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Preparation and evaluation of self-microemulsifying drug delivery systems (SMEDDS) containing atorvastatin

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Abstract

Atorvastatin is insoluble in aqueous solution and the bioavailability after oral administration is low. Self-microemulsifying drug delivery systems (SMEDDS) containing atorvastatin have been successfully prepared to improve its bioavailability. SMEDDS is a mixture of lipid, surfactant, and cosurfactant, which are emulsified in aqueous medium under gentle digestive motility in the gastrointestinal tract. Pseudo-ternary phase diagrams composed of various excipients were plotted. Droplet size, zeta-potential and long-term physical stability of the formulations were investigated. The release of atorvastatin from SMEDDS capsules was studied using the dialysis bag method in 0.1 M HCl and phosphate buffer (pH 7.4), compared with the release of atorvastatin from a conventional tablet. A pharmacokinetic study was performed in 6 beagle dogs after oral administration of 6 mg kg^{-1} atorvastatin. The bioavailability of atorvastatin SMEDDS capsules was significantly increased compared with that of the conventional tablet. SMEDDS capsules consisting of Labrafil, propylene glycol and Cremophor RH40 provided the greatest bioavailability. Our studies indicate that the use of SMEDDS for the delivery of atorvastatin can improve its bioavailability.

Introduction

Some drugs with a good clinical therapeutic effect often have low systemic availability because of poor water solubility and poor absorption. Many approaches to improve oral bioavailability have been researched, such as salt forming techniques, complexation (i.e. cyclodextrins), particle size reduction, solubilization based on cosolvent or surfactant, etc. Self-microemusifying drug delivery systems (SMEDDS) have received great attention recently for its potential in improving oral bioavailability for the delivery of poorly water-soluble drugs (Charman et al 1992; Humberstone & Charman 1997; Lawrence & Rees 2000; Gursoy et al 2004).

SMEDDS formulation is composed of lipid, surfactant and cosurfactant, with or without water. The system has the ability of forming oil-in-water (o/w) microemulsion when dispersed by aqueous phase under gentle agitation. The agitation required for self-emulsification comes from the digestive motility provided by the movement of stomach and intestine in the gastrointestinal tract (Pouton 1985, 1997, 2000; Holm et al 2003). SMEDDS present drugs in small droplet size and well-proportioned distribution, and increase the dissolution and permeability (Constantinides 1995; New & Kirby 1997; Kawakami et al 2002). Furthermore, since drugs can be loaded in the inner-phase and delivered by lymphatic bypass share, SMEDDS protect drugs against hydrolysis by enzymes in the gastrointestinal tract and reduce the presystemic clearance in the gastrointestinal mucosa and hepatic first-pass metabolism (Embleton & Pouton 1997; Porter & Charman 1997; Wilson et al 1997; Gershanik et al 1998; Gershanik & Benita 2000; Westesen 2000; Hu et al 2001; O'Driscoll 2002; Kossena et al 2004). The principle of self-emulsification is still the subject of speculation. Various models, and speculation about the absorption in the gastrointestinal tract, have been proposed to improve bioavailability of water-insoluble compounds caused by lipidic excipients, such as altering the gastrointestinal motility, increasing bile flow and mesenteric lymph flow, etc. (MacGregor et al 1997; Charman 2000; Agoram et al 2001; Shen et al 2001; Wagner et al 2001). Lipid-based drug delivery systems have been developed to overcome

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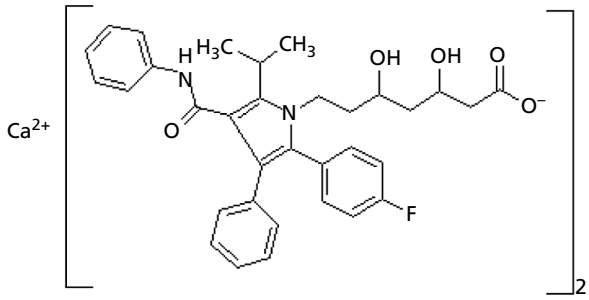

Figure 1 Structure of atorvastatin calcium.

the possible adverse influence of P-glycoprotein (Porter & Charman 2001; Wasan 2001). On all accounts, SMEDDS can improve oral bioavailability significantly.

Atorvastatin is an inhibitor of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase, which catalyses the conversion of HMG-CoA to mevalonate, an early and rate-limiting step in sterol biosynthesis (Malhotra & Goa 2001). Atorvastatin is a monocarboxylic acid with a pK_a of 4.46 and is commonly used as atorvastatin calcium. Atorvastatin calcium is [R-(R*,R*)]-2-(4-fluorophenyl)-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1*H*-pyrrole-1-heptanoic acid hemicalcium salt (Figure 1). Atorvastatin calcium is a crystalline powder and is insoluble in aqueous solution of pH 4 and below; it is very slightly soluble in water and pH 7.4 phosphate buffer. The solubility in aqueous solution of pH 2.1 is about 20.4 μg mL^{-1}, while the solubility in aqueous solution of pH 6.0 is about 1.23 mg mL^{-1} (Kearney et al 1993). Atorvastatin is rapidly absorbed after oral administration; the T_{max} is about 1–3 h and the bioavailability is just 14%. The low systemic availability is attributed to presystemic clearance in gastrointestinal mucosa and hepatic first-pass metabolism (Cilla et al 1996; Lennernas 2003).

