 ± 0.036
SCT	190.0 ± 4.2	0.095 ± 0.026

[a] Intensity mean particle size, values are mean ± standard error of mean.

Table 3
T_{50} of six formulations.

	SME			Oil solution		
	LCT	MCT	SCT	LCT	MCT	SCT
T_{50} (s)	50 ± 5	27 ± 3	19 ± 2	2100 ± 153	540 ± 29	190 ± 15

3.2. In vitro digestion

The cumulative volume of NaOH (1 M) required for titration at different time-points, and the extent of lipid digestion (%) versus time are shown in Fig. 2. The T_{50} values of six lipid-based formulations are shown in Table 3. In SME formulations, lipids were digested rapidly while in oil solutions, they were digested significantly slower.

The impact of lipid composition and formulation on drug distribution after 35 min of digestion in vitro are shown in Table 4. About 83% of ATT precipitated from both SCT-SME and SCT-oil solution, about 76% from MCT-SME and MCT-oil solution, and less than 5% from LCT-SME and LCT-oil solution. Approximately 50% of lipid was digested from LCT-oil solution after 35 min, whereas 72.9% of the

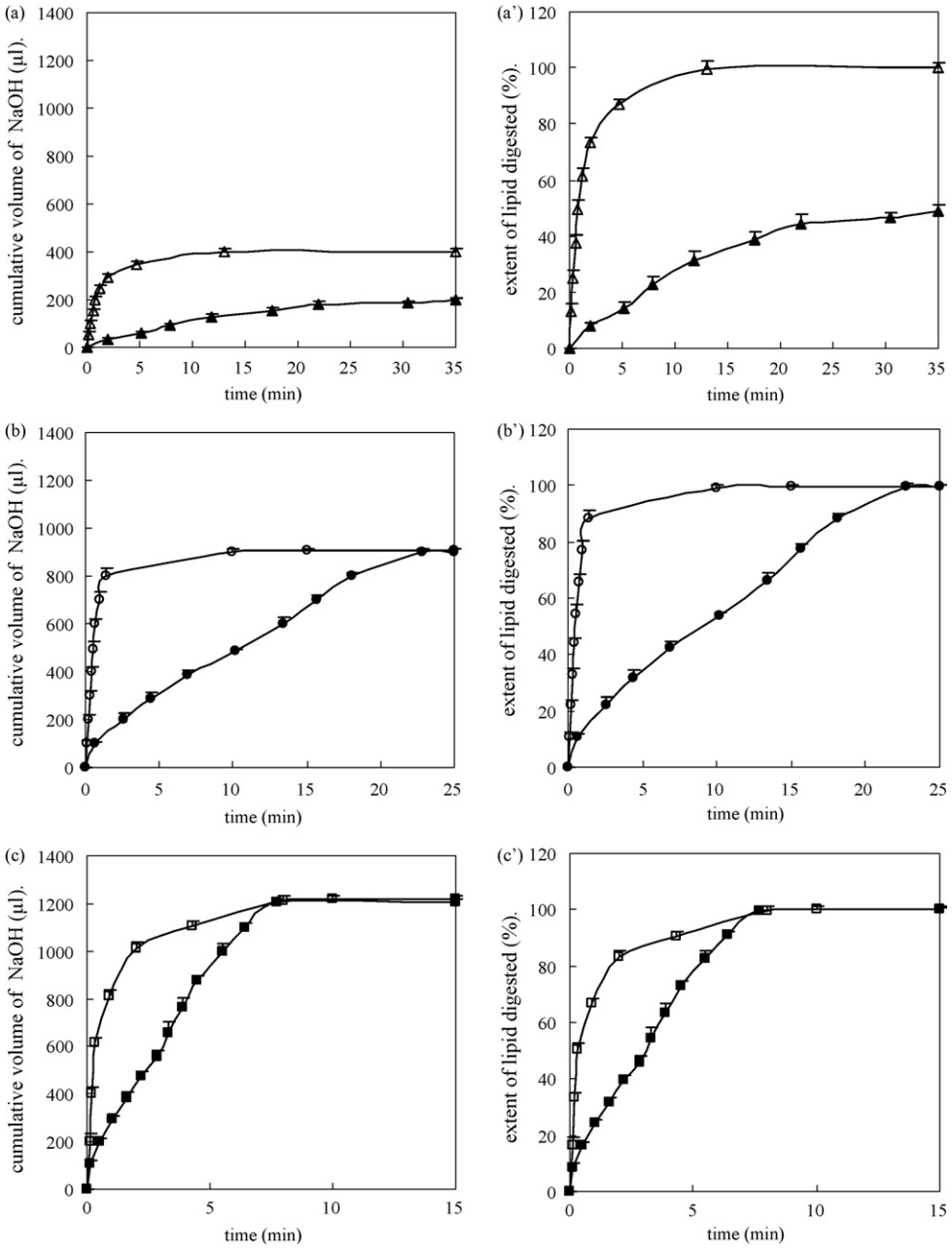

Fig. 2. Digestion profile of six lipid formulations of ATT, cumulative volume (μl) of NaOH (1 M) titrated versus time (min) (a, b, c), and extent of lipid digested (%) versus time (min) (a′, b′, c′). (a, a′): (△) LCT-SME, (▲) LCT-oil solution; (b, b′): (○) MCT-SME; (●) MCT-oil solution; (c, c′): (□) SCT-SME, (■) SCT-oil solution. Each system contained 2.1 mg ATT and 210 mg lipids (n = 3).

Table 4
Assessment of the impact of formulation and lipid component in lipid digestion experiments *in vitro*.

Parameter	SME			Oil solution		
	LCT	MCT	SCT	LCT	MCT	SCT
% in AP	95.1 ± 2.7	23.5 ± 2.5	16.6 ± 2.2	26.6 ± 3.0	24.4 ± 2.9	16.5 ± 2.8
% in pellet	4.9 ± 2.7	76.5 ± 2.5	83.4 ± 2.2	0.5 ± 0.2	75.6 ± 2.9	83.5 ± 2.8
