Journal of Pharmaceutical Investigation
Vol. 41, No. 2, 103-109 (2011)

Development of Self-microemulsifying Drug Delivery System for Enhancing the Bioavailability of Atorvastatin

Shun-Ji Jin*, Wonkyung Cho*, Heejun Park, Kwang-Ho Cha, Junsung Park, Jaseong Koo, Hunsik Wang, Jeong-Soo Kim, Min-Soo Kim and Sung-Joo Hwang†

Center for Nanotechnology-based New Drug Dosage Form, College of Pharmacy, Chungnam National University, 220, Gung-dong, Yuseong-gu, Daejeon 305-764, Korea

(Received March 25, 2011 · Revised March 29, 2011 · Accepted March 31, 2011)

ABSTRACT – The objective of the study was to prepare self-microeulsifying drug delivery system (SMEDDS) incorporating atorvastatin calcium and evaluate its properties and oral bioavailability. Solubility of atorvastatin in various vehicles was determined. Pseudo-ternary phase diagrams were constructed to identify the good self-emulsification region. The droplet size distributions of the resultant emulsions were determined by dynamic light scattering measurement. The mean droplet size of chosen formulation (20% ethyl oleate, 40% tween-80, 40% Carbitol®) was 23.4±1.3 nm. The SMEDDS incorporating atorvastatin calcium appeared to be associated with better performance in dissolution and pharmacokinetic studies, compared with raw atorvastatin calcium. In dissolution test, the release percentage of atorvastatin from SMEDDS mixture could rapidly reach more than 95% within 3 min. Oral $AUC_{0\rightarrow 8hr}$values in SD rats was 1994±335 ng·hr/mL, which significantly increased ($P<0.05$) compared with raw atorvastatin calcium. The SMEDDS formulation was relatively stable when stored at 4°C during 3 months. Our studies illustrated the potential use of SMEDDS for the delivery of hydrophobic compounds, such as atorvastatin, by the oral route.

Key words – Atorvastatin calcium, SMEDDS, Phase diagram, Bioavailability, Stability

Atorvastatin, as a synthetic lipid-lowering agent, is an inhibitor of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase which catalyzes the conversion of HMG-CoA to mevalonate, an early rate-limiting step in cholesterol biosynthesis(Lennernäs, 2003). Atorvastatin is currently used as calcium salt for the treatment of hypercholesterolemia. Atorvastatin calcium ([R-(R*,R*)]-2-(4-fluorophenyl)-b,d-dihydroxy-5-(1-metyl-etyl)-3-phenyl-4[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid hemi calcium salt, Figure 1) is insoluble in aqueous solution of pH 4 and below; it is very slightly soluble in water and pH 7.4 phosphate buffer. The intestinal permeability of atorvastatin is high at the physiologically relevant intestinal pH(Lennerns, 1997, Wu et al., 2000). However, it is reported that the absolute bioavailability (F) of atorvastatin is 12% after a 40 mg oral dose(Corsini et al., 1999).

However, these chronic disease therapy like hypercholesterolemia, the oral route is especially more pleasant, preferred and convenient than injection or some other forms. Recently, the oral delivery of drugs could be made possibly by SMEDDS, which had been shown to improve oral bioavailability substantially(Khoo et al., 1998, Lattuada et al., 1998, Kang et al., 2004, Attama and Nkemnele, 2005).SMEDDS was a mixture of oils, surfactants, and co-surfactants, which were emulsified and formed fine oil-in-water (o/w) microemulsions in aqueous media under conditions of gentle agitation and digestive motility that would be encountered in the gastro-intestinal (GI) tract(Neslihan Gursoy and Benita, 2004). Compared with emulsion or microemulsion, it is put little or no energy, has improved physical stability, can be filled into soft or hard capsules for convenient oral administration.

Moreover, Kang et al(Kang et al., 2004) had successfully formed SMEDDS containing statin (e.g. simvastatin et al), but atorvastatin has the difference characteristic with other statins because of its water-soluble polarity; it is also a challenge to formulate SMEDDS incorporating atorvastatin. Therefore, our researches aimed at developing and characterizing the optimal formulations of SMEDDS incorporating atorvastatin. And it is always another big challenge to solve the stability of liquid state formulation, so our researches also investigate the stability of SMEDDS incorporating atorvastatin.