In this study, atorvastatin SMEDDS formulations containing lipid, surfactant and cosurfactant were developed successfully and the physicochemical characteristics were evaluated in-vitro and in-vivo. The solubility of atorvastatin in various excipients was analysed. Pseudo-ternary phase diagrams composed of lipid, cosurfactant, surfactant and water were mapped and the region of microemulsion occurring was plotted. Droplet size and size distribution, zeta-potential and long-term physical stability were investigated in detail. The morphology and droplet size/distribution of atorvastatin microemulsion were observed by transmission electron microscope photograph. The release profile of atorvastatin from SMEDDS capsules in 0.1 M HCl and phosphate buffer (pH 7.4) was studied using the dialysis bag method, which was compared with the release profile of atorvastatin from the conventional tablet. The pharmacokinetic study was performed by oral administration of 6 mg kg^{-1} atorvastatin to 6 beagle dogs in different formulations. The oral bioavailability of atorvastatin in SMEDDS capsules was significantly more than that of the conventional tablet. Our study indicates that SMEDDS formulations consisting of labrafil, propylene glycol and Cremophor RH40 are optimal. SMEDDS show good potential to improve oral bioavailability for the delivery of atorvastatin.

Materials and Methods

Materials

Atorvastatin calcium was supplied by Honghui Biopharmaceutical Co. (Beijing, China). Indometacin was provided by the National Institute for the Control of Pharmaceuticals and Biological Products of China (Beijing, China). Lipitor was from Pfizer Pharmceutical Co. (Dalian, China). Caprylocaproyl macrogol-8 glyceride (Labrasol), diethylene glycol monoethyl ether (Transcutol), oleoyl macrogol-6 glycerides (Labrafil), caprylic/capric triglyceride (Labrafac) and glyceryl monolinoleate (Maisine) were supplied by Gattefosse Co. (France). Cremophor RH40 and Cremophor EL were supplied by BASF Co. (Germany). Propylene glycol dicaprylate/caprate (Estol) and isopropyl myristate were supplied by Uniqema Co. (France). Tween 80, castor oil, glycerol, PEG 400, ethanol, propylene glycol, n-butanol and ammonium acetate were purchased from Shanghai Reagent Inc. (Shanghai, China). Acetonitrile and methanol were HPLC grade and purchased from Burdick & Jackson Co. (Muskegon, MA). The purified water was filtered through the Milli-Q UV-Plus purification system from Millipore Inc. (18MV-cm, Milford, MA). All other chemicals and solvents were of analytical grade.

HPLC analysis of atorvastatin in-vitro

The concentration of atorvastatin was determined by HPLC analysis. This was carried out using a Waters 2690 system consisting of an Alliance 2690 pump, Waters 2487 UV detector, Millenium 32 chromatography work station and autosampler (Waters Inc, MA). The chromatographic column was Kromasil C_{18} (150 mm × 4.6 mm, 5 μm) at ambient temperature (25°C). The mobile phase was acetonitrile–0.05% acetic acid solution (65:35). A UV detector was set at λ 248 nm.

The SMEDDS capsule dispersions were dissolved in methanol to precipitate the excipients sufficiently. After the sample was centrifuged at 12 000 rev min^{-1} for 15 min, the concentration of atorvastatin in the supernatants was determined by the above-mentioned HPLC analysis.

Solubility studies of atorvastatin in various excipients

The solubility of atorvastatin in various lipids, surfactants and cosurfactants was determined. An excess amount of atorvastatin was introduced to 2 mL of each excipient and the mixture in a capped cuvette was stirred in a water-bath at 25°C for 48 h; a vortex mixer was used to facilitate the solubilization if necessary. After standing for 24 h and reaching equilibrium at ambient temperature, each cuvette was centrifuged at 3000 rev min^{-1} for 10 min using a centrifuge (Sigma 3K15; Sigma Co., USA). Undissolved atorvastatin was removed by filtering with a membrane filter (0.45 μm). The concentration of atorvastatin was determined by the above-mentioned HPLC analysis.

Preparation of pseudo-ternary phase diagram

The pseudo-ternary phase diagrams consisting of lipid, surfactant, cosurfactant and water were developed using the water

titration method. We selected 4 types of non-ionic surfactant, namely Cremophor EL, Cremophor RH40, Tween 80 and Labrasol, combined with 4 types of solubilizer as cosurfactants (ethanol, propylene glycol, PEG 400 and Transcutol). Lipids employed were Labrafil, Labrafac, Estol and IPM. Surfactant was blended with cosurfactant in the ratio of 1:2, 1:1, 2:1, 3:1 (i.e. K_m, w/w). Volumes of each surfactant and cosurfactant mixture (S_{mix}) were blended with lipid in a ratio of 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 (w/w), then water was folded in a drop-wise manner to each lipid–S_{mix} mixture under gentle shake at 37°C. After equilibrium, the appearance and dispersibility of the formulation was observed and droplet size/distribution and zeta-potential were analysed. So it was distinguishable between the microemulsion which was clear and slight blue and the crude emulsion which had a white appearance. The amount of water, lipid, surfactant and cosurfactant folded was noted down and calculated. The pseudo-ternary phase diagrams were mapped with Origin 7.0 according to the data. The microemulsion region in the diagrams were plotted and the wider region indicated the better self-microemulsification efficiency.

