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The characteristics and mechanism of simvastatin loaded lipid nanoparticles to increase oral bioavailability in rats

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ABSTRACT

Simvastatin (SV), a cholesterol-lowering agent, has been widely used in the treatment of hypercholesterolemia, dyslipidemia and coronary heart disease, but SV shows the low oral bioavailability due to its poor aqueous solubility and extensive metabolism by cytochrome-3A system in intestinal guts and liver. In this work, SV loaded lipid nanoparticles (SLNs) with different components were designed to enhance its oral bioavailability, and the plasma concentration of SV and its active metabolite (simvastatin acid, SVA) was determined by LC–MS–MS method. The experimental results showed that SLNs were spherical nano-sized particles with high encapsulation efficiency (>95%). The in situ intestinal absorption indicated that the absorption of SLNs was greatly improved compared with that of free SV, and the absorption was changed with the site of the intestinal segments. SLNs could be uptaken into the enterocytes through both clathrin and caveolae mediated endocytosis pathways. The oral bioavailability of SV after its incorporation into the lipid nanoparticles was improved by 3.37-fold for SLNs I and 2.55-fold for SLNs II compared with that from free SV in rats, and that of the SVA was significantly enhanced as well. As a result, lipid nanoparticles could be a promising delivery system to enhance the oral bioavailability of simvastatin.

1. Introduction

Oral drug delivery is the simplest and major route for the treatment of many diseases, in particular, for cardiovascular or cerebral diseases. However, approximately 30% of selling medicines and 40% of new chemical entities (NCEs) entering development programs showed too low aqueous solubility or oral bioavailability to bring about satisfactory therapeutic efficacy (Gursoy and Benita, 2004). To overcome above problems, many strategies for delivery of poorly water-soluble drugs were exploited, such as designing prodrug (Stella and Nti-Addae, 2007), reducing particle size by micronization (Chaumeil, 1998), using cyclodextrins inclusion and solid dispersion (Brewster and Loftsson, 2007; Vasconcelos et al., 2007), utilizing permeation enhancers or surfactants and entrapping drug in lipid-based formulation (Saha and Kou, 2000; Pouton and Porter, 2008). Recently, more attention has been paid to nanotechnology-based drug delivery systems including biodegradable polymeric nanoparticles (Kumari et al., 2010), smart polymeric micelles (Nishiyama et al., 2005), nanocrystals (Gao et al., 2008) or nanosuspension (Rabinow, 2004), nanoemulsion (Tagne et al., 2008) and solid lipid nanoparticles (Manjunath et al., 2005). In particular, lipid nanoparticles have been especially considered because they are composed of natural or synthetic lipids, and show good biocompatibility, high bioavailability and controlled release of drugs (Jannin et al., 2008; Chen, 2008; Li et al., 2009).

Simvastatin (SV), a cholesterol-lowering agent, has been widely used in the treatment of hypercholesterolemia, dyslipidemia and coronary heart disease. SV is also a potent inhibitor of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase which catalyzes the conversion of HMG-CoA to mevalonate. In addition, as an inactive lactone, SV is rapidly metabolized to its corresponding β-hydroxy acid (simvastatin acid, SVA) after oral administration (Nirogi et al., 2007; De Angelis, 2004). However, SV shows low bioavailability after oral administration due to its poor aqueous solubility and extensive metabolism by cytochrome-3A (CYP3A) system in intestinal guts and liver (De Angelis, 2004). Although several approaches to enhance the dissolution rate and bioavailability of SV have been investigated (Patil et al., 2007; Kang et al., 2004; Lai et al., 2009), the results were not quite satisfying. Recently, it has been reported that Solutol HS-15 could be used to enhance the oral bioavailability of hydrophobic drugs (González et al., 2002; Peltier et al., 2006), and the enzyme activity of CYP3A could be inhibited by Tween 20 or oleic acid (Mountfield et al., 2000; Ren et al., 2008). According to our knowledge, it has not been reported whether the oral bioavailability of SV could be increased by lipid nanoparticles up to now.

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We are interested in enhancing the oral bioavailability of SV by lipid nanoparticles and investigating its intestinal absorption mechanism. In this work, SV loaded lipid nanoparticles (SLNs) composed of HS-15 or Tween 20 and oleic acid were developed, respectively, and their physicochemical characteristics were determined. The in situ intestinal absorption of SLNs was performed in rats, and the intestinal absorption mechanism was investigated as well. The pharmacokinetics was further performed to evaluate the potential of lipid nanoparticles as an oral delivery carrier for SV.

2. Materials and methods

2.1. Materials

Simvastatin was purchased from Apeloa Kangyu Pharmaceuticals (Zhejiang, China). Solutol® HS-15 was purchased from BASF (Germany), Tween 20 and oleic acid (OA) were supplied by the Sinopharm Chemical Reagent Co, Ltd., Miglyol 812 (M812) was kindly gifted by Sasol (Germany), lecithin (S-75) was provided by Lipoid (Germany), Acetonitrile for HPLC and LC–MS–MS was obtained from Merck Co Ltd. All other chemicals and solvents were of analytical reagent grade.

