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Enhanced bioavailability of the poorly water-soluble drug fenofibrate by using liposomes containing a bile salt

Yaping Chen[a], Yi Lu[a], Jianming Chen[b], Jie Lai[a], Jing Sun[b], Fuqiang Hu[c], Wei Wu[a,*]

[a] School of Pharmacy, Fudan University, Shanghai 201203, China
[b] School of Pharmacy, Second Military Medical University, Shanghai 200433, China
[c] School of Pharmacy, Zhejiang University, Hangzhou 310058, China

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ABSTRACT

The main purpose of this study was to evaluate oral bioavailability of the poorly water-soluble drug fenofibrate when liposomes containing a bile salt were used as oral drug delivery systems. Liposomes composed of soybean phosphotidylcholine (SPC) and sodium deoxycholate (SDC) were prepared by a dry-film dispersing method coupled with sonication and homogenization. Several properties of the liposomes, including particle size, entrapment efficiency and membrane fluidity, were extensively characterized. In vitro release experiments indicated that no more than 20% of total fenofibrate was released from SPC/cholesterol (CL) and SPC/SDC liposomes at 2 h, in contrast with near complete release for micronized fenofibrate capsules. Strikingly, in vivo measurements of pharmacokinetics and bioavailability demonstrated higher rates of fenofibrate absorption from both SPC/SDC and SPC/CL liposomes than micronized fenofibrate. The bioavailability of SPC/SDC and SPC/CL liposomes was 5.13- and 3.28-fold higher, respectively, than that of the micronized fenofibrate. The disparity between oral bioavailability and in vitro release for liposomes strongly suggests alternative absorption mechanisms rather than enhanced release. Importantly, SPC/SDC liposomes exhibited a 1.57-fold increase in bioavailability relative to SPC/CL liposomes, indicating that liposomes containing bile salts may be used to enhance oral bioavailability of poorly water-soluble drugs.

1. Introduction

Liposomes are enclosed vesicles composed of phosphotidylcholine and cholesterol lipid bilayers, and have been extensively studied as drug carriers. Hydrophilic drugs can be loaded into the inner aqueous phase, whereas hydrophobic drugs can be inserted into the hydrophobic lipid bilayers. Although drug-loaded liposomes have been mainly used for the parenteral administrations, their potential application as oral drug delivery systems has also been studied. Oral liposomes may provide increased solubility of their load and protection from the hostile environment in the gastrointestinal tract (Ariën et al., 1993, 1994). Most importantly, the similarity between liposomal lipid bilayers and biomembranes and the relatively small size of liposomes significantly facilitate oral absorption (Aungust, 1993). For example, oral administration of protein- or polypeptide-loaded liposomes has been shown to give rise to enhanced absorption of active biomacromolecules (Ariën et al., 1993; Dapergolas and Gregoriadis, 1976; Degim et al., 2004). Incorporation of poorly permeable small molecule drugs into liposomes also yielded improved oral absorption (Sun et al., 2008; Moutardier et al., 2003; Deshmukh et al., 2008). In particular, for poorly water-soluble drugs, substantial enhancement in bioavailability or in vivo efficacy has been observed following liposomal encapsulation (Mourão et al., 2005; Guo et al., 2001; Xiao et al., 2006).

The underlying mechanism for the facilitated oral absorption by liposomes has been partly elucidated by studies on the phase transition behavior of liposomal vesicles under simulated gastrointestinal environment, especially in the presence of bile salts. Physiological bile salts can interact with phospholipids in the gastrointestinal tract to form mixed micelles that play important roles in enhancing absorption of digestive lipids and poorly water-soluble drugs and alleviating membrane toxicity induced by bile salt monomers (Dial et al., 2008; Hildebrand et al., 2003; Martin and Marriott, 1981; Porter et al., 2007). Likewise, bile salt monomers can penetrate into liposomal lipid bilayers and disrupt the vesicular structure, and further increase in bile salt concentration can induce liposomes to undergo vesicle–micelle transition (Hildebrand et al., 2003; Andrieux et al., 2009). The resultant mixed micelles have been shown to function as excellent vehicles for poorly water-soluble drug molecules and one of the most important mesophases before absorption (Porter et al., 2007). Therefore, liposomes containing

* Corresponding author. Tel.: +86 21 51980002; fax: +86 21 51980002.
E-mail address: wuwei@shmu.edu.cn (W. Wu).

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bile salts, also called transfersomes when originally developed for transdermal delivery (Cevc, 2004; Cevc et al., 1998, 2008), can readily transform into mixed micelles in the gastrointestinal environment, thus enhancing transmembrane absorption.

Fenofibrate is a highly lipophilic drug clinically used to lower lipid levels. However, its therapeutic efficacy has been compromised for years due to the virtual insolubility in water and physiological fluids (Guay, 2002). More recently, micronized fenofibrate showed significantly improved dissolution and enhanced oral bioavailability (Keating and Ormrod, 2002). Furthermore, the presence of food increased absorption of fenofibrate by ~35% compared with fasting conditions (Balfour et al., 1990). Although the clinical role of food on fenofibrate pharmacokinetics remains unclear, induced secretion of physiological bile salts has been implicated as a result of food digestion (Guivarc'h et al., 2004).