Preparation of atorvastatin SMEDDS formulations

After the pseudo-ternary phase diagrams were plotted and compared, optimal surfactant, cosurfactant and lipid combinations were selected. Atorvastatin SMEDDS formulations were prepared by firstly dissolving atorvastatin into cosurfactant or S_{mix} in a glass cuvette, heating at 37°C in a water-bath or using a vortex mixer to facilitate the solubilization if necessary, then adding the required weight of lipid into the cuvette and mixing. The mixture was filled in capsules (Licaps; Suzhou Capsugel Ltd, Suzhou, China). The capsules were tightly sealed and stored at ambient temperature (25°C) until used.

Preliminary assessment of self-microemulsification efficiency in-vitro

SMEDDS concentration was diluted with medium and then visualized. Purified water (20 mL) or 0.1 M HCl (20 mL) was added in a drop-wise manner to SMEDDS concentration (0.2 mL) in a volumetric flask at 25 or 37°C under gentle shaking. After equilibrium, the time of self-microemulsification, dispersibility, appearance and flow ability was observed and scored according to the five grading systems shown in Table 1 (Pouton 1985; Khoo et al 1998; Gershanik & Benita 2000; Kang et al 2004). Water compared with 0.1 M HCl was chosen as diluting medium to evaluate the effect of pH on the self-microemulsification efficiency. Furthermore, the self-microemulsification efficiency dispersing at 37°C was compared with that at 25°C.

After SMEDDS concentration was dispersed, the stability was assessed by visualizing periodically the phase separation or precipitation occurring, and analysing the change in droplet size.

Determination of droplet size/distribution and zeta-potential

Atorvastatin SMEDDS concentration (approximately 0.2 mL) was diluted with purified water (20 mL) or 0.1 M HCl (20 mL) and gently shaken in a volumetric flask at 25 or 37°C. The droplet size/distribution and zeta-potential were analysed by dynamic light scattering with particle sizing apparatus (Nicomp 388 ZLS; PSS Nicomp Particle Sizing Systems, USA).

Table 1 Visual assessment of efficiency of self-microemulsification

Grade	Dispersibility and appearance	Time of self-microemulsification
I	Rapid forming microemulsion which is clear or slightly bluish in appearance	<1 min
II	Rapid forming, slightly less clear emulsion which has a bluish white appearance	<2 min
III	Bright white emulsion (similar to milk in appearance)	<3 min
IV	Dull, greyish white emulsion with a slightly oily appearance that is slow to emulsify	>3 min
V	Exhibits poor or minimal emulsification with large oil droplets present on the surface	>3 min

Transmission electron microscopy photograph of atorvastatin microemulsion

After the atorvastatin SMEDDS concentration was dispersed with water and turned into microemulsion, the sample was negatively stained and the morphology of the microemulsion was photographed by transmission electron microscopy (TEM); the droplet size/distribution was also observed (performed by the Electron Microscope Laboratory of Fudan University).

Stability study

The stability was assessed by analysing droplet size and distribution at 0.2, 1, 10, and 24 h after SMEDDS formulation was dispersed. The optimal atorvastatin SMEDDS formulations filled in capsules were tightly sealed for storage at ambient temperature (25°C) for one year. The content of atorvastatin and droplet size were determined at predetermined intervals.

Drug release profile in-vitro

The release profile of atorvastatin SMEDDS capsules was performed using the dialysis bag method according to dissolution apparatus 2 in USP 24. Atorvastatin SMEDDS capsules (atorvastatin 10 mg) placed in the dialysis bag (MWCO 12 000; Spectrum, USA) were fixed in a dissolution vessel (ZRS-8G dissolution apparatus; Tianjin University Radio Factory, Tianjin, China) and shaken at 100 rev min^{-1} in a 37 ± 0.5°C water bath. Release medium (900 mL) was added outside the dialysis bag. At predetermined intervals, a 1-mL sample was withdrawn and the concentration of atorvastatin in the filtrate was determined by the HPLC analysis of atorvastatin in-vitro as mentioned above; the removed volume was replaced by fresh medium. Simulated gastric fluids (0.1 M HCl) and simulated intestinal fluids (pH 7.4 phosphate buffer) were used as release medium to evaluate the effect of pH on the release profile. The release profile of SMEDDS

was compared with that of the conventional tablet (Lipitor, atorvastatin 10 mg).