*Co-first Author
†Corresponding Author :
Tel : +82-42-821-5922, E-mail : sjhwang@cnu.ac.kr
DOI : 10.4333/KPS.2011.41.2.103

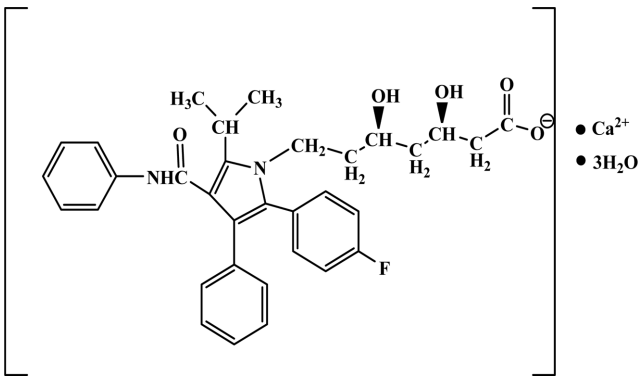

Figure 1. Chemical structure of atorvastatin $(C_{77}H_{34}FN_2O_5)_2$ $Ca{\cdot}3H_2O$

Materials and Methods

Material

Captex 300 and Capmul MCM were obtained from Korea Abitec Corp., Diethylene glycol monoethyl ether were from SIGMA (St. Louis, MO, USA), Labrasol, Labrafill M 1944 CS, and Capryol PGMC were from Gattefosse (Lyon, France), Cremophor EL were from BASF (Ludwigshafen, Germany). The HPLC grade acetonitrile, methanol, sodium dihydrogen phosphate, di-sodium hydrogen phosphate, sodium hydroxide and phosphoric acid were purchased from Merck (Darmstadt, Germany). And all other chemicals in this study were analytical reagent grade.

Solubility test of atorvastatin in various excipient

The saturation solubility of atorvastatin in various excipients was determined. Excess amounts of samples were dissolved in 10 mL of various oils or surfactant or co-surfactants. The sample were sonicated for 10 min and then shaken in water bath maintained at 37.0±0.1°C for 24 hr. A portion of solution was withdrawn and then filtered through a 0.1 μm nylon syringe filter. The filtrate was diluted with methanol, and the quantification of atorvastatin was determined by HPLC. All experiments were triplicated.

Preparation of SEDDS/SMEDDS mixtures

Active drug (Atorvastatin 75 mg) was dissolved in co-surfactant. Oil and surfactant were accurately weighed into glass vials. Then, the components were mixed by gentle stirring and vortex mixing, and heated at 37°C in incubator, until drugs perfectly had dissolved. The mixtures were stored at room temperature until used.

Visually assessment of efficiency of self-emulsification

The *in vitro* performance of formulations was visually assessed as following descriptions(Khoo et al., 1998, Kommuru et al., 2001). SMEDDS mixtures (20 μL) were introduced into 20 mL of water into glass cap vials. Gently mixing with a magnetic stir bar, the tendency to form an emulsion was judged as "good" when droplets spread easily in water and formed a fine milky emulsion and it was judged "bad" when there was poor or no emulsion formation with immediate coalescence of oil droplets, especially when stirring was stopped.

Pseudo-ternary phase diagram investigation

In the study of pseudo-ternary phase diagram, SMEDDS mixtures (20 μL) were diluted with water (20 mL) in vials and gently mixed by vortex mixing. And the time of emulsifying and the phase clarity of these mixtures were observed. These pseudo-ternary phases diagrams were constructed identifying the good self-emulsifying region (the areas represented the microemulsfying areas having clear or slightly bluish).

Emulsion droplet size analysis

In the study of emulsion droplet size analysis, SMEDDS mixtures (20 μL) were diluted with water (20 mL) in vials and gently mixed by glass bar. Then, the droplet size distributions of resultant emulsions were determined by dynamic light scattering measurement using electrophoretic light scattering spectrophotometer (ELS-8000, Potal, Japan).