% in oil	No oil phase	No oil phase	No oil phase	72.9 ± 3.1	No oil phase	No oil phase
% digestion	~100	~100	~100	~50	~100	~100

The solubilization patterns of ATT after 35 min digestion of 210 mg LCT, MCT or SCT from SMEs or oil solutions (containing ATT 2.1 mg in respective formulations) under simulated fasted state conditions (5 mM sodium taurodeoxycholate (NaTC)/1.25 mM phosphatidylcholine (PC)) (mean ± SD, $n = 3$).

drug still remained in the oil phase. For the other 5 lipid formulations, all the lipids were digested (the titration curve in Fig. 2 reach stagnation phase, and no oil phase exists after centrifugation) after 35 min; the drug therefore distributed between the aqueous phase and the pellet phase.

3.3. Pharmacokinetics

Fig. 3 plots mean plasma ATT + ATX concentrations against time for seven formulations containing equal amounts of ATT. The corresponding pharmacokinetic parameters are presented in Table 5. The $AUC_{0-8\,h}$ and C_{max} from all lipid-based formulations were higher than that of the suspension. The $AUC_{0-8\,h}$ of ATT + ATX from the LCT-SME was significantly greater than that from MCT-SME (1.5-fold, $P < 0.05$) and SCT-SME (2.1-fold, $P < 0.05$); there was no significant difference in the extents of drug absorption from the three oil solutions and LCT-SME. The $AUC_{0-8\,h}$ of ATT + ATX from MCT-oil and SCT-oil solution was higher than that from MCT-SME (1.3-fold, $P > 0.05$) and SCT-SME (1.7-fold, $P < 0.05$) respectively. The C_{max} of ATT + ATX from LCT-SME was also higher than that from either MCT-SME (1.6-fold, $P < 0.05$) or SCT-SME (2.4-fold, $P < 0.05$). The T_{max} and $MRT_{0-8\,h}$ of ATT + ATX from SMEs were smaller than that from oil solutions.