HPLC analysis of atorvastatin in beagle dog plasma

The concentration of atorvastatin in beagle dog plasma was determined by HPLC. The HPLC system (Shimadzu Inc., Japan) consisted of a LC-10AD pump, SIL-10A autoinjector and SPD-10A UV detector. Data were collected and analysed by Class LC-10 software (version 1.63; Shimadzu, Japan). The chromatographic column was Kromasil C_{18} (150 mm × 4.6 mm, 5 μm) at ambient temperature (25°C). The mobile phase consisted of acetonitrile–0.1 M ammonium acetate buffer, pH 4.0 (50:50). The detection wavelength was 270 nm. Indometacin was used as the internal standard.

Oral bioavailability study in beagle dogs

Oral bioavailability study in beagle dogs was performed by determining the concentration of atorvastatin in blood samples following oral administration. Six healthy male beagle dogs (supplied by the Laboratory Animal Center of Fudan University), 12–14 kg, fasted for 24 h before the experiment, were allocated to four groups at random. Beagle dogs were administered atorvastatin SMEDDS capsule A, C and E and the conventional tablet within four periods of experiment; the washout interval between the administrations was kept at 7 days.

Blood samples (3 mL) were collected from the vein of four limbs into heparinized tubes at the following times: immediately before administration, and 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12 and 24 h after administration. The blood samples were immediately centrifuged at 3000 rev min^{-1} for 10 min at 4°C. Plasma samples were collected in capped tubes and stored at −20°C until assay. Frozen plasma samples were thawed at room temperature just before assay. Indometacin (200 μL; 0.1 μg mL^{-1} in acetonitrile) used as the internal standard was added into 0.5 mL plasma sample, 2 mL ice-cold acetonitrile was then added and vibrated for 2 min vigorously to precipitate protein. After centrifugation, the upper acetonitrile layer was transferred to a clean tube and the separated organic mixture was taken to near dryness under a stream of nitrogen at room temperature. The residue was reconstituted in 200 μL of 50% acetonitrile (v/v) and a 25-μL fraction was injected for HPLC analysis. Ethical committee approval for the study was granted by the host institution.

Statistical analysis of the pharmacokinetic data

Statistical analysis of the pharmacokinetic data was performed based on a non-compartmental model with WinNonlin (version 4.1; Pharsight Inc., Mountain, CA). Data from the plasma concentration–time curve within 24 h after drug intake were used to obtain the peak plasma concentration (C_{max}, ng mL^{-1}) and time of peak plasma concentration (T_{max}, h) for individual formulations. The area under the plasma concentration–time curve ($AUC_{0\rightarrow 24\,h}$) was calculated using the linear trapezoidal method. The relative bioavailability (F_r) of the SMEDDS capsules to the conventional tablet with the same dose was calculated as: $F_r = [AUC_{cap\ (0\rightarrow 24\,h)}/AUC_{tab\ (0\rightarrow 24\,h)}] \times 100\%$. The pharmacokinetic parameters were analysed statistically by Kruskal–Wallis test using SPSS software (version 12.0; SPSS Inc., USA). Data were expressed as means ± s.d.

Results and Discussion

Solubility of atorvastatin in excipients

The concentration of atorvastatin in various excipients at 25°C was determined by HPLC and presented in Table 2. Atorvastatin should be soluble in the excipient of SMEDDS formulation. Since the solubility of atorvastatin in propylene glycol was much more than that in other excipients, propylene glycol was taken as a good cosurfactant to prepare atorvastatin SMEDDS formulations.

Study of excipients in SMEDDS formulations

Generally, lipids with smaller molecular volume can penetrate the interfacial monolayer of the surfactant in the same way as that of the cosurfactant. Lipids commonly used in lipid-based formulations are medium- or long-chain glycerides (such as mono-, di- and triglycerides, namely MCT, MCM and LCT), such as Labrafac (i.e. C_8:C_{10} triglyceride), Estol (i.e. C_8:C_{10}), Labrafil (i.e. C_{18} mono-, di- and triglyceride with oleic fatty acids) and Maisine (C_{18} glycerol monolinoleate), which have often been used in studies (Charman et al 1992; Malcolmson et al 1998). Although the decrease in the interfacial tension plays an important role in forming a microemulsion, the surface tension of the lipids are nearly the same; the polarity of the lipids decrease with an increase in the number and length of the alkyl chain. Lipids with high polarity seem to be adequate to form a microemulsion (Kawakami et al 2002).