Dissolution test

Dissolution profiles of the self-microemulsified formulations (atorvastatin 10 mg) and raw atorvastatin powder were performed using VK 7000 dissolution testing station and VK 750d heater/circulator (Vankel, USA) according to the USP XXXIV paddle method. The stirring speed used was 50 rpm, and the temperature was maintained at 37.0±0.1°C. Each test was carried out in 900 mL of distilled water. It was accurately weighted SMEDDS mixtures and raw atorvastatin powders into soft capsule.Then they were placed in the dissolution medium. After that, 4 mL of aliquot samples were withdrawn in certain time intervals and filtered using a 0.1 μm nylon syringe filter. At each sampling time, an equal volume of the test medium was replaced. Filtered samples were appropriately diluted with methanol and assayed for drug concentration by HPLC.

Pharmacokinetic study in rats

Animals

Male SD rats (6-7 weeks old) weighing between 180 and 200 g were purchased from Samtaco Bio Korea Inc. (Korea). All rats had free access to tap water and pelleted diet. The rats

were housed in a cage and maintained on a 12 hr light/dark at room temperature (25°C) and relative humidity of 55±10%. General and environmental conditions were strictly monitored. All animal experiments were performed according to the "Guidelines for the Care and Use of Laboratory Animals" at Chungnam National University. The rats were deprived of food 24 hr before the experiment and food was reoffered 4 hr post-dosing.

Experimental design

The rats were divided into three groups of five animals each. Each group was administrated orally 1 mL of 0.2 (w/v)% methylcellulose suspensions containing raw atorvastatin calcium (Group 1), 1 mL of microemulsion containing SMEDDS mixture (1) (ethyl oleate:Tween-80:Carbitol®=20:40:40) (Group 2), 1 mL of microemulsion containing SMEDDS mixture (2)[Capryol PGMC: (CremphorEL:Tween-20=1:1):Carbitol®= 20:40:40] (Group 3), which equivalent to 25 mg/kg of atorvastatin. Blood samples (approximately 300 μL each) were collected by tail vein; predose, 15, 60, 120, 180, 240, 480 min post-dosing. Blood samples were held on ice (+4°C) until centrifuged at 10000 rpm, 4°C for 10 min. Plasma was transferred to individual Eppendorfs tube and stored at 20°C until analyzed.

Plasma concentration of atorvastatin by LC/MS analysis

A plasma sample (100 μL) was spiked with 50 μL of internal standard solution (methaqualone, 100 ng/mL) and 350 μL of acetonitrile was added. After vortex mixing for 30 s, 400 μL of supernatant was carefully transferred to a glass test tube and evaporated to dryness using a centrifugal evaporator (CVE-2000, Eyela, Japan). Then the dried residual was reconstituted in 100 μL of methanol and a 5 μL aliquot was injected into the LC/MS system for the quantification of atorvastatin. The LC/MS system consisted of a LC-10ADvp pump, SIL-10A autoinjector, SPD-10ADvp UV detector, and LCMS-2010A mass spectrometer (Shimadzu, Japan). The mobile phase consisted of 0.1 M ammonium acetate buffer (pH 4.0)/acetonitrile (50:50, v/v) was pumped at a flow rate of 1.0 mL/min. The analytical column was Kromasil C18 (150×4.6 mm, 5 μm) and the detection wavelength was 270 nm. The mass spectrometer was operated in positive ion mode and connected to the chromatographic system using an API electrospray interface. The $[M+H]^+$, m/z 251.00 for methaqualone and $[M+H]^+$, m/z 559.00 for atorvastatin were selected as detecting ions, respectively (Hermann et al., 2005, Nirogi et al., 2006). The MS operating conditions were optimized as follows: drying gas 1.5 L/min, CDL temperature 250°C, block temperature 200°C and probe voltage: +4.5 kV. Data processing was performed using the LC/MS solution software (Shimadzu, Japan).

Pharmacokinetic data analysis

The area under the drug concentration-time curve from zero to 12 hr ($AUC_{0\rightarrow12hr}$) was calculated using the noncompartmental analysis (WinNonlin 2.1, Pharsight Corp., Mountain View, CA). The maximal plasma concentration of drug (C_{max}) and the time to reach maximum plasma concentration (T_{max}) were directly obtained from plasma data. One-way analysis of variance (ANOVA) test and Tukey's HSD test were performed to demonstrate statistical differences, using SPSS 12.0 software (SPSS, Chicago, IL, USA).

Stability of SMEDDS

In the stability test, three batches of SMEDDS formulations were prepared and stored at 4°C for periods up to 3 months. The stability was determined by HPLC method assay (same as the dissolution test), and was expressed as the percent of initial concentration measured.