Liposomes containing bile salts have been shown to increase intestinal absorption of the model macromolecule salmon calcitonin (Song et al., 2002, 2005); however, their potential usage as oral drug delivery carriers for poorly water-soluble drugs has not been investigated. In this study, fenofibrate-loaded soybean phosphotidylcholine/sodium deoxycholate liposomes were prepared and evaluated both in vitro and in vivo. Oral bioavailability of fenofibrate for bile salt-containing liposomes, conventional phosphotidylcholine/cholesterol liposomes and micronized fenofibrate was measured and compared. Our results clearly indicate a significant role of bile salts in the enhanced oral bioavailability of a poorly water-soluble drug.

2. Materials and methods

2.1. Materials

Fenofibrate (FB) was purchased from Enhua Pharma. Corporation (Xuzhou, China). Soybean phosphotidylcholine (SPC) was supplied by Lipoid (Germany). Sephadex G-50 was purchased from Pharmacia. Sodium deoxycholate (SDC) and cholesterol (CL) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Deionized water was prepared by a Milli-Q purification system (Millipore, USA). HPLC-grade methanol was supplied by Merck (Darmstadt, Germany). Lipanthyl, a micronized fenofibrate capsule formulation (200 mg) of Solvay China (Suzhou, China), was purchased from a local distributor. All other chemicals used in the study were of analytical grade.

2.2. Preparation of fenofibrate SPC/SDC and SPC/CL liposomes

SPC/SDC liposomes were prepared by an improved dry-film dispersing method (Lu et al., 2007). FB (10 mg) and SPC (300 mg) were first dissolved in diethyl ether anhydrous, and evaporated for at least 3 h (RE-52AA rotatory evaporator, Shanghai, China) to remove the solvent in a 50 ml round-bottom flask under vacuum in a water bath at 30 °C. A thin film of SPC formed on the wall of the flask. 10 ml phosphate buffer (pH 7.4) containing SDC in different amount was added to the lipid film and rotated for 30 min in a water bath at 30 °C to obtain a crude dispersion of liposomes. The liposome dispersion was then sonicated for 10 min by intermittent probe sonication (JYD-650, Shanghai, China) under cooling in a water bath, and finally homogenized by passing five times through a high-pressure homogenizer (Avestin Em-C3) at 20,000 psi and 25 °C to obtain an opalescent dispersion of liposomes. The liposome dispersion was stored at room temperature until analysis.

For the preparation of SPC/CL liposomes, similar procedures were conducted using CL. Briefly, FB (10 mg), CL (75 mg) and SPC (300 mg) were first dissolved in diethyl ether anhydrous, and evaporated to remove diethyl ether to form a thin-film layer on the wall of the flask. Blank phosphate buffer was added to hydralize the thin film. Other experimental details were the same as in the SPC/SDC liposome preparation.

2.3. Particle size analysis

Particle size measurements were performed at 25 °C by photon correlation spectroscopy on Malvern Zetasizer Nano® instrument (Malvern, UK) equipped with a 4 mW He–Ne laser (633 nm). Samples were diluted with deionized water prior to measurement, and dispersant viscosity was set to 0.8872 cP at 25 °C. Particle size was analyzed by the Dispersion Technology Software provided by Malvern Instruments.

2.4. Entrapment efficiency

The percentage of drug incorporated into liposomes was measured after separating free drugs from liposomes by Sephadex G-50 chromatography. About 2 g Sephadex G-50 was packed in a 30 cm glass column with an inner diameter of 1.5 cm. After preconditioning with 30 ml water, fenofibrate-loaded SPC/SDC or SPC/CL liposomes was mounted and subsequently eluted with 10 ml of water. The elution was monitored through turbidity measurement at a wavelength of 500 nm by using a UV-2401 spectrophotometer (Shimadzu, Japan). The eluent containing liposomes was treated with 10% triton-X100 to disrupt the vesicular structure to release fenofibrate. The content of fenofibrate was determined by using an Agilent 1100 HPLC system. Acetonitrile/water (90/10, v/v), adjusted by dilute acetic acid to pH 4.0, was used as the mobile phase at a flow rate of 1.0 ml/min. Fenofibrate was determined at 40 °C by C18 column (Diamonsil, 5 μm, 4.6 mm × 150 mm, Dikma, China) guarded with a refillable precolumn (C18, 2.0 mm × 20 mm, Alltech, USA) and detected at 287 nm. Linearity range of the calibration curve was within 0.01–10 μg/ml with a correlation coefficient of 0.9997 ± 0.0002. Entrapment efficiency (*EE%*) was calculated as:

$$EE\% = W_c/W_t \times 100$$

where W_c and W_t denote drug content in liposomes and total drug in dispersion.