The efficiency of self-microemulsification is much related to the HLB hydrophilic–lipophilic balance value of the surfactant. Generally, surfactants with HLB 12–15 are regarded as being of good efficiency. Considering the safety and biocompatibility of the excipient, we selected several nonionic surfactants, namely Cremophor EL (HLB 12–14), Cremophor RH40 (HLB 12–14), Tween 80 (HLB 15) and Labrasol (HLB 14), combined with ethanol, propylene glycol, glycerol, PEG 400, mannitol, n-butanol or Transcutol as cosurfactant. In our preliminary study, although

Table 2 Solubility of atorvastatin in various excipients

Excipient	Solubility (mg mL^{-1})	Excipient	Solubility (mg mL^{-1})
H_2O (pH 2.1)	0.02 ± 0.52	Transcutol	141.12 ± 3.67
H_2O (pH 6.0)	1.23 ± 0.81	Propylene glycol	175.99 ± 2.08
Maisine	1.02 ± 1.44	Glycerol	4.54 ± 2.50
Castor oil	8.35 ± 2.50	PEG 400	41.92 ± 2.42
Labrafil	15.14 ± 2.55	Ethanol	48.89 ± 1.43
Labrafac	11.16 ± 3.21	Cremophor EL	14.92 ± 2.21
Estol	12.69 ± 1.47	Cremophor RH40	20.62 ± 3.78
IPM	16.83 ± 3.25	Tween 80	40.80 ± 3.42

Data are means ± s.d., n = 6.

Labrasol qualified by the HLB value, when it was mixed with other lipids and cosurfactants used in our test there were a lot of large oil droplets presenting on the surface of the mixture and each component of the formulation was separated after standing for a while. Cremophor EL and Cremophor RH40 exhibited better microemulsification efficiency than Tween 80 and were selected to prepare the formulation. When mixed with the aforementioned lipids and cosurfactants, the mixture containing Tween 80 as surfactant showed a coarse emulsion with a white appearance and bulky sized droplets in most cases.

The microemulsification efficiency was also affected by cosurfactant. For example, the microemulsification efficiency changed with the chain length of cosurfactant. n-Butanol is not fit for oral administration because of unpleasant odour, although it has good microemulsification efficiency with many excipients. The formulations composed of Cremophor EL or Cremophor RH40 as surfactants, combined with ethanol, propylene glycol or PEG 400 used as cosurfactants, and Labrafil, Estol, or Labrafac as lipids, having good microemulsification efficiency, could form microemulsions and were selected for the following study.

Preparation of pseudo-ternary phase diagrams

The pseudo-ternary phase diagrams were mapped with the water titration method to identify the area of microemulsion regions at 37°C. The purified water was used as diluting medium and added into the formulation. The proper ratio of one excipient to another in the SMEDDS formulation was analysed. Several formulations with different lipid and K_m values (the ratio of surfactant to cosurfactant) were dispersed with water at 37°C. The pseudo-ternary phase diagrams of the formulation composed of Labrafil, Cremophor RH40 and propylene glycol, scaling with different K_m, are shown Figure 2. The shadow area represents the o/w microemulsion existence region. The size of the microemulsion region in the diagrams was compared, the larger the size the greater the self-microemulsification efficiency. The size of the micro-