2.2. Animals

Male Sprague–Dawley rats (180–200 g) and male ICR mice (18–20 g) were purchased from Sino-British Sippr/BK Lab Animal Ltd. (Shanghai, China). Animals were maintained on standard food with water freely available under a 12 h light/dark cycle at the Animal Care Facility and acclimatized for at least 3 days before experiments. All animal procedures were performed according to the protocol approved by the Institutional Animal Care and Use Committee of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

2.3. Preparation of SLNs

SLNs were prepared by the emulsification solvent evaporation technology. Briefly, the surfactant, oil, lecithin and SV according to SV:HS-15:M812:S-75 (5:28:14:20, w/w) or SV:Tween 20:OA:S-75 (5:40:20:20, w/w) were dissolved in dichloromethane, and then dropped into double-distilled water (1:3, v/v) under agitation. The mixture was emulsified by sonication with probe (JYD-650, China) for 90 s. Finally, the organic solvent was removed under reduced pressure (Heidolph Laborata 4000, Germany) until the solution was clear.

2.4. Physicochemical characteristics of SLNs

The morphology of SLNs was characterized by atomic force microscopy (AFM, MultiMode™ Veeco) with tapping mode. Briefly, 20 μL of SLNs (1 mg/mL) was pipetted onto freshly cleaved muscovite mica and settled onto the mica for 10 min at room temperature. After the excessive nanoparticles were rinsed in double-distilled water, SLNs were imaged directly in liquid. The particle size distribution and zeta potential were measured by laser light scattering using the NICOMP™ZLS 380 instrument (Particle Sizing System, USA).

The amount of SV incorporated in SLNs was determined by HPLC to evaluate the entrapment efficiency. The free drug was separated from SLNs by ultrafilteration (Molecular cutoff 10,000 Da, Milipore). Meanwhile, a certain volume of SLNs suspension was accurately taken and diluted with methanol to obtain the total amount of SV. The concentration of SV was determined by HPLC system (Waters 2695-2489) with Eclipse XDB-C_{18} column (150 mm × 4.6 mm, I.D. 5 μm, Agilent), the mobile phase: acetonitrile–25 mM monosodium phosphate buffer (pH 4.8) (70:30, v/v); detection wavelength: 238 nm; the flow rate, 1.0 mL/min. The drug loaded in SLNs was calculated with the total amount of SV subtracting that of free drug. The drug entrapment efficiency (EE) and the drug loading (DL) were obtained by the following equations:

$$\mathrm{EE}(\%) = \frac{W_{lp}}{W_{total}} \times 100\%$$

$$\mathrm{DL}(\%) = \frac{W_{lp}}{W_{lipid}} \times 100\%$$

W_{lp}: amount of SV loaded in lipid nanoparticles; W_{total}: total amount of SV; W_{lipid}: weight of lipid in SLNs.

2.5. In vitro release experiment

The *in vitro* release of SV from SLNs was evaluated by dialysis method at 37 °C. Briefly, 1 mL of freshly prepared SLNs suspension was put into the dialysis bags (⌀16 mm, molecular cutoff 14,000 Da) and sealed. The dialysis bags were put into a container with 100 mL of release media (PBS, pH 6.8) and shaken at 200 rpm. Subsequently, 1 mL of release media was withdrawn at certain time intervals and analyzed by HPLC method as described above.

2.6. In situ single-pass intestinal perfusion of SLNs in rats

The rats were abstained from solid food for 16–18 h before experiment and anesthetized with pentobarbital sodium (20 mg/mL, 60 mg/kg) by intraperitoneal injection. SLNs were dispersed in the perfusion fluid (Kerbs Ringer's buffer solution, 7.8 g NaCl, 0.35 g KCl, 1.37 g $NaHCO_3$, 0.02 g $MgCl_2$, 0.22 g NaH_2PO_4 and 1.48 g glucose in 1000 mL purified water), filled in the donor vial bottles and weighted accurately, then maintained at 37 °C for intestinal perfusion. The empty receptor vials was also accurately weighted.

After the abdomen was operated by laparatomy, 10 cm-long segments of duodenum (1 cm distal to pyloric sphincter), jejunum (15 cm to pyloric sphincter) and ileum (20 cm proximal to cecum) were immediately cannulated and ligated with both sides. The intestinal segments were rinsed with pre-warmed perfusion fluid and then equilibrated with SLNs suspension (10 μg/mL) for 45 min at a constant flow rate of 0.22 mL/min with a peristaltic pump (BT100-1F, Longer). Each perfusion experiment lasted for 120 min. At an interval of 15 min, the pre-weighted donor vials and the receptor ones were simultaneously replaced and collected. At the end of experiment, the length and perimeter of the perfused intestinal segments were accurately measured. Each sample was accurately weighted and centrifuged at 15,000 × *g* for 15 min (Biofuge Stratos, Thermo). The supernatant was accurately diluted with methanol and measured by HPLC method. The perfusion of free SV solution (SV dissolved in ethanol and diluted with Kerbs Ringer's buffer) was performed as control. The absorption rate (K_a) and apparent permeability (P_{app}) of SLNs in the intestinal segments were calculated according to the following equations:

$$Ka = \left(1 - \frac{X_{out}}{X_{in}}\right) \frac{v}{\pi r^2 l}$$

$$Papp = \frac{-v \ln(X_{out}/X_{in})}{2\pi r l}$$

where X_{in} was the amount of SV in the donor perfusate, X_{out} was that of SV in the receptor perfusate, v was the flow rate and l was the length of the intestinal segments perfused, while r was the radius of the intestinal segments.