Results and Discussion

Solubility test of atorvastatin in various excipients

For selecting a suitable self-emulsifying vehicle, it was important to assess; (a) the drug solubility in various com-

Table 1. *Solubility of atorvastatin in various excipients (n = 3, mean ± S.D.)*

Excipients		Solubility (mg/mL)
Oils	ethyl oleate	6.39 ± 4.26
	capryolPGMC	5.64 ± 4.31
	campul MCM	2.70 ± 0.84
	captex 300	5.64 ± 1.36
	soybean oil	3.73 ± 1.72
	olive oil	4.00 ± 2.30
Surfactants	Tween80	1.66 ± 0.47
	labrasol	1.40 ± 0.22
	Brij 92	4.08 ± 0.61
	Span 80	0.78 ± 0.15
	Cremphor EL	1.29 ± 0.20
	CremphorEL:Tween-20 = 1:1	4.93 ± 0.38
Cosurfactants	Carbitol®	194.51 ± 7.66
	PEG 400	19.50 ± 0.86
	PEG 200	13.39 ± 3.20
	n-butanol	45.28 ± 7.01
	propylene glycol	99.66 ± 13.70
	labrafil M 1944CS	152.44 ± 49.81

ponents, (b) the area of self-emulsifying region in the phase diagram, and (c) droplet size distribution following self-emulsification(Kommuru et al., 2001). In this study, various types of self-emulsifying formulations were prepared using six oils (ethyl oleate, capryol PGMC, campul MCM, captex 300, soybean oil, and olive oil), six surfactants (labrasol, Tween-80, Brij 92, Span 80, Cremphor EL, and Cremphor EL:Tween-20=1:1), and six co-surfactants (PEG 400, PEG 200, *n*-butanol, propylene glycol, Carbital, and labrafil M 1944CS). The solubility of atorvastatin in various vehicles was presented in Table I. From the table, oils (ethyl oleate, captex 300 and capryol PGMC),Surfactants (Cremphor EL:Tween-20=1:1, Brij 92, Tween-80) and Co-surfactants (Carbitol®, labrafil M 1944CS) were exhibited the relatively higher solubility than other vehicles. Especially Carbitol® provided significant solubility than all the others. So as a cosurfactant, it was selected for the optimal SMEDDS formulation resulting in improved drug loading capabilities.

Pseudo-ternary phase diagrams

Phase diagrams were constructed in the presence of atorvastatin to obtain the optimum concentrations of oil, surfactant, and cosurfactant. The visual test was measured the apparent spontaneity of emulsion formation. The series of SMEDDS were prepared and their self-emulsifying properties were observed visually. Pseudo-ternary phase diagrams were constructed to identify the self-emulsifying regions (Figure 2). The closed areas represented the microemulsfying areas having clear or slightly bluish. As a result, it could be seen that Figure 2 (a) and Figure 2 (d) gave the larger range for preparing the self-emulsification.

And it also could be seen that the use of surfactants (CremphorEL: Tween-20=1:1, Tween-80) were better for the form of clear appearance microemulsions rapidly within 1 min. It demonstrated that the emulsifying efficiency of surfactant was important which increase the drug diffusion/absorption by increasing the dissolution rate of the drug. Thus, some nonionic surfactants with a relatively high hydrophiliclipophilic balance (HLB) were widely recommended and selected(Gershanik and Benita, 2000).

Emulsion droplet size analysis

The droplet size exhibited the influence on the rate and the extent of drug release. The smaller droplet size improved drug