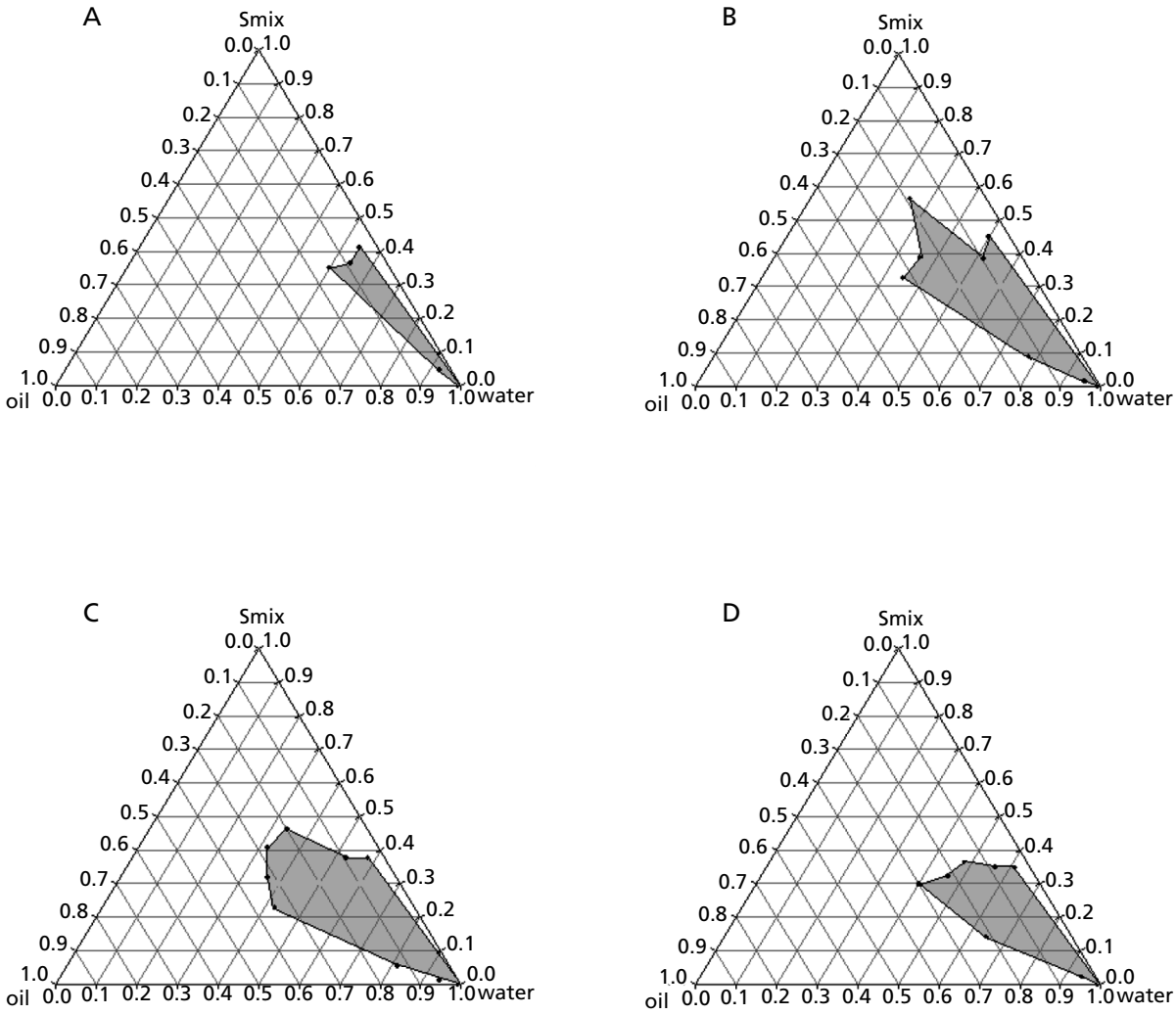

Figure 2 Pseudo-ternary phase diagrams of the formulation composed of Labrafil:Cremophor RH40:propylene glycol dispersed with water at 37°C. K_m = 1:2 (A), 1:1 (B), 2:1 (C) and 3:1 (D) (w/w). The shadow area represents o/w microemulsion region.

emulsion region of the formulation with Labrafil as lipid was larger than that of the others. The optimal ratio of the excipients in the formulation was also determined from the phase diagrams. When K_m was 1, the microemulsion region had the largest size.

Assessment of self-microemulsification efficiency in-vitro

After the pseudo-ternary phase diagrams were plotted and the size of the microemulsion region was compared, Cremophor EL and Cremophor RH40 were selected as surfactant, combined with propylene glycol as cosurfactant; the lipids employed were Labrafil, Labrafac or Estol. SMEDDS formulations A–F and the assessment in-vitro after dispersing with medium are represented in Table 3.

The self-microemulsification efficiency in-vitro was evaluated based on the time of self-microemulsification, dispersibility, the droplet size/distribution, zeta-potential and formulation stability. Generally speaking, the droplet size and zeta-potential are the important parameters of the colloid system, which indicate the static electricity repulsion and congregation of the droplets (Pouton 1985; Gershanik et al 2000; Kang et al 2004). The droplet size of the microemulsion containing Cremophor RH40 was smaller than that of the microemulsion containing Cremophor EL. The droplet size of the microemulsion containing Labrafil as lipid, Cremophor RH40 as surfactant and propylene glycol as cosurfactant was within 70 nm and showed Gaussian distribution, which was the smallest of all the formulations. There were minor differences in mean droplet size between diluting with water and with 0.1 M HCl.

The effect of the concentration of atorvastatin on the droplet size was investigated after SMEDDS formulation A was dispersed with purified water at 37°C. The droplet size increased from 30 nm to 270 nm when the concentration of atorvastatin added increased from 2% to 12%. The droplet size changed a little with the concentration of atorvastatin when it was less than 4%. The droplet size increased greatly when the concentration of atorvastatin was more than 5%. When the concentration of atorvastatin was more than 9%, the droplet size was larger than 200 nm and the mixture showed a bluish white appearance.

The effect of the concentration of lipid on the droplet size was investigated after SMEDDS formulation A was dispersed with purified water at 37°C. The droplet size increased from 28 nm to 260 nm when the concentration of lipid added increased from 10% to 45%. When the concentration of lipid was more than 35%, the droplet size of the mixture was larger than 110 nm.

The effect of the dispersing medium on zeta-potential was investigated when SMEDDS formulations A–F were dispersed with water and 0.1 M HCl. There were minor difference in zeta-potential between the two when dispersed at the same dilution times. When the dilution time was more than 500-fold, zeta-potential seemed to be unchanged. However, when the dilution time was less than 500-fold, zeta-potential changed with the dilution time. So the determination of zeta-potential should be performed at the same dilution times.

It was seen from Table 3 that formulations A, C and E, consisting of Cremophor RH40 as surfactant and propylene glycol as cosurfactant, with Labrafil, Estol or Labrafac as lipid, had good self-microemulsification efficiency. Besides, the droplet size of formulation A was the smallest among the three after dispersing.