2.7. Uptake of SLNs in the everted intestinal rings

The everted intestinal rings was prepared as previously described methods (Leppert and Fix, 1994; Molina et al., 2007) and applied to evaluate the uptake of SLNs in rat intestine. Briefly, the rats were anesthetized with sodium pentobarbital (60 mg/kg, i.p.). After midline laparotomy, an intestinal segment (15–20 cm) was immediately taken, rinsed in cold Krebs Ringer's buffer saturated with 95% O_2 and 5% CO_2, everted and cut into 30–50 mg rings. The everted rings were placed in the wells of a 24-well plate containing 1 mL of DMEM cell culture media without phenol red (Gibco) saturated with 95% O_2 and 5% CO_2, and incubated for 30 min in a gently shaking atmosphere bath, prior to the absorption experiments. To identify the uptake mechanism of SLNs in rat intestine, the everted intestinal rings were incubated in DMEM at 37 and 4 °C, or in the presence of chlorpromazine (Chlor, 10 μg/mL) or nystatin (25 μg/mL) at 37 °C. Then, SLNs suspension was dropped into the medium with the terminal concentration of SV (10 μg/mL). After 10 min, the everted rings was taken and rinsed with cold Krebs Ringer's buffer. After blotted with filter paper, the everted rings were accurately weighted, and homogenized with cell culture lysis reagent (Promega). SV in the intestinal homogenates was extracted with acetonitrile and determined by HPLC.

To evaluate the damage of the everted intestinal rings, the lactate dehydrogenase (LDH) activity in the culture media was measured by LDH release-assays kit (GMS10073, Genmed), and the leaked protein in the culture media was also determined by BCA protein assay kit (Pierce) to normalize the protein content. The everted rings incubated in PBS (pH 7.4) containing 1.0% Trixton was set as the positive control, and the relative activity of the everted rings was set as the baseline.

2.8. Observation of SLNs in the proximal intestine

To visualize the absorption of SLNs in the proximal intestine, DiI, a hydrophobic fluorescent dye was incorporated into label SLNs. The fluorescently labeled SLNs were given to mice by oral administration (20 mg/kg). After 40 min, mice were sacrificed and the intestine segments (1.5–2.5 cm) were taken, flushed with cold PBS, and frozen in cryoembedding media (OCT) for subsequent cryostat sectioning at 20 μm (CM3050S, Leica). Sections were applied to glass slides, washed twice with cold PBS, fixed with 4% buffered formalin for 10 min at room temperature, stained with DAPI (5 μg/mL) for 10 min, and rinsed in cold PBS. Subsequently, the sections were observed under fluorescence microscopy (Olympus X51, Japan) (Nassar et al., 2008; Benny et al., 2008).

2.9. Pharmacokinetics of SLNs in rats

The rats were fasted overnight (12–18 h) prior to dosing with free access to water. The free SV and two SLNs were administrated orally at a dose of 20 mg/kg. 0.2 mL of blood was withdrawn into heparinized tubes at specified times intervals. Plasma was separated immediately by centrifugation (10,000 × *g* for 10 min at 4 °C) and stored in polypropylene vials below −20 °C until analysis. Methanol–water (1:1, v/v) (25 μL), internal standard solution (lovastatin, 100 ng/mL) (25 μL) and 200 μL of phosphoric acid solution (pH 6.0) were added to rat plasma (25 μL) and mixed. Then, 3 mL of extraction solvent (diethyl ether and dichloromethane, 3:2, v/v) was added to the mixed solution and vortexed for 1 min, reciprocatingly shaken for 10 min (240 rpm), and centrifuged for 5 min (3500 rpm). The organic supernatant was removed and evaporated to dryness under nitrogen at 40 °C. The residue was reconstituted with 100 μL of mobile phase, and an aliquot of 20 μL was injected into the LC–mass spectrometer with Agilent 1100 series model HPLC pump plus auto-sampler for analysis. The concentration of SV and SVA in rat plasma was determined by LC–MS–MS.

For the measurement of SV in rat plasma, the LC separation was achieved on Capcell PAK MG column (100 mm × 4.6 mm, I.D. 5 μm) with a C_{18} guard column (4 mm × 3.0 mm, Phenomenex). The mobile phase consisted of acetonitrile–5 mM ammonium acetate–formic acid (60:40:0.1, v/v/v) with a flow rate of 0.4 mL/min. The column temperature was maintained at 30 °C. The mass spectrometer was operated in positive electrospray ionization (ESI) mode with the nebulization voltage of 4.0 kV and connected to the chromatographic system. Nitrogen was used as the sheath gas (35 Arb) and auxiliary gas (5 Arb) for nebulization. Argon was used as the collision gas at a pressure of 1.2 mTorr and the heated capillary temperature was set to 320 °C. The optimized energies for collision-induced dissociation (CID) of 15 eV were chosen for the determination of SV. Quantification was carried out using selected reaction monitoring (SRM) mode to monitor the precursor → product ion transitions of *m/z* 436.2 ($[M+NH_4]^+$ 436.2) → *m/z* (199 + 285) for SV and *m/z* 422.2 ($[M+NH_4]^+$ 422.2) → *m/z* (199 + 285) for lovastatin (internal standard), with a scan time of 0.2 s.