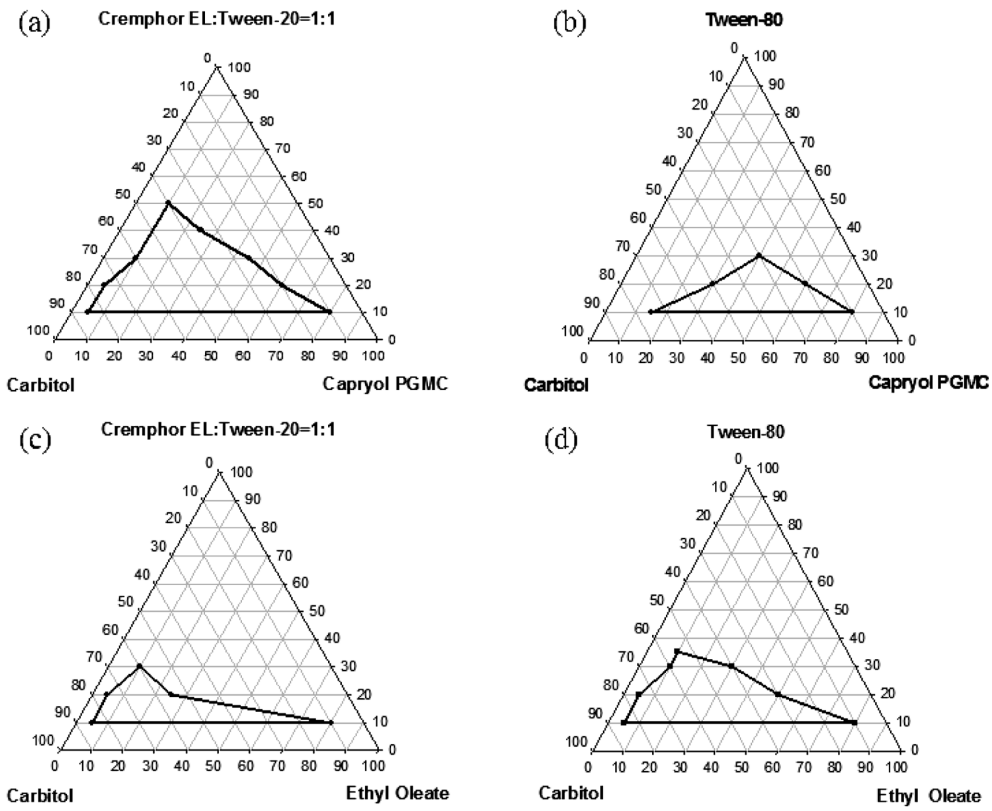

Figure 2. Pseudo-ternary phase diagrams indicated the efficient self-emulsification region (a), CapryolPGMC−(CremphorEL: Tween-20=1:1)−Carbitol®; (b), Capryol PGMC−Tween-80−Carbitol®; (c), ethyl oleate−(CremphorEL: Tween-20=1:1)−Carbitol®; (d), ethyl oleate−Tween-80−Carbitol®. (The closed areas represented the microemulsfying areas having clear or slightly bluish.)

Table II. *Droplet size data for the emulsification system. The concentration of the drug (atorvastatin) was 7% (n=3)*

Composition	Ratio	Droplet size (nm)	
		0 hr	1 hr
CapryolPGMC:(CremphorEL: Tween-20=1:1):Carbitol®	30:30:40	71.5±2.1	101.5±6.7
	30:40:30	71.6±3.4	109.1±13.0
	20:20:60	73.1±4.0	101.4±3.8
	20:30:50	76.9±2.1	108.5±7.4
	20:40:40	73.4±1.0	101.7±5.0
ethyl oleate:Tween-80: Carbitol®	20:20:60	285.7±19.0	282.8±6.7
	20:30:50	244.2±18.9	241.9±12.3
	20:40:40	23.4±1.3	36.6±4.1

Table III. *Droplet size data for the emulsification system (20% ethyl oleate- 40% Tween-80- 40% Carbitol®) (n=3)*

Drug concentration	4%	7%	10%	12%	12.5%
Droplet size (nm)	24.2±2.1	23.4±3.6	25.6±4.0	26.6±6.2	26.1±3.8

release and provided larger interfacial area across which drug could diffuse into the gastrointestinal fluids and thus increased drug absorption(Constantinides, 1995). In this study, in the middle of the closed area of Figure 2 (a) and Figure 2 (d), five points or three points of compositions were chosen respectively for incorporating atorvastatin. The concentration of the drug (atorvastatin) was determined by 7%. The effect of the formulation of SMEDDS on the droplet size distribution was shown in Table II. In case of the formulation of SMEDDS containing ethyl oleate as oil, Tween-80 as surfactant, and Carbitol® as cosurfactant, the mean droplet size was smaller than the other formulations. And maximum drug incorporation could reach 12.5% at the composition of ethyl oleate: Tween 80: Carbitol® (20:40:40).