4. Discussion

4.1. In vitro digestion

Lipid digestion test results show that the type of lipid used in a drug formulation directly impacts the drug's solubilization during in vitro digestion. Many previous studies have explored the lipid digestion process and the drug solubilization/distribution profile following in vitro digestion of different lipids. Lipolysis of triglyceride produces di-glyceride, mono-glyceride, fatty acid and glycerol. When mixed with bile salts and phospholipids, the mono-glyceride and fatty acid can constitute mixed micelles, vesicles, etc. When different type and/or quantity of lipids are digested, dissimilar mixed micelles and vesicles with different drug solubilization power are generated (Porter et al., 2004b). In our study, the results show that the post-digestion system of LCT can solubilize more ATT than that of MCT and SCT, irrespective of whether SMEs or oil solutions are used. Although the lipids of LCT-oil solution are not completely lipolyzed after 35 min of digestion (the drug precipitated little) while the lipids of other five formulations have already been lipolyzed, we believe that the LCT-oil solution will have similar solubilization power as LCT-SME when it is completely digested in vivo.

In the in vitro digestion test, T_{50} was used to assess the lipid digestion rates. The results indicate that SMEs are digested much more rapidly than oil solutions. This may be attributed to the smaller particle size of SMEs that increases the surface area of lipids interacting with the enzyme. In comparison with the LCT oil solution, the rapid digestion rates of SCT and MCT oil solutions prove that lipolysis rate is dependent on the length of the lipid side-chains. Another reason for the slow and uncompleted digestion of LCT might be the inhibition effect of the lipolysis product of LCT on pancreatic lipase mediated lipolysis. But in our in vivo experiments, we assume that the absorption of the lipolysis product makes the lipolysis react continuously, and the LCT-oil (containing 200 mg of lipids) is digested completely in the intestine.

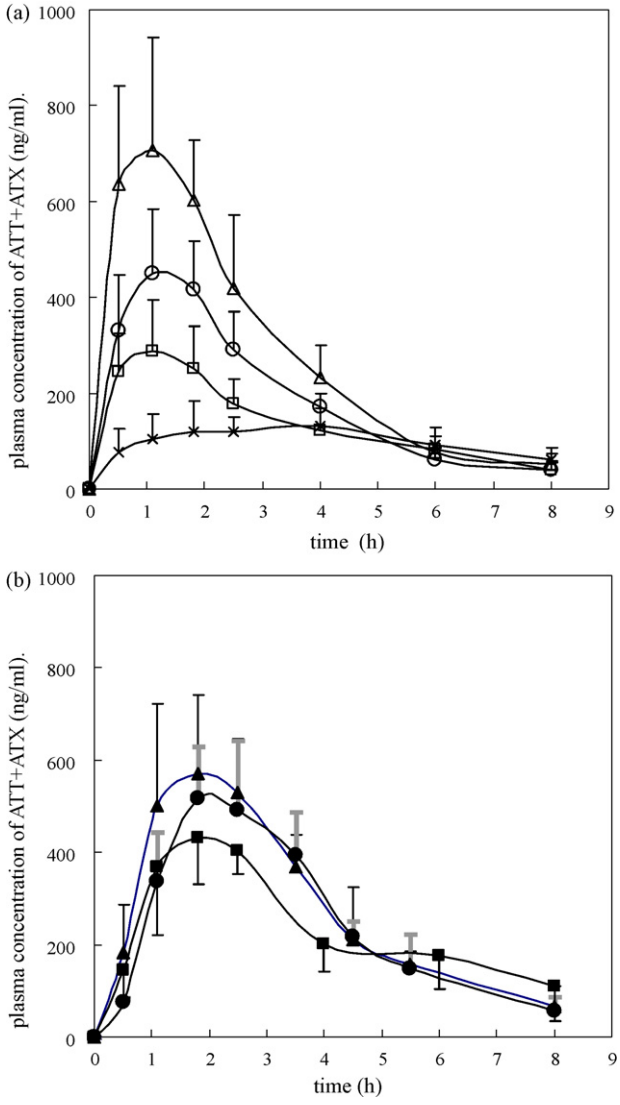

Fig. 3. Mean plasma concentration versus time profiles (mean ± SD, $n = 6$) for ATT + ATX following oral administration of ATT formulations to rats. (△) LCT-SME (a), (○) MCT-SME (a), (□) SCT-SME (a), (×) aqueous suspension (a); (▲) LCT-oil solution (b), (●) MCT-oil solution (b), (■) SCT-oil solution (b). The total administered ATT dose was 6.75 mg/kg.