Table 3 Composition and assessment of SMEDDS formulations

	A	B	C	D	E	F
Composition of SMEDDS formulations						
Atorvastatin (g)	0.05	0.05	0.05	0.05	0.05	0.05
Labrafil (g)	0.31	0.31	—	—	—	—
Estol (g)	—	—	0.31	0.31	—	—
Labrafac (g)	—	—	—	—	0.31	0.31
Cremophor RH40 (g)	0.32	—	0.32	—	0.32	—
Cremophor EL (g)	—	0.32	—	0.32	—	0.32
Propylene glycol (g)	0.32	0.32	0.32	0.32	0.32	0.32
Assessment of SMEDDS diluted with purified water						
Grade	I	II	I	I	I	II
Droplet size (nm) (after 0.15 h)	72 ± 4.7	105 ± 3.3	84 ± 4.0	99 ± 2.6	75 ± 3.9	106 ± 2.8
Droplet size (nm) (after 10 h)	75 ± 3.8	103 ± 3.5	88 ± 4.2	105 ± 4.7	77 ± 3.3	102 ± 4.1
Zeta-potential (mV) (after 0.15 h)	−12.2 ± 1.4	−9.3 ± 1.0	−10.8 ± 2.7	−9.9 ± 2.1	−9.5 ± 1.2	−6.9 ± 1.3
Zeta-potential (mV) (after 10 h)	−10.8 ± 1.1	−7.9 ± 1.3	−12.3 ± 2.4	−8.3 ± 1.5	−8.4 ± 1.5	−7.6 ± 0.9
Assessment of SMEDDS diluted with 0.1 M HCl						
Grade	I	I	I	II	I	II
Droplet size (nm) (after 0.15 h)	70 ± 2.4	99 ± 3.6	82 ± 2.5	104 ± 2.9	72 ± 1.2	110 ± 3.8
Droplet size (nm) (after 10 h)	72 ± 2.0	98 ± 1.4	80 ± 1.9	117 ± 3.0	74 ± 2.8	113 ± 1.6
Zeta-potential (mV) (after 0.15 h)	−12.6 ± 0.7	−8.3 ± 1.2	−10.8 ± 0.4	−7.7 ± 0.6	−8.6 ± 0.9	−7.7 ± 1.6
Zeta-potential (mV) (after 10 h)	−11.4 ± 1.8	−9.6 ± 0.5	−9.9 ± 1.1	−6.9 ± 1.0	−9.3 ± 1.7	−9.0 ± 1.3

Data are means ± s.d., n = 3.

Transmission electron microscopy (TEM) photograph of atorvastatin microemulsion

When atorvastatin SMEDDS was dispersed with water, it turned into atorvastatin microemulsion. The morphology of the microemulsion was photographed with a transmission electron microscope. Droplet size and distribution of the microemulsion was also examined from the TEM photograph. The average droplet size of microemulsion dispersed from formulation A was within 100 nm and showed Gaussian distribution. It was consistent with the data analysed using particle sizing apparatus.

Stability study

There was no obvious change in the droplet size of SMEDDS formulations dispersed with the medium after standing for 24 h. The data of droplet size and zeta-potential after standing for 0.15 h and 10 h are listed in Table 3. After 1 year's storage, there was no major change in the content of atorvastatin and droplet size of microemulsion dispersed from the above formulations (data was not listed).

In-vitro dissolution study

Based on the aforementioned study, dissolution study of capsules filled with three optimal SMEDDS (i.e. formulations A, C and E) and the conventional tablets was performed. The release profile of atorvastatin was investigated in simulated gastric (pH 1.2) and intestinal fluid (pH 7.4) to evaluate the effect of pH on the release of atorvastatin. Dissolution profiles of atorvastatin from formulation A, C and E capsules and the conventional tablets in simulated intestinal fluid (pH 7.4) are represented in Figure 3. The release of formulation A was a little faster than that of formulations C and E. There were minor differences in the release among the three SMEDDS formulations, which released more and faster than the conventional tablet. It was estimated that atorvastatin was dissolved perfectly in SMEDDS formulations with very small droplet size, so it could be released much more rapidly than from the tablet. It was suggested that dissolving atorvastatin in SMEDDS and reducing droplet size could facilitate the release of atorvastatin from the formulation. However, the content of atorvastatin in the SMEDDS was greater than that in the tablet after 24 h, when the dissolution had nearly reached equilibrium. The drug release was less than 80% at 8 h in all formulations, which was a complicated process, especially for the SMEDDS. It might be produced by the release rate and the design of the dissolution study, which needs more research.

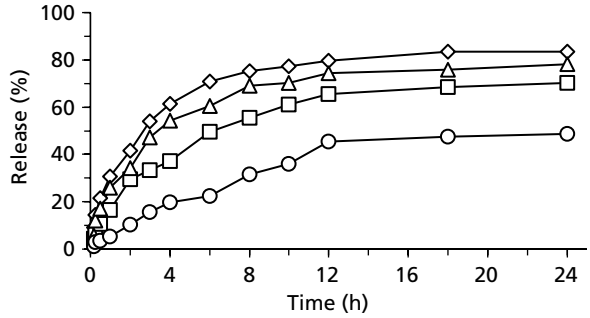

Figure 3 Dissolution profile of atorvastatin from SMEDDS formulation A (diamonds), C (squares), E (triangles) and the conventional tablet (circles) in simulated intestinal fluid (pH 7.4).