For the determination of SVA in rat plasma, the LC separation was carried out using a Capcell PAK MG column (100 mm × 4.6 mm, I.D. 5 μm) with a C_{18} guard column (4 mm × 3.0 mm, Phenomenex). The mobile phase was composed of acetonitrile and 5 mM ammonium acetate (90:10, v/v) with a flow rate of 0.5 mL/min. The column temperature was 30 °C. The mass spectrometer was operated in negative ESI mode with the nebulization voltage of 4.0 kV. Nitrogen was used as the sheath gas (35 Arb) and auxiliary gas (5 Arb) for nebulization. Argon was used as the collision gas at a pressure of 1.2 mTorr and the heated capillary temperature was set to 300 °C. The optimized energies for CID of 20 eV were chosen for the determination of SVA. Quantification was carried out using SRM mode to monitor the precursor → product ion transitions of *m/z* 435 ($[M-H]^-$ 436.2) → *m/z* 319 for SVA with a scan time of 0.2 s.

The peak concentration (C_{max}) and the time to C_{max} (T_{max}) were obtained directly from the individual concentration–time profiles, but the area under the concentration–time curve (AUC_{0-t}) was calculated by trapezoidal methods. The relative bioavailability (*F*%) was defined as the AUC_{0-t} of SLNs compared to that of free SV suspension.

2.10. Statistical analysis

The results were presented as mean ± standard deviation (SD). Statistical analysis was performed using a Student's *t*-test. The differences were considered significant for $p < 0.05$, and very significant for $p < 0.01$.

3. Results and discussion

3.1. Preparation and physicochemical characteristics of SLNs

In order to increase the oral bioavailability of SV, two SLNs with different components were prepared by emulsification solvent evaporation technology. SLNs I were consisting of SV:HS-15:M812:S-75 (5:28:14:20, w/w) to evaluate the effect of HS-15 on enhancing the oral bioavailability of SV, and SLNs II were composed of SV:Tween 20:OA:S-75 (5:40:20:20, w/w) to investigate the effect of Tween 20 and OA on the oral delivery of SV because reports demonstrated that Tween 20 and OA could inhibit the enzyme activity of CYP3A system (Mountfield et al., 2000; Ren et al., 2008).

The morphology of SLNs observed by AFM showed that SLNs were spherical or hemispherical nano-sized particles (Fig. 1) with

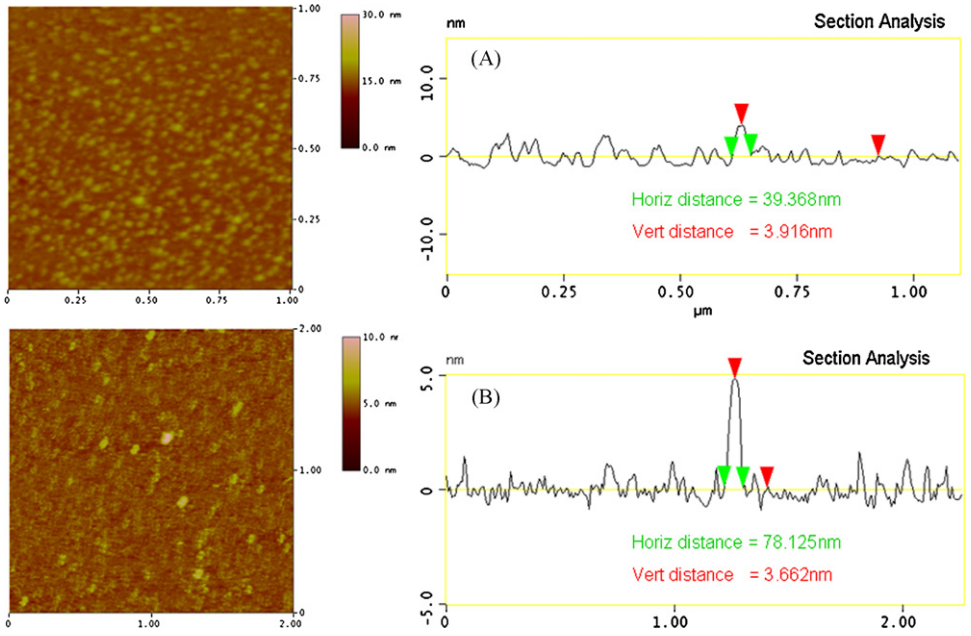

Fig. 1. The morphology of SLNs under atomic force microscopy (AFM). A: SLNs I; B: SLNs II.

the particle size of 39.4 nm (SLNs I) and 78.1 nm (SLNs II), which was in accord with the data measured by laser light scattering with the mean diameter of 48.9 nm (SLNs I) and 68.3 nm (SLNs II). The zeta potential of two SLNs was around −30 mV, and the drug loading was 7.46% (SLNs I) and 5.88% (SLNs II) with the encapsulation efficiency 97.2 and 99.2% for SLNs I and SLNs II, respectively. These results indicated that the physicochemical properties except the particle size were not significantly influenced by the ingredients of SLNs. The solubility of SV in water after its incorporation into SLNs was at least 2 mg/mL, which was improved over 1000-fold than that in water (1.5 ng/mL) (Serajuddin et al., 1991), which could be beneficial for enhancing its oral bioavailability. In addition, no drug crystal was visible under AFM, which could be attributed to the high entrapment efficiency of the lipid nanoparticles. The *in vitro* release profiles of SLNs indicated that the release of SV from SLNs was slow, and less than 10% of SV was released into the media within 12 h (Fig. 2), which could result from the high lipophilicity and good compatibility of SV with the lipid materials in SLNs.

3.2. *In situ* absorption of SLNs in rat intestinal segments

The effects of lipid nanoparticles on the permeability of SV through rat small intestine were evaluated by in situ perfusion of SLNs. It has been reported that phenol red could interfere with the absorption and transport of poor water-soluble drugs in rat intestinal perfusion due to its partial absorption in the intestine (Hu et al., 1996). Gravimetric method was used to calibrate the volume change of the perfusate. The absorption rate (K_a) of SLNs I showed the highest in duodenum, slightly decreased in jejunum, and significantly reduced in ileum compared with the absorption in jejunum ($p < 0.05$) (Fig. 3A). On the contrary, the apparent permeability (P_{app}) of SLNs I did not show significant difference in duodenum, jejunum and ileum (Fig. 3B). To SLNs II, the K_a was maximal in duodenum, and obviously decreased in jejunum and ileum ($p < 0.05$) (Fig. 3A), so was the P_{app} (Fig. 3B). Compared with free SV solution, the K_a of SLNs was significantly enhanced in duodenum and jejunum ($p < 0.05$). The P_{app} of SLNs I was remarkably

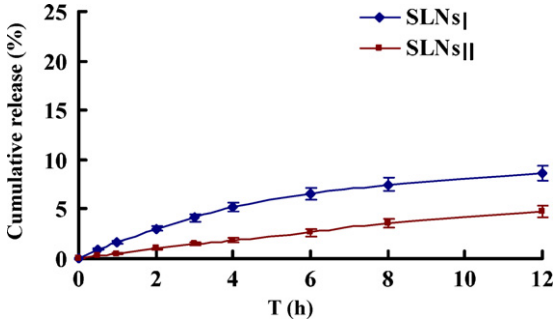

Fig. 2. The *in vitro* drug release of SLNs in PBS (pH6.8).

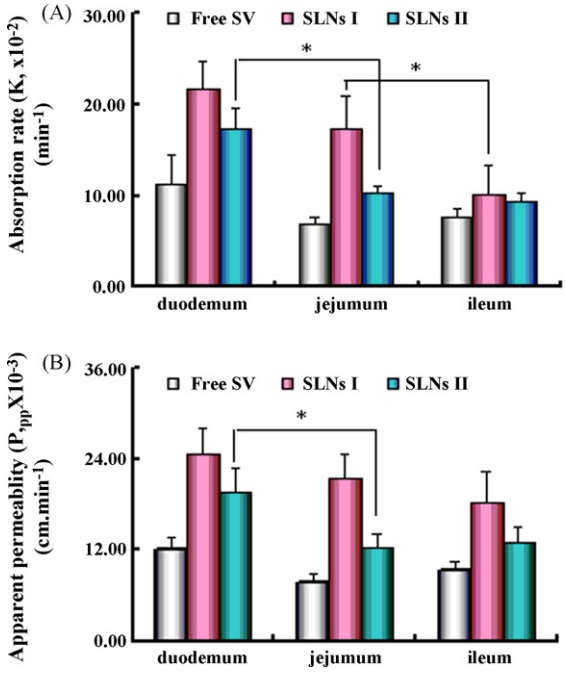

Fig. 3. The in situ absorption of SLNs in rat intestinal segments compared with free SV. A: the absorption rate (K_a); B: the apparent permeability (P_{app}) (*$p < 0.05$).

improved in total intestinal segments in comparison with that of free SV solution ($p < 0.05$). Meanwhile, the P_{app} of SLNs II was evidently increased in duodenum and jejunum, but not statistically enhanced in ileum ($p > 0.05$). In addition, the absorption of SLNs I in rat intestine was slightly better than that of SLNs II, which could be resulted from the particles size difference between them. As a result, it could be deduced that the absorption of SV in rat intestine was greatly enhanced after SV was incorporated into SLNs, and its absorption was changed with the site of the intestinal segments.

3.3. Uptake of SLNs in rat intestine

It has been reported that the absorption of many drugs (Leppert and Fix, 1994; Molina et al., 2007; Iñigo et al., 2006) or lipid-based proliposome (Deshmukh et al., 2008; Potluri and Betageri, 2006) in the intestine could be evaluated by everted intestinal rings or sacs models. Moreover, these reports demonstrated that lipid nanocapsules could be transported through human intestinal membrane (Caco-2 cells) by vesicle-mediated transcytosis including calthrin and caveolae mediated endocytosis (Roger et al., 2009). Therefore, the uptake of SLNs in the intestine was investigated. Firstly, the absorption of SLNs in the everted intestinal rings was performed at 37 and 4 °C because the endocytosis process could be blocked at 4 °C. The results showed that the absorption of SLNs in the everted intestinal rings at 4 °C was only about 10% of that at 37 °C (Fig. 4A and C), which indicated that SLNs could be endocytosed into the enterocytes during the absorption in rat intestine. Secondly, the absorption experiments were performed in the presence of specific inhibitors to know the endocytosis pathway of SLNs in enterocytes. It was reported that chlorpromazine could disturb the intracellular clathrin processing and inhibit the clathrin-mediated endocytosis (Von Gersdorff et al., 2006; Roger et al., 2009), and nystatin could inhibit the caveolae-mediated endocytosis (Mcfarland et al., 2004). The experimental results showed that the absorption of SLNs I was reduced by 44% after the treatment of chlorpromazine, and 30% when the everted rings were incubated with nystatin (Fig. 4B). Similarly, the uptake of SLNs II decreased over 40% in the presence of chlorpromazine, and about 30% after nystatin was added into the incubation media (Fig. 4D). As a result, the uptake of SLNs was significantly reduced when the everted intestinal rings were treated with chlorpromazine or nystatin, which could be speculated that both clathrin and caveolae mediated endocytosis pathways were involved in the uptake of SLNs in rat intestine.

The viability of the everted intestinal rings was also evaluated by measuring the LDH activity and the protein content in the culture media. The results indicated that the viability of the everted rings incubated at 4 °C or in the presence of different inhibitors did not show significant difference in comparison with that at 37 °C (Fig. 4E), which implied that the intestinal tissues had high activity and could assure the effective uptake of SLNs in the everted intestinal rings.

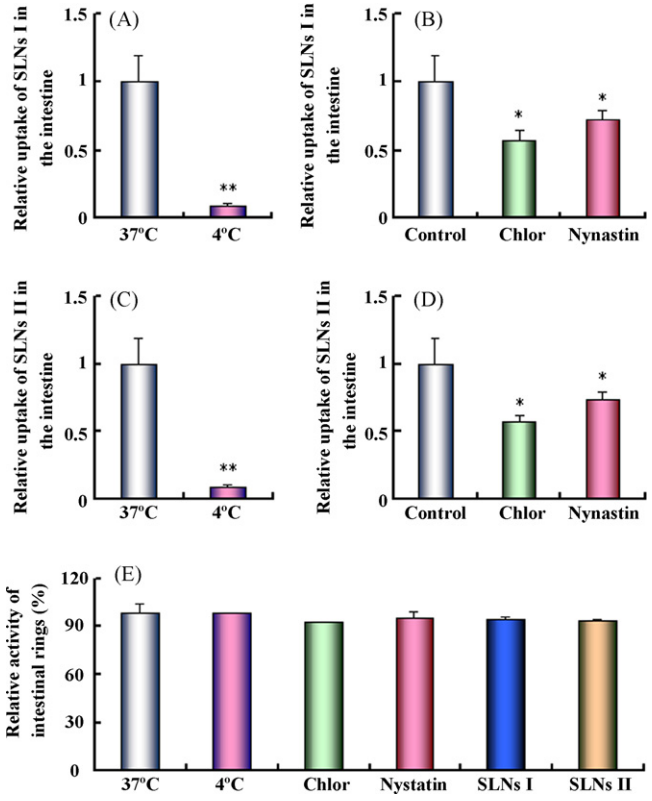

Fig. 4. Uptake of SLNs in everted intestinal rings in different conditions. A: Uptake of SLNs I at 37 and 4 °C (**$p < 0.01$); B: uptake of SLNs I in everted intestinal rings treated with specific inhibitors (10 μg/mL chlorpromazine or 25 μg/mL nystatin) (*$p < 0.05$); C: effect of temperature on the uptake of SLNs II (**$p < 0.01$); D: effect of specific inhibitors on the uptake of SLNs II; E: the tissue activities of the everted intestinal rings (*$p < 0.05$).

3.4. Absorption observation of SLNs in the proximal intestine

It has been reported that the absorption of polymeric micelles or lipid nanocapsules in the intestine could be observed by the incorporation of fluorescent dyes in the nano-delivery system (Nassar et al., 2008; Benny et al., 2008). In order to visualize the absorption of SLNs in the proximal intestine by microscopy, DiI was used to label SLNs. Meanwhile, DAPI was utilized to label the nucleus of the intestine section as a contrast. The experimental results showed that numerous SLNs (red) (for interpretation of the references to color in this sentence, the reader is referred to the web version of the article) could be detected in the cytoplasma of the enterocytes (Fig. 5), which indicated that the orally administrated SLNs were rapidly absorbed into the enterocytes from the intestine tract, even though the diffusion of the fluorescent marker could not be excluded. In addition, the above results (Section 3.3) also showed that SLNs could be absorbed into the enterocytes by endocytosis. Consequently, due to the rapid absorption, it could be suggested that SLNs were absorbed into the intestine before degradation in the intestine lumen, which could be beneficial for enhancing the oral bioavailability of SV.

3.5. Pharmacokinetics of SLNs in rats

It has been reported that SV must be biotransformed into its active metabolite SVA before it produced pharmacological activity in antihyperlipidemics (Nirogi et al., 2007). As a result, both SV and SVA in rat plasma were determined to evaluate *in vivo* pharmacokinetic behavior. After two SLNs and free SV suspension were orally administered, the concentration of SV and SVA in rat plasma was determined by LC–MS–MS method. The concentration–time curve was showed in Fig. 6, and the pharmacokinetic parameters were summarized in Table 1. The results showed that the significant difference occurred between the pharmacokinetic profiles of two SLNs and free SV suspension (Fig. 6A and C). The peak concentration (C_{max}) of SV in rats plasma after oral administration of SLNs I and SLNs II was 2.83 and 3.53 times of that from free SV suspension, while the T_{max} did not show statistical difference ($p > 0.05$). In particular, the area under the concentration–time curve (AUC_{0-t}) of SV was 24.05 ng h/mL for SLNs I and 18.23 ng h/mL for SLNs II, which was 3.37- and 2.55-fold compared with that of free SV suspension (7.14 ng h/mL) (Fig. 6B). On the other hand, the C_{max} of SVA after oral administration of SLNs I and SLNs II increased by 3.02 and 4.31 times, and the AUC_{0-t} of SVA was improved over 2-fold

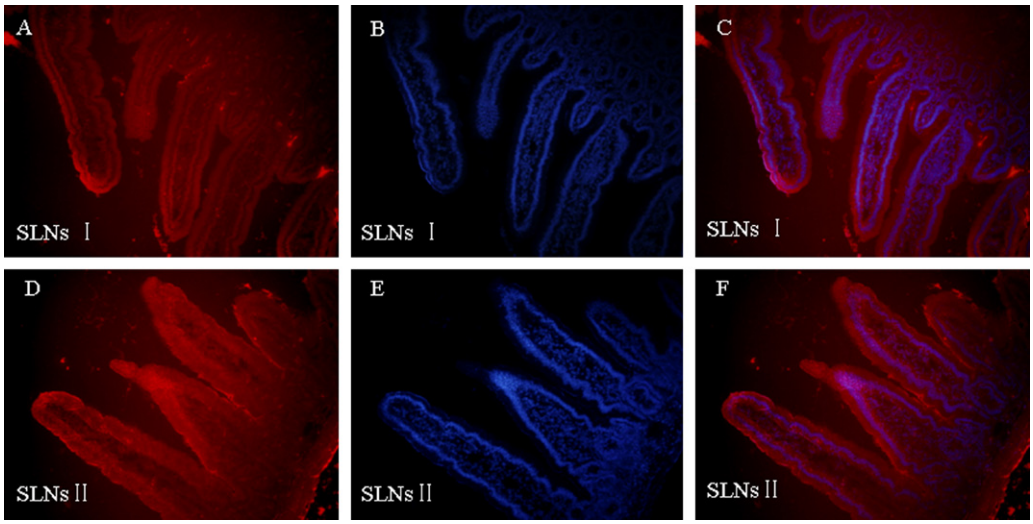

Fig. 5. Fluorescence photomicrographs of histological section of mice proximal intestine at 40 min after oral gavage of SLNs (1 mg/mL) loaded with DiI (0.06 mg/mL) (A and D). B and E: The nucleus of the section dyed with DAPI. C and F: The merged photomicrographs (20×).