Table 5
Pharmacokinetics parameters (mean ± SD, $n = 6$) for ATT + ATX.

Parameters	SME			Oil solution			Suspension
	LCT	MCT	SCT	LCT	MCT	SCT	
$AUC_{0-8\,h}$ (ng h/ml)	2370 ± 619	1596 ± 263[a,b]	1112 ± 293[a]	2236 ± 598[b]	2062 ± 389[b]	1881 ± 304[b,c]	801 ± 169[a]
T_{max} (h)	1.2 ± 0.5	1.2 ± 0.5[b]	1.2 ± 0.8[b]	1.9 ± 0.5[b]	2.0 ± 0.4[b]	1.8 ± 0.6[b]	3.3 ± 1.1[a]
C_{max} (ng/ml)	753 ± 221	480 ± 118[a,b]	316 ± 103[a]	610 ± 153[b]	545 ± 142[b]	458 ± 105[b]	158 ± 46[a]
$MRT_{0-8\,h}$ (h)	2.4 ± 0.2	2.6 ± 0.3[b]	2.9 ± 0.5[b]	3.0 ± 0.2[b]	3.1 ± 0.3[b]	3.4 ± 0.5	3.9 ± 0.5[a]

[a] Statistically different when compared with the data of LCT-SME ($P < 0.05$ vs. LCT-SME).
[b] Statistically different when compared with the data of Suspension ($P < 0.05$ vs. suspension).
[c] Statistically different when compared with the data of SCT-SME ($P < 0.05$ vs. SCT-SME).

4.2. Drug absorption and lipid digestion in the intestine of animals

4.2.1. Comparison of lipid-based formulations and suspensions

Lipid-based formulations have been used for many years to improve solubility and enhance the bioavailability of many poorly water-soluble drugs (Porter et al., 2007; Sachs-Barrable et al., 2008). Several earlier studies demonstrated that lipid component can significantly influence the absorption of certain drugs (Dahan and Hoffman, 2007; Porter and Charman, 2001). In our study, the ATT aqueous suspension most likely displayed low drug bioavailability due to the low aqueous solubility of ATT and thus propensity of ATT to precipitate from the suspension formulation. The 'lipid strategy' is successful in enhancing the absorption of the drug: The AUC_{0-8} and C_{max} from the lipid formulations are 1.4–3.0-fold and 2.0–4.8-fold higher than the $AUC_{0-8\,h}$ and C_{max} from suspensions, respectively. Compared with aqueous suspension, all six lipid-based formulations confirm improved drug bioavailability. However, of the six formulations, some perform noticeably better than others. Through our studies, we found that both the formulation and the lipid type play a role in determining ATT bioavailability.

4.2.2. SMEs

Plasma profiles (Fig. 3) and pharmacokinetic parameters (Table 5) suggest increase in AUC_{0-8} and C_{max} following oral administration of LCT-SME when compared with MCT-SME or SCT-SME. The increase in ATT bioavailability following administration of LCT-SME is consistent with the solubilization patterns observed from in vitro digestion. Due to smaller particle size, SME formulations demonstrate rapid lipid digestion and drug dissolution after entry into the rat intestine. The drug also precipitates rapidly from MCT-SME and SCT-SME, similar to the precipitation profile during in vitro digestion. Overall, ATT absorption was lower from MCT-SME and SCT-SME than from LCT-SME.