Bioavailability study

The conventional tablets and the capsules filled with the three SMEDDS formulations, consisting of Cremophor RH40 and propylene glycol, with three lipids, Labrafil, Estol or Labrafac, respectively, were used for the bioavailability study. Pharmacokinetic parameters and the relative bioavailability (F_r) of atorvastatin after oral administration of the four formulations to beagle dogs are shown in Table 4. The plasma profiles of atorvastatin in beagle dogs following oral administration of the different formulations are represented in Figure 4. The C_{max} and $AUC_{0\rightarrow24\,h}$ of the SMEDDS were significantly higher than those of the tablet. The T_{max} of the SMEDDS was less than that of the tablet. The relative bioavailability of atorvastatin in formulation A was about 1.5 fold compared with the tablet. This might be the solubilization and droplet-size reduction produced by SMEDDS. SMEDDS could increase the oral bioavailability of atorvastatin. It might be a promising approach for rapid onset and effective absorption with oral administration of atorvastatin. Although the effect of SMEDDS on the absorption of drug has not been clarified, and evaluating methods in-vitro are still in its infancy, the progress of SMEDDS has been greatly advanced by the achievements involved in models simulating the release and absorption in the gastrointestinal tract (Charman et al 1992; Constantinides 1995; Gursoy & Benita 2004). However, further discussion on the relationship between bioavailability and droplet size is needed; maybe the increase in bioavailability was caused by the decrease in droplet size in this study.

Table 4 Pharmacokinetic parameters and bioavailability of atorvastatin after oral administration of different formulations to beagle dogs

Parameter	Formulation A	Formulation C	Formulation E	Conventional tablet
T_{max} (h)	1.25 ± 0.38	1.33 ± 0.37	1.17 ± 0.24	2.17 ± 0.37
C_{max} (ng mL^{-1})	512.98 ± 52.60	446.03 ± 55.91	435.56 ± 62.43	230.88 ± 30.87
$AUC_{0\rightarrow24\,h}$ (ng h mL^{-1})	2612.96 ± 367.64	2568.28 ± 407.96	2520.81 ± 308.40	1738.04 ± 207.86
F_r (%)	150.34 ± 24.35	147.77 ± 27.47	145.04 ± 19.72	100

Data are means ± s.d., n = 6.

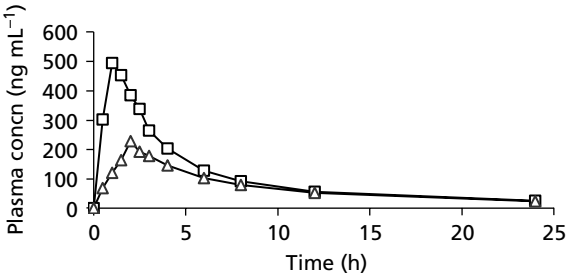

Figure 4 Plasma concentration profile of atorvastatin acid after oral administration of SMEDDS formulation A (squares) and the conventional tablet (triangles) in beagle dogs (n = 6 and 6 mg kg^{-1}).

Conclusion

SMEDDS formulations consist of lipids, surfactants and cosurfactants, which are emulsified by aqueous medium under gentle digestive motility in the gastrointestinal tract. It is considered that the excipients in SMEDDS could increase the dissolution and permeability of drug by significantly decreasing droplet size and restraining the secretion of drug efflux transporter P-gp. Atorvastatin is poorly aqueous soluble. The low bioavailability of atorvastatin is produced by the poor solubility and extensive first-pass metabolism in the gut wall and liver. The use of SMEDDS for the delivery of atorvastatin could improve its solubility and permeability through mucous membranes significantly. In this paper, we prepared atorvastatin SMEDDS formulations and assessed the dissolution in-vitro; an oral bioavailability study in beagle dogs was also performed. We found that SMEDDS might have the potential to advance the oral bioavailability of atorvastatin. The concentration of atorvastatin in various excipients was analysed. Pseudo-ternary phase diagrams composed of lipid–cosurfactant–surfactant–water were mapped, the microemulsion region in each diagram was plotted and compared. The morphology and the droplet size/distribution of atorvastatin microemulsion was observed by transmission electron microscope photograph. Droplet size and distribution, zeta-potential and long-term stability were investigated in detail. Optimal formulations that contained Cremophor RH40 as surfactant, propylene glycol as cosurfactant and Labrafil as lipid can become microemulsions when dispersed with medium. The average droplet size of the optimal formulation is within 100 nm and shows Gaussian distribution. The rate and amount of the release of atorvastatin from SMEDDS capsules were more than those from the conventional tablets in 0.1 M HCl and phosphate buffer (pH 7.4). After oral administration of 6 mg kg^{-1} atorvastatin to 6 beagle dogs, the oral bioavailability of SMEDDS capsules was increased by nearly one and a half times compared with that of the conventional tablets. Our study indicates that the potential use of SMEDDS for the oral delivery of atorvastatin can be an alternative to improve its systemic availability. The development of SMEDDS is promising for improving the oral bioavailability of poorly soluble drugs.

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