2.5. Membrane fluidity measurement

The membrane fluidity of SPC/SDC liposomes was studied by fluorescence anisotropy measurement as reported previously (Park et al., 2006). The lipid bilayers of SPC/SDC liposomes were labeled by a fluorescent probe, 1,6-diphenyl-1,3,5-hexatriene (DPH). Steady-state fluorescent anisotropy (r) was calculated according to the following equation:

$$r = \frac{I_{VV} - I_{VH}}{I_{VV} + 2I_{VH}}$$

where I_{VV} and I_{VH} are the light intensity emitted in the vertical and horizontal directions relative to the beam of excitation, respectively. The membrane fluidity correlates negatively to the r value.

For labeling, 2 ml of 2 μM DPH was added to 2 ml SPC/SDC liposomes suspension and incubated in a thermostatic water bath at 25 °C for 30 min in the dark. The fluorescence anisotropy of DPH in SPC/SDC liposomes was measured with a fluorescence spectrophotometer (FLS 920, Edinburgh Instruments Ltd., England) at room temperature. The excitation and emission wavelengths used for DPH were 362 and 432 nm, respectively. SPC/CL liposomes and SPC/CL liposomes containing benzyl alcohol were used as the negative and positive control, respectively (Lee et al., 1999).

Table 1
Formulation details of SPC/SDC and SPC/CL liposomes for in vitro release and bioavailability studies.

Liposome type	SPC/CL (SDC) ratio	Theoretical fenofibrate concentration (mg/ml)	Actual fenofibrate concentration (mg/ml)	Theoretical SPC concentration (mg/ml)	Theoretical SDC concentration (mg/ml)	Theoretical CL concentration (mg/ml)	Particle size (nm)	Entrapment efficiency (%)
SPC/CL	4/1	1	0.69	30	0	7.5	175	84.91
SPC/SDC	4/1	1	0.73	30	7.5	0	159	88.23

2.6. Cryo-transmission electron microscopy (Cryo-TEM)

A drop of 3 μl liposome dispersion was placed on a carbon-coated holey film (Quantifoil) supported by a copper grid and gently blotted with filter paper to obtain a thin liquid film (20–400 nm) on the grid, which was subsequently covered by another carbon-coated holey film. The grid was plunged into liquid ethane. Excess ethane was removed and the sample was transferred into a Cryo-TEM (JOEL JEM-2010, Japan). Samples were viewed under low-dose conditions at a constant temperature around −170 °C. The images were recorded digitally with a CCD camera (Gatan 832) under low-dose conditions with an underfocus of approximately 3 μm and an acceleration voltage of 120 kV.

2.7. In vitro release study

In vitro release of fenofibrate from SPC/CL, SPC/SDC liposomes and micronized powder was evaluated by a dynamic dialysis method. The fundamental properties of both SPC/CL and SPC/SDC liposomes are given in Table 1. Before release test, 3 ml of SPC/SDC and SPC/CL liposome dispersion were instilled into a dialysis bag (MWCO 14,000, Spectrum Medical Industries Inc., USA), whereas micronized fenofibrate powder was suspended with 3 ml of release medium. Release test was carried out in a ZRS-8G release tester (Tianjin, China) according to the Chinese Pharmacopoeia Method III (the small beaker method). The dialysis bags were put in a beaker containing 100 ml 2% Cremophor EL in pH 7.4 distilled water as release medium, and maintained at 37 °C with a paddle revolution speed of 100 r/min. At the indicated time intervals, 200 μl of release sample was withdrawn and centrifuged at 10,000 r/min for 10 min. The concentrations of fenofibrate were analyzed by Agilent 1100 series HPLC system (Agilent, USA).

2.8. Determination of fenofibric acid in dog plasma by RP-HPLC

As a prodrug, fenofibrate is rapidly metabolized to its major active metabolite, fenofibric acid (FA), after absorption. No intact fenofibrate can be detected in the plasma after oral administration. Therefore, pharmacokinetic evaluation of fenofibrate is based on the quantification of fenofibric acid in plasma (Balfour et al., 1990; Straka et al., 2007; Streel et al., 2000).

In this study, fenofibric acid in dog plasma was determined by an HPLC-UV method. The Agilent 1100 series HPLC system (Agilent, USA) was composed of a quaternary pump, a degasser, an autosampler, a column heater and a tunable ultraviolet detector. Fenofibric acid was separated at 30 °C by a C18 column (Diamonsil, 5 μm, 4.6 mm × 250 mm, Dikma, China) guarded with a refillable C18 pre-column (2.0 mm × 20 mm, Alltech, USA) and detected at 287 nm. The mobile phase was composed of methanol and 0.01 M KH_2PO_4 (adjusted to pH 2.5 with hydrochloric acid) in a volume ratio of 75/25(v/v) at a flow rate of 1.0 ml/min.