In a previous study on griseofulvin (Dahan and Hoffman, 2007), LCT, MCT and SCT were used to prepare three lipid-based formulations, and there was a strong positive correlation ($R^2 = 0.98$) between the drug content in aqueous phase and in vivo AUC values. However, in our study on ATT, the linear correlation is not very strong between in vivo and in vitro results. The performance rank order of ATT in the aqueous phase during in vitro digestion was in accord with the $AUC_{0-8\,h}$ ($R^2 = 0.91$) and C_{max} ($R^2 = 0.91$) of ATT + ATX obtained from in vivo study. We thought that the high permeability of ATT may be the reason for weaker correlation. In the present study, the high permeability of ATT indicates that after dissolution from SMEs, the drug molecule diffuses into enterocyte rapidly. Though the digestion of SMEs and drug dissolution are rapid, the rapid drug absorption will make the drug precipitation profile in vivo a little different from drug precipitation in vitro (there is no drug absorption during in vitro digestion), i.e., the drug may precipitate less in vivo. Nevertheless, our result of the relationship between in vivo drug bioavailability and in vitro drug solubilization is comparable to earlier studies (Cuine et al., 2008; Dahan and Hoffman, 2007; Grove et al., 2005; Porter et al., 2004a, 2004b).

4.2.3. Oil solutions

In contrast to the results obtained with SMEs, in vivo data obtained from the three oil solutions show no statistical differences in $AUC_{0-8\,h}$ and C_{max} of ATT + ATX. The in vivo drug performance of oil solutions is not in line with the in vitro drug distribution profiles. We discovered that the T_{max} values of ATT + ATX from oil solutions were longer than from SMEs, indicating that lipid digestion and drug dissolution from oil solutions are slower than from SMEs. This result is in accord with in vitro digestion data. We think that the low digestion rate of oil solutions may explain the similar in vivo behavior of the different oil solutions. Low lipid digestion rates are likely to retard drug dissolution from oil solutions whereas drug diffusion into enterocytes can be rapid (the drug is highly permeable). Slow dissolution and rapid absorption combine to reduce drug concentration and precipitation in the intestine. We believe that there may be little drug precipitated from oil solutions. Ultimately, all drug distributes into the aqueous phase and can be absorbed, leading to similar bioavailability from the three formulations. The difference between SME and oil solution formulations may be explained by differences in lipid digestion rates.

These considerations may also explain our finding that drug absorption from MCT and SCT oil solutions was better than from the corresponding SME formulations. It may not be concluded that low particle size inevitably leads to better bioavailability because the performance of lipid-based delivery systems is governed by their fate in the gastrointestinal tract, rather than purely by the particle size of the initial dispersion (Cuine et al., 2008). It remains possible, in some cases, that reduced precipitation could increase drug absorption.

4.3. Limitations of using rats to predict drug absorption from lipid-based formulation

There are some limitations of using rats to predict the effect of lipid-based formulations on oral bioavailability of poorly water-soluble drugs. Lipid digestion is a very important process for the drug dissolution and absorption of lipid-based formulations. Rats do not have a gall bladder which stores and releases bile to facilitate lipid digestion and drug solubilization after administration of lipid. This causes the digestion profile in rats different from humans. Consequently, the effect of lipid-based formulation on drug absorption in rats may be different from humans. Another notable point is the lipid dose. Studies in rats often administer 100–500 mg lipids (Porter et al., 2004b). In this study, the lipid dose administered to rats is about 200 mg, which is a reasonable dose, but if calculated by body weight, 200 mg to rats is equivalent to 50 g of lipid to humans. This is greater than the amount of lipid generally included in lipid formulations for human use. Overall, advanced study may be needed to get better extrapolation results for humans.

5. Conclusion

Lipid-based formulations significantly improved the oral bioavailability of the poorly water-soluble and lipophilic drug ATT. The study also revealed, for this BCS class II drug, that both lipid composition and formulation influence the drug bioavailability. Lipid composition was found to influence drug solubilization behavior, while formulation affected the lipid digestion rate. These two factors may influence the drug precipitation profile in vivo, and therefore directly influence drug absorption. We conclude that both lipid type and formulation need to be taken into account when developing preparations that maximize the bioavailability of lipophilic drugs.

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