The effect of the drug concentration on droplet size in distilled water was represented in Table III. Under the same composition, at the drug composition of 4%, 7%, 10%, 12%, 12.5% respectively, the droplet size had little differences, all of them were under 50 nm. It could be thought that undissolved drugs or dissolved drugs in the formulation had no big affects on the mean droplet size to increase. The droplet size distribution (*e.g.* at the composition of ethyl oleate: Tween-80: Carbitol® = 20:40:40) were all in the uniform dispense behavior (shown in Figure 3).

Dissolution test

The experimental conditions of *in vitro* release studies

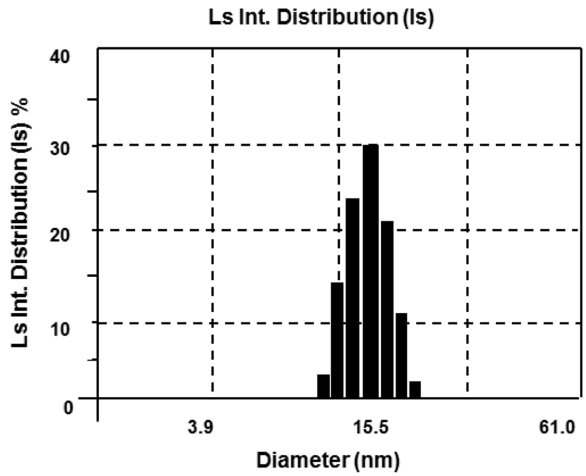

Figure 3. The droplet size distribution of microemulsionwith the composition of ethyl oleate:Tween-80:Carbitol®=20:40:40 (the drug concentration was 7%)

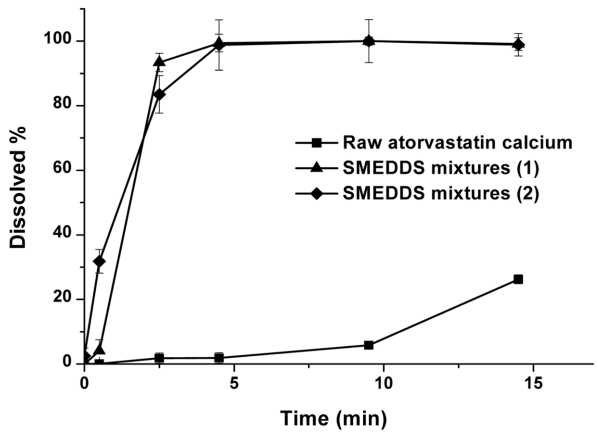

Figure 4. Dissolution profiles of atorvastatin in water from SMEDDS mixtures(1) (-▲-), SMEDDS mixtures(2) (-◆-) and raw atorvastatin calcium(-■-) (n=3)

played a significant role in governing the release behavior. Experimental conditions *in vitro* studies that could reflect the possible *in vivo* behavior of dosage form. For the purpose, dissolution studies were performed for SMEDDSmixtures (1) (20% ethyl oleate- 40% Tween-80- 40% Carbitol® with 7% drug concentration), SMEDDS mixtures(2) (20% CapryolPGMC- 40%(CremphorEL:Tween-20=1:1)- 40% Carbitol® with 7% drug concentration) and raw atorvastatin powder. The release of atorvastatin from these forms was evaluated in distilled water; the release percentage of atorvastatin from the SMEDDS mixtureswere significantly higher than that of atorvastatin from raw material powder. And the release percentage of atorvastatin from SMEDDS mixtures could rapidly reach more than 95% within 3 min (Figure 4). It could suggest that atorvastatin dissolved perfectly in SMEDDS form could be released due to the small droplet size, which permited a faster

rate of drug release into aqueous phase, faster than raw atorvastatin powder, and it could affect the bioavailability.