Fenofibric acid in dog plasma was extracted by liquid–liquid extraction procedures. To 0.8 ml dog plasma, 30 μl of 1 mol/l hydrochloric acid were added, followed by 40 μl of internal standard solution (indomethacin, 4 μg/ml in methanol). After vortex mixing for 30 s, 5 ml of diethyl ether anhydrous was added and vortex mixed for 10 min. After centrifugation at 5000 r/min for 10 min, the organic layer was transferred to another tube and evaporated under a light stream of nitrogen at 40 °C. The residue was dissolved by 100 μl of the mobile phase, and centrifuged at 10,000 r/min for 10 min, 60 μl of the supernatant was injected for HPLC analysis. Quantification was based on peak area ratio R (A_{FA}/A_{IS}). The linearity was observed over the concentration range of 0.003125–1.875 μg/ml with correlation coefficients of over 0.99. A typical calibration curve was as follows: $R = 3.5075C - 0.013$ ($r = 0.9984$, $n = 7$). The lower limit of quantification (LLOQ) for the determination of fenofibric acid in dog plasma was found to be 3.1 ng/ml. Accuracy of the determination of fenofibric acid in dog plasma ($n = 9$) is 94.86 ± 3.33%. Within-day and between-day precisions were all below 4.0%. Extraction recovery of fenofibric acid in dog plasma ($n = 9$) was 74.67 ± 2.46%.

2.9. Bioavailability studies

Bioavailability of fenofibrate-loaded SPC/SDC liposomes was compared with those of fenofibrate-loaded SPC/CL liposomes and micronized fenofibrate capsules (Lipanthyl, Laboratories Fournier S.A., France). The formulation details of both SPC/SDC and SPC/CL liposomes are given in Table 1. Six beagle dogs weighing 10 ± 2 kg, purchased from the Experimental Animal Center of Fudan University, were allocated at random to three treatment groups and given SPC/SDC, SPC/CL liposomes and micronized capsule in a three-period crossover experimental design. The washout period between two consecutive treatments was 7 days. Guidelines on experiments involving use of animals issued by the Ethical Committee of Fudan University were strictly followed.

Beagle dogs were kept in an environmentally controlled breeding room for one week and fasted for 12 h prior to the experiment. A dose of each formulation (30 mg, expressed as fenofibrate equivalents) was given through gavage administration. Both SPC/SDC and SPC/CL liposome dispersions were given without dilution, whereas micronized fenofibrate was given in capsules with 50 ml of water. After administration, about 2 ml of blood sample was collected through the hind leg vein into heparinized tubes at 0, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 4, 6, 8, and 10 h. Blood samples were centrifuged at 3000 × g for 10 min using a high speed centrifuge (TGL-16, Shanghai, China) and plasma samples were withdrawn and stored at −18 °C.

Pharmacokinetic analysis was performed by a model-independent method using the 3P87 computer program (issued by the State Food and Drug Administration of China for pharmacokinetic studies). C_{max} and T_{max} were observed as raw data. Area under the curve to the last measurable concentration (AUC_{0-t}) was calculated by the linear trapezoidal rule.

2.10. Statistical analysis

Raw data were analyzed using the SPSS statistical software (version 11.0, SPSS, Inc.). Post hoc multiple comparisons were done using one-way ANOVA and the Student–Newman–Keuls test (q test) to determine the significance of differences between groups; a P value less than 0.05 was considered statistically significant.

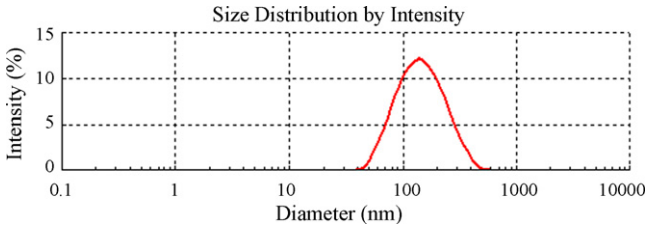

Fig. 1. Size distribution of fenofibrate SPC/SDC liposomes.

3. Results and discussion

3.1. Formation and characterization of SPC/SDC liposomes

Spontaneous formation of SPC/SDC liposomes was observed upon hydration of the SPC film with phosphate buffer containing SDC. The primary SPC/SDC liposomes before sonication and homogenization were typically a few microns in size, yet exhibited a wide distribution as observed under photomicroscopy. These liposomes were likely to be multilamellar, similar to the structure of other liposomes prepared by an analogous film-dispersion method (Amselem et al., 1990). After sonication and homogenization, the particle size was reduced dramatically, suggestive of a small unilamellar structure (Yang et al., 2006). Under homogenization at 20,000 psi for 5 circles, the particle size of SPC/SDC liposomes was further reduced, with a minimum size of 120 nm and a typical lognormal distribution (Fig. 1). The recovery, calculated through monitoring fenofibrate in the liposome dispersion, was about 70% (Table 1).