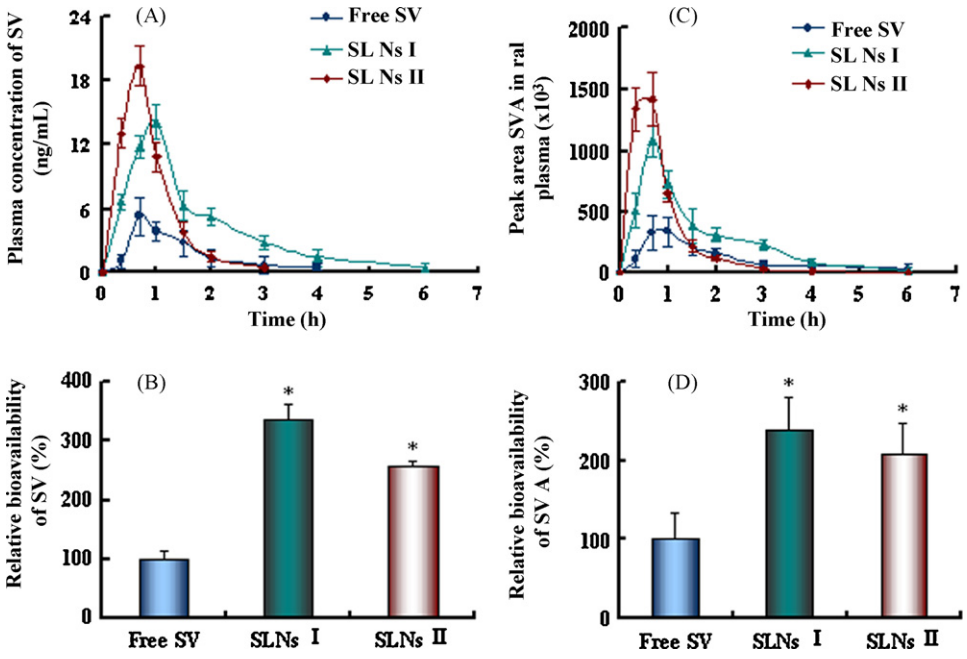

Fig. 6. The concentration–time profiles of SV (A) and SVA (C) in rats plasma after a single oral dose (20 mg/kg) of free SV suspension and SLNs ($n = 4–5$). The relative oral bioavailability of SV (B) and SVA (D) from SLNs in rats compared with free SV suspension was calculated as well (*$p < 0.05$).

Table 1
Pharmacokinetics of SV and SVA after oral administration of SLNs and free SV in rats (20 mg/kg).

	SV			SVA		
	Free SV	SLNs I	SLNs II	Free SV	SLNs I	SLNs II
T_{max} (h)	0.78 ± 0.19	1.06 ± 0.42	0.56 ± 0.20	0.92 ± 0.17	0.56 ± 0.20	0.56 ± 0.20
C_{max} (ng/mL)	5.68 ± 2.43	16.11 ± 2.76	20.07 ± 1.32	357 ± 138[a]	1079 ± 148[a]	1541 ± 332[a]
AUC_{0-t} (ng h/mL)	7.14 ± 1.03	24.05 ± 1.63	18.23 ± 0.68	692 ± 233[a]	1640 ± 304[a]	1442 ± 274[a]
$AUC_{0-\infty}$ (ng h/mL)	8.11 ± 1.19	24.85 ± 2.19	18.57 ± 0.81	717 ± 248[a]	1668 ± 329[a]	1443 ± 273[a]
MRT (h)	1.96 ± 0.73	1.80 ± 0.66	0.88 ± 0.13	2.14 ± 0.08	1.72 ± 0.18	0.88 ± 0.08
$T_{1/2}$ (h)	1.09 ± 0.42	1.22 ± 0.68	0.39 ± 0.07	1.17 ± 0.05	1.24 ± 0.51	0.58 ± 0.15
F (%)	–	336.8	255.3	–	237.0	208.3

Each value represents the mean ± SD ($n = 4–5$).
[a] Calculated as the peak area (×10^3) of SVA in rat plasma.

compared with that of free SV suspension (Fig. 6D). Reports have demonstrated that the low oral bioavailability of SV mainly resulted from its poor solubility and the extensive metabolism by the CYP3A system in intestinal guts and liver (Nirogi et al., 2007; De Angelis, 2004). In this work, the obtained results indicated that the aqueous solubility (see Section 3.1) and intestinal absorption (see Section 3.2) was significantly improved after SV was incorporated in SLNs. Moreover, as the results showed that SV was almost encapsulated in SLNs (see Section 3.1) and could be absorbed into the enterocytes by endocytosis (see Sections 3.3 and 3.4), the exposure of SV to the CYP3A system in the intestinal guts could be partially reduced during the absorption process, which could result in the enhancement of C_{max} and oral bioavailability of SV and SVA. As a result, the relative oral bioavailability of SV and SVA from SLNs was greatly improved after the incorporation of SV into the lipid nanoparticles. In addition, the C_{max} of SV and SVA from SLNs II increased over 20% compared with that from SLNs I, which could attribute to the different components of SLNs because the enzyme activity of CYP3A could be inhibited by Tween 20 and OA (Mountfield et al., 2000; Ren et al., 2008). On the contrary, the AUC_{0-t} of SV and SVA from SLNs II was not improved in comparison with that from SLNs I. The reason may lie in the more rapid elimination of SV from SLNs II than that from SLNs I, as the $T_{1/2}$ of SV and SVA from SLNs II was significantly reduced compared with that of SLNs I ($p < 0.05$), as well as the higher particle size (see Section 3.1) and lower intestinal absorption (see Section 3.2) of SLNs II.

4. Conclusions

The lipid nanoparticles containing simvastatin were developed and characterized. The SLNs exhibited nanometer range spherical structure characterized by AFM and laser light scattering. The absorption of SLNs in rat intestine was greatly improved compared with that from free SV, and the absorption was changed with the site of the intestinal segments. Both clatrhin and caveolae mediated endocytosis could be included in the uptake of SLNs in rat intestine. The relative oral bioavailability of SV and SVA was significantly enhanced compared with that from free SV suspension. These results suggested that lipid nanoparticles could be a promising delivery system to enhance the oral bioavailability of simvastatin.

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