Pharmacokinetic study in rats

SMEDDS mixturesof ethyl oleate-(Tween-80)-Carbitol® and CapryolPGMC-(CremphorEL:Tween-20=1:1)-Carbitol® were selected for bioavailability studies. The *in vivo* study was performed to quantify atorvastatin (25 mg/kg) afteradministration of atorvastatin calcium. The plasma profiles of atorvastatin in rats following oral administration of raw atorvastatin powders and SMEDDS mixtureswere compared. Figure 5 showed that plasma concentration profiles of atorvastatin for both SMEDDS mixtures represented significantly greater improvement of drug absorption than raw atorvastatin powder. Pharmacokinetic parameters of the maximum plasma concentration (C_{max}) and the corresponding time (T_{max}) for atorvastatin following oral administration were shown in Table IV. The area under the concentration-time curve ($AUC_{0\rightarrow 8hr}$), C_{max} and T_{max} were estimated. In pharmacokinetic parameters of SMEDDS-mixtures (1), $AUC_{0\rightarrow 8hr}$ and C_{max} were 1994 ±335 ng·hr/mL and 1749±448 ng/mL, SMEDDS mixtures (2), $AUC_{0\rightarrow 8hr}$ and C_{max} were 1699±556 ng·hr/mL and 1723±517 ng/mL, compared to raw atorvastatin powder which were 936±149 ng·hr/mL and 589±198 ng/mL, respectively. The $AUC_{0\rightarrow 8hr}$ of SMEDDS mixtures (1) were nearly 2.1 times higher than that of raw atorvastatin powder.While the $AUC_{0\rightarrow 8hr}$ of SMEDDS-mixtures(2) wereabout 1.7-times higher than that of raw atorvastatin calcium powder.

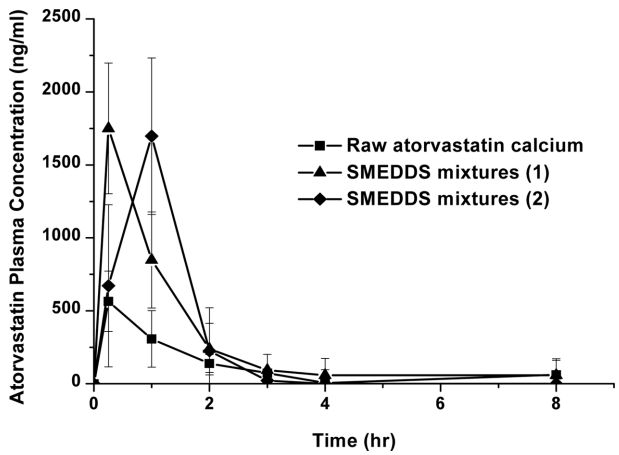

Figure 5. Plasma concentration-time curves of SMEDDSmixtures(1) (-▲-), SMEDDSmixtures (2)(-◆-)and raw atorvastatin calcium (-■-), (n=5, mean±S.D.)

Table IV. *Pharmacokinetic parameters after oral administration of SMEDDS mixtures and raw atorvastatin calcium to rats (n=5)*

		$AUC_{0\rightarrow 8hr}$ (ng·hr/mL)	C_{max} (ng/mL)	T_{max} (hr)
Raw atorvastatin calcium		936±149	589±199	0.4±0.3
SMEDDS mixtures	(1)	1994±335[a]	1749±448[a]	0.25±0.0
	(2)	1699±556[a]	1723±517[a]	0.7±0.4

[a]Indicates $P<0.05$ between raw atorvastatin powder and SMEDDS mixtures

SMEDDS form enhanced the values of $AUC_{0\rightarrow 8hr}$ and C_{max} of drug compared to raw material. The T_{max} of SMEDDS showed the fairly rapid onset compared to raw material. Therefore, SMEDDS might be a promising approach for the rapid onset and the effective absorption into oral administration delivery of atorvastatin and could increase bioavailability for the other poorly water-soluble drug.

Stability of SMEDDS

Liquid formulation stability is critical to their performance. If formulation is unstable, the dose emitted and particle size characteristics may be unpredictable and will lead to poor therapy. In the stability test, three batches of SMEDDS formulations (20% Ethyl oleate- 40% Tween-80- 40% Carbitol® with less than 7% drug concentration) were prepared and stored at 4°C for periods up to 3 months. The results were shown in Table V. It showed that the SMEDDS formulation was relatively stable when stored at 4°C during 3 months. It is suggested that the formulation would have clinical perspective.