Since particle size exerts significant influence on the in vitro and in vivo performance of liposomes (Sun et al., 2008), we next examined the effect of several variables on particle size. The SDC/SPC ratio, the hydration phosphate buffer pH and homogenization parameters were found to affect particle size and size distribution significantly (Fig. 2). Specifically, a substantial decrease in particle size correlated with increased SDC in the bilayers, about 120 nm at a SDC/SPC ratio of 1/3. In contrast, at the SDC/PC ratio of 1/6 or 1/7, the particle size was increased to over 200 nm. The reduction in particle size as a function of SDC content in lipid bilayers may be ascribed to increased flexibility and reduced surface tension of the vesicles. The pH of the hydration medium also influenced particle size significantly. It is possible that as the pH increased, SDC dissociation and ionization became more active, which in turn increased its surface activity. Particles with a diameter of over 300 nm were obtained at pH 6.5, while a slight increase of pH to 7.4 yielded a sharp reduction in the particle size (down to 100 nm). Paradoxically, at a higher pH of 8.0, the particles again were enlarged, possibly due to the increased viscosity of the hydration phosphate buffer in the presence of SDC. Therefore, the optimal pH of the hydration medium should approximate physiological conditions. The particle size also correlated with the homogenization conditions. Increases in either homogenization pressure or the number of homogenization circles served to reduce the diameter of SPC/SDC liposomes. On the other hand, the fenofibrate/SPC ratio showed no significant effects on particle size. The particle size fluctuated within 150–200 nm when the fenofibrate/SPC ratio varied from 1/50 to 1/25, with higher drug loading corresponding to a slight increase in particle size. The effect of drug loading needs to be further studied with a broader drug loading range.

Entrapment efficiency of fenofibrate into SPC/SDC liposomes was primarily dependent on the SDC/SPC ratio and the hydration medium pH. As shown in Fig. 3, robust entrapment efficiency was observed, likely as a result of the lipophilic affinity of fenofibrate to the hydrophobic region within the lipid bilayers. SDC in the lipid bilayers was supposed to be able to “solubilize” and “hold” fenofibrate. Near complete entrapment was achieved at a very high SDC

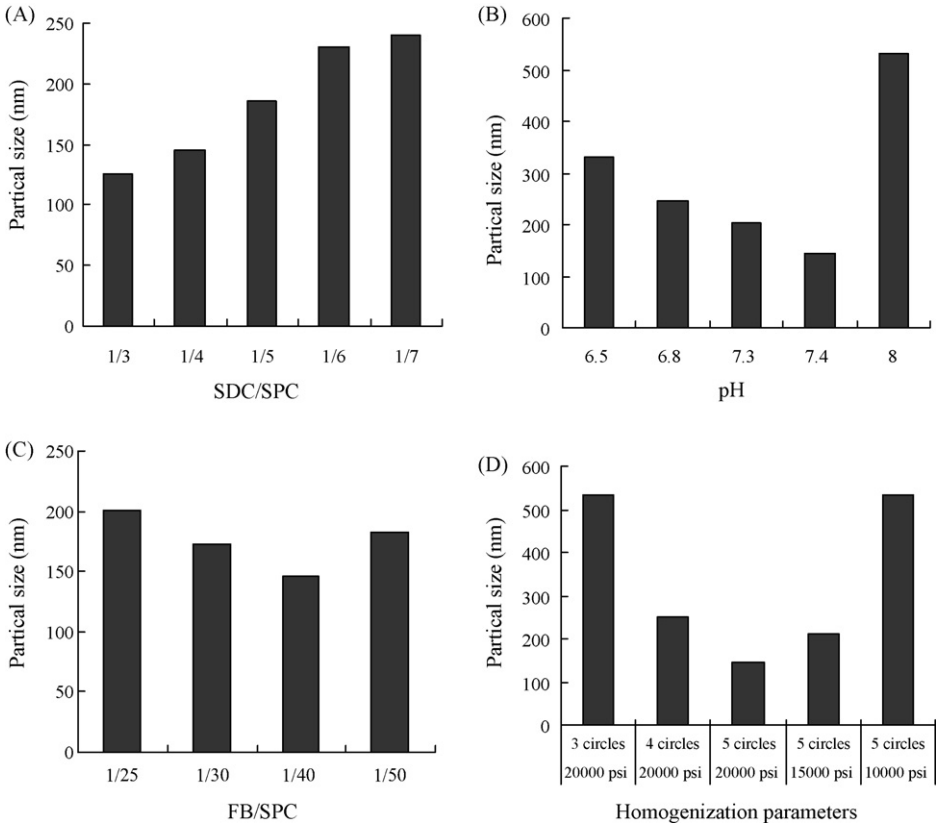

Fig. 2. Effects of the SDC/SPC ratio (A), the hydration phosphate buffer pH (B), the FB/SPC ratio (C) and homogenization parameters (D) on liposome particle size.

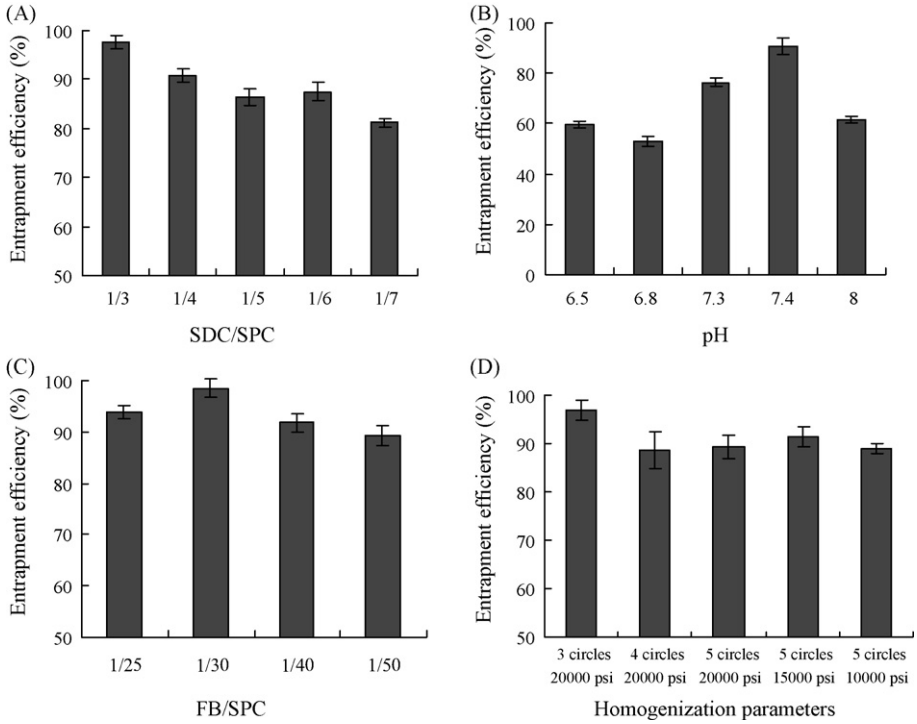

Fig. 3. Effects of the SDC/SPC ratio (A), the hydration phosphate buffer pH (B), the FB/SPC ratio (C) and homogenization parameters (D) on fenofibrate entrapment efficiency in liposomes. Data expressed as mean ± S.D. ($n = 3$).

content (SDC/SPC = 1/3). Entrapment efficiency decreased when SDS content increased, and significant difference was observed between each SDC/SPC ratios ($P < 0.01$) except the two ratios of 1/5 and 1/6 ($P > 0.05$). The pH may initially modulate SDC dissociation, consequently altering the particle size and the ability of SDC to hold fenofibrate in lipid bilayers. Highest entrapment efficiency was observed at pH 7.4. There was significant difference in entrapment efficiency between each pH groups ($P < 0.05$) except the two pH groups of 6.5 and 8.0 ($P > 0.05$). Drug loading and homogenization parameters seemed to have minimal effects on entrapment efficiency within the observed range.

Cryo-TEM photographs demonstrated that blank and fenofibrate-loaded SPC/SDC liposomes retained hydrated morphology (Fig. 4), as near spherical morphology was observed for both. Vesicular structure was discernable, whereas the inner lamellar could not be unambiguously observed. Comparison of the photographs indicated no obvious changes in SPC/SDC liposome morphology as a result of drug loading.

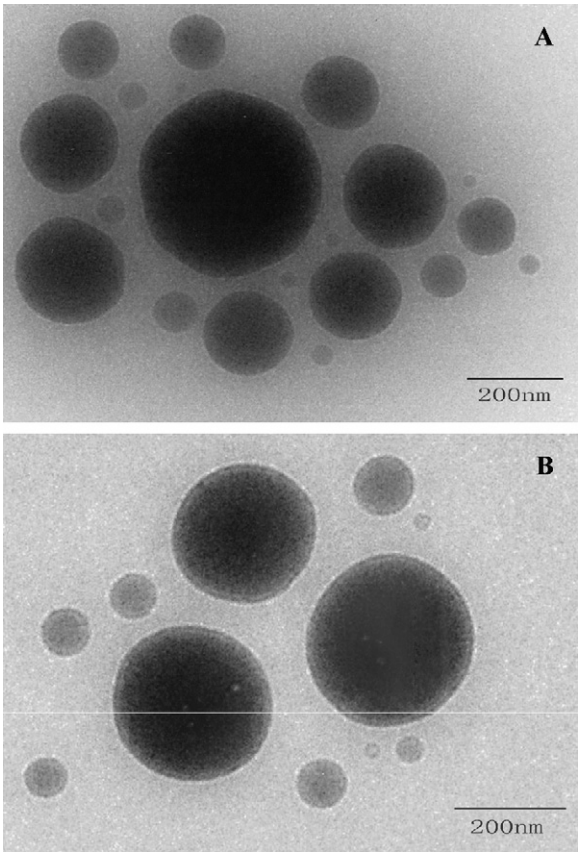

Fig. 4. Cryo-TEM photographs of blank (A) and fenofibrate-loaded (B) SPC/SDC liposomes.

3.2. Membrane fluidity

As a result of the "softening" effect of bile salts, membrane fluidity serves to distinguish liposomes containing bile salts from conventional cholesterol-based counterparts. The ultra-flexibility of SPC/SDC liposomes may impart them with unique in vitro and in vivo performances. Consistent with this notion, whereas SPC/CL liposomes, the negative control, showed the highest r value (Fig. 5), incorporation of SDC resulted in decreases in the r value in a dose-dependent manner, especially at higher SDC/SPC ratios of 1/3 and 1/4 ($P < 0.05$). These results indicated that increases in the SDC content in lipid bilayers concomitantly enhanced membrane fluidity.

Increased membrane fluidity has wide-ranging effects on liposome properties and functions, such as particle size and distribution, leakage of incorporated drugs (Kawano et al., 2009; Lim et al., 2000), passage through cellular tight junctions (Cevc et al., 1998;

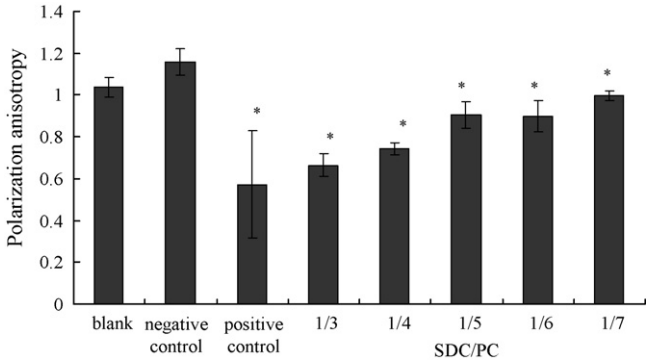

Fig. 5. Polarization anisotropy of SPC/SDC liposomes at different SDC/SPC ratios compared with SPC/CL liposomes (negative control) and liposomes containing benzyl alcohol (positive control). Data expressed as mean ± S.D. (n = 3). *Significant at P < 0.05 vs. negative control.

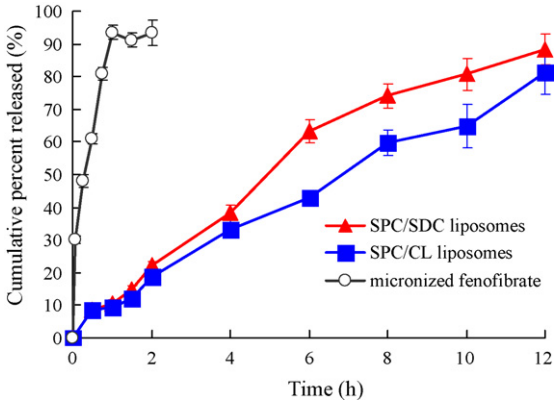

Fig. 6. In vitro release of fenofibrate from SPC/SDC and SPC/CL liposomes and micronized fenofibrate capsules. Data expressed as mean ± S.D. (n = 3).

Cevc, 2004) and possibly in vivo behavior. As mentioned above, particle size was reduced in response to increases in the SDC content in lipid bilayers. It is likely that increased membrane fluidity at least in part facilitated disruption of primary multilamellar vesicles and thus promoted formation of small unilamellar ones. Other effects of increased membrane fluidity are discussed below.

3.3. In vitro release

In vitro release has been used as a very important surrogate indicator of in vivo performance, especially for poorly water-insoluble drugs. As shown in Fig. 6, the in vitro release profiles of three fenofibrate formulations were compared. In order to separate free fenofibrate molecules from those associated with SPC/SDC liposomes, a dynamic dialysis method was employed to circumvent the escape of SPC/SDC liposomal particles into the outer release medium.

Release of micronized fenofibrate capsules proceeded at a high rate, with approximately 95% release at 1 h. Release of fenofibrate from both SPC/CL and SPC/SDC liposomes appeared to be much slower, with no more than 20% at 2 h and less than 85% at 12 h. SPC/SDC liposomes released fenofibrate somewhat faster than SPC/CL liposomes, possibly due to the presence of SDC that enhanced lipid bilayer fluidity and thus allowed leakage of more drugs (Kawano et al., 2009). These findings indicated that incorporation of fenofibrate into liposomes did not give rise to enhanced release, prompting us to perform the pharmacokinetic studies below.

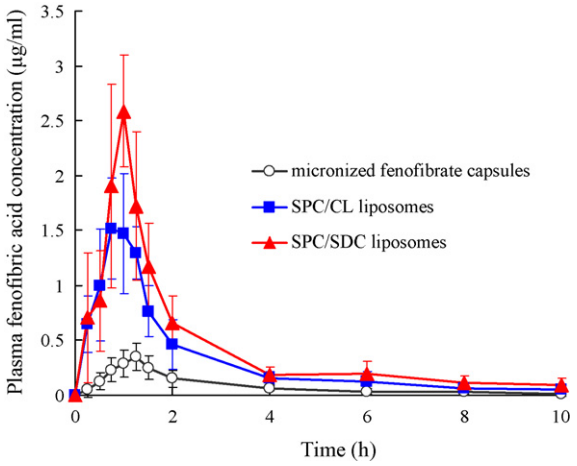

Fig. 7. Plot of plasma fenofibric acid concentration as a function of time after a single oral dose of 30 mg equivalent SPC/SDC, SPC/CL liposomes and micronized fenofibrate capsules. Data expressed as mean ± S.D. (n = 6).

3.4. Bioavailability studies

To further investigate the role of bile salts, oral bioavailability of fenofibrate-loaded SPC/SDC liposomes in beagle dogs was compared with those of micronized fenofibrate and SPC/CL liposomes. The details of the three formulations are given in Table 1. Mean plasma fenofibric acid concentration versus time plots of three fenofibrate formulations are shown in Fig. 7, and pharmacokinetic parameters obtained by the non-compartmental method are shown in Table 2.

After gavage administration, SPC/SDC and SPC/CL liposomes showed similar absorption and elimination profiles, yet significantly differed from micronized fenofibrate. More rapid absorption with T_{max} of 0.79 and 0.74 h was observed for SPC/SDC and SPC/CL liposomes (P > 0.05), respectively. As shown in Fig. 7, plasma fenofibric acid from micronized fenofibrate peaked at 1.14 h, indicating a significant delay relative to the liposome formulations (P < 0.01) despite a greater in vitro release rate. The absorption of the liposome formulations was substantially greater, as indicated by the values of C_{max} and AUC. Furthermore, SPC/SDC liposomes exhibited the highest absorption, with greater C_{max} and AUC_{0-t} values (2.51 μg/ml vs. 1.40 μg/ml (P < 0.01) and 3.54 μg h/ml vs. 2.26 μg h/ml (P < 0.01)) than SPC/CL liposomes. Upon normalization

Table 2
Pharmacokinetic parameters after oral administration of fenofibrate-loaded SPC/SDC and SPC/CL liposomes and micronized fenofibrate capsules.

Formulation	T_{max} (h)	C_{max} (μg/ml)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (μg·h/ml)	AUC_{0-t} (μg·h/ml)	Relative Bioavailability (%)[a]
SPC/SDC liposomes	0.79 ± 0.21[b]	2.51 ± 0.52[b,c]	14.28	4.70 ± 0.82[b,c]	3.54 ± 0.48[b,c]	513.04
SPC/CL liposomes	0.74 ± 0.16[b]	1.40 ± 0.61[b]	5.44	2.54 ± 0.44[b]	2.26 ± 0.38[b]	327.54
Micronized fenofibrate capsules	1.14 ± 0.13	0.26 ± 0.05	4.48	0.75 ± 0.35	0.69 ± 0.31	

[a] Calculated on AUC_{0-t} with micronized fenofibrate capsules as reference.
[b] Significant at P < 0.01 vs. micronized fenofibrate capsules.
[c] Significant at P < 0.01 vs. SPC/CL liposomes.

with the AUC_{0-t} value of micronized fenofibrate, the bioavailability of SPC/SDC and SPC/CL liposomes was approximately 5.13- and 3.28-fold higher, respectively.

Given the inferior release rates of liposomes relative to micronized fenofibrate in vitro, it is remarkable that the two liposome formulations exhibited greatly enhanced oral bioavailability of fenofibrate. Previously, excellent in vivo/in vitro correlation (IVIVC) has been observed for fenofibrate fast release formulations by Buch et al. (2008) and our group (data not shown). In this study, one potential drawback of the in vitro assay is that the media contained only low levels of surfactants, thus may not faithfully mimic in vivo digestion. Furthermore, the in vitro assay failed to take into account the possible disruption of liposomal vesicular structure in physiological conditions. Thus, the in vitro release test of oral liposomes should be further optimized to accommodate destabilization of the vesicles and phase transition into mixed micelles (Ariën et al., 1993, 1994; Andrieux et al., 2009).

SPC/SDC liposomes showed 1.57-fold higher oral bioavailability than SPC/CL liposomes. Although both liposome formulations may undergo vesicle–micelle phase transition in the gastrointestinal tract, the superior oral bioavailability of SPC/SDC liposomes indicates an enhancing effect of sodium deoxycholate. Perhaps sodium deoxycholate in lipid bilayers facilitates fast transition from vesicles to mixed micelles. However, other mechanisms likely exist to account for the enhanced absorption. For example, fast release formulations of poorly water-soluble drugs have been shown to promote mixed micelle formation with physiological phosphotidylcholine and bile salts before absorption (Li et al., 1996; Ilardia-Arana et al., 2006), which did not result in enhanced oral bioavailability as observed for fenofibrate in this study. If the vesicles remain stable in the gastrointestinal tract for prolonged periods of time, facilitated uptake by M cells in the Peyer's patch may occur (Rogers and Anderson, 1998; Aramaki et al., 1993). In addition, the ultra-deformability of liposomes containing bile salts may allow carrier-mediated transmembrane absorption. Both mechanisms may contribute to improved bioavailability. Work is currently under way to elucidate the functional mechanisms of oral deformable liposomes containing bile salts.

4. Conclusions

The oral bioavailability of fenofibrate was significantly enhanced by using liposomes containing a bile salt, sodium deoxycholate, compared with the fast release formulation of micronized fenofibrate. The apparent disparity between slow release of incorporated fenofibrate and quick peak time indicates mechanisms other than enhanced release by liposomes that contribute to improved bioavailability. Comparison between SPC/SDC and SPC/CL liposomes highlights the desirable effect of bile salt incorporation on the oral bioavailability of poorly water-soluble drugs.

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