Conclusion

In summary, the obtained results indicated that the self-microemulsifying system incorporating atorvastatin was established. The optimal combination of SMEDDS mixturescontaining atorvastatin (relatively high drug loading and small particle size) was as following: 20% of ethyl oleate, 40% of Tween-80 and 40% of Cabitol®. *In vitro* dissolution studies revealed that release of atorvastatin from SMEDDS was faster

Table V. *Stability test was performed (20% Ethyl oleate- 40% Tween-80- 40% Carbitol® with 7% drug concentration) at 4°C for periods up to 3 months*

Temperature	Initial concentration	Concentration (% initial concentration)			
		14 days	1 month	2 month	3 month
4℃	100 %	99.70 ± 3.41	99.55± 2.26	98.21± 1.88	99.06± 1.09

than raw material particles with the first 3 min. Also, *in vivo* studies in rats, SMEDDS showed significantly greater extent of absorption than raw materials. In stability test, the SMEDDS formulation was relatively stable when stored at 4°C during 3 months. Our studies illustrated the potential use of SMEDDS for the delivery of hydrophobic compounds, such as atorvastatin, by the oral route.

Acknowledgements

This work was supported by a grant of the Korean Health Technology R&D Project, Ministry for Health, Welfare & Family Affairs, Republic of Korea (A092018) and by Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0093815)

Reference

Attama, A.A., Nkemnele, M.O. 2005. In vitro evaluation of drug release from self micro-emulsifying drug delivery systems using a biodegradable homolipid from Capra hircus. International Journal of Pharmaceutics, 304, 4-10.

Constantinides, P.P. 1995. Lipid Microemulsions for Improving Drug Dissolution and Oral Absorption: Physical and Biopharmaceutical Aspects. Pharmaceutical Research, 12, 1561-1572.

Corsini, A., Bellosta, S., Baetta, R., Fumagalli, R., Paoletti, R., Bernini, F. 1999. New insights into the pharmacodynamic and pharmacokinetic properties of statins. Pharmacology & Therapeutics, 84, 413-428.

Gershanik, T., Benita, S. 2000. Self-dispersing lipid formulations for improving oral absorption of lipophilic drugs. European Journal of Pharmaceutics and Biopharmaceutics, 50, 179-188.

Hermann, M., Christensen, H., Reubsaet, J.L.E. 2005. Determination of atorvastatin and metabolites in human plasma with solid-phase extraction followed by LC-tandem MS. Analytical and Bioanalytical Chemistry, 382, 1242-1249.

Kang, B.K., Lee, J.S., Chon, S.K., Jeong, S.Y., Yuk, S.H., Khang, G., Lee, H.B., Cho, S.H. 2004. Development of self-microemulsifying drug delivery systems (SMEDDS) for oral bioavailability enhancement of simvastatin in beagle dogs. International Journal of Pharmaceutics, 274, 65-73.

Khoo, S.-M., Humberstone, A.J., Porter, C.J.H., Edwards, G.A., Charman, W.N. 1998. Formulation design and bioavailability assessment of lipidic self-emulsifying formulations of halofantrine. International Journal of Pharmaceutics, 167, 155-164.

Kommuru, T.R., Gurley, B., Khan, M.A., Reddy, I.K. 2001. Self-emulsifying drug delivery systems (SEDDS) of coenzyme Q10: formulation development and bioavailability assessment. International Journal of Pharmaceutics, 212, 233-246.

Lattuada, R., Martini, A., Muggetti, L. 1998. Smedds® (self microemulsifying drug delivery systems) for oral administration of an immunosuppressive drug. European Journal of Pharmaceutical Sciences, 6, S67-S67.

Lennern, S.H. 1997. Human jejunal effective permeability and its correlation with preclinical drug absorption models. Journal of Pharmacy and Pharmacology 49, 627-638.

Lennern, S.H. 2003. Clinical pharmacokinetics of atorvastatin. Clin Pharmacokinet, 42, 1141-1160.

Neslihan Gursoy, R., Benita, S. 2004. Self-emulsifying drug delivery systems (SEDDS) for improved oral delivery of lipophilic drugs. Biomedecine & Pharmacotherapy, 58, 173-182.

Nirogi, R.V.S., Kandikere, V.N., Shukla, M., Mudigonda, K., Maurya, S., Boosi, R., Anjaneyulu, Y. 2006. Simultaneous quantification of atorvastatin and active metabolites in human plasma by liquid chromatography–tandem mass spectrometry using rosuvastatin as internal standard. Biomedical Chromatography, 20, 924-936.

Wu, X., Whitfield, L.R., Stewart, B.H. 2000. Atorvastatin Transport in the Caco-2 Cell Model: Contributions of P-Glycoprotein and the Proton-Monocarboxylic Acid Co-Transporter. Pharmaceutical Research, 17, 